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Processive Ubiquitin Chain Formation by the Anaphase-Promoting Complex

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Abstract

Progression through mitosis requires the sequential ubiquitination of cell cycle regulators by the anaphase-promoting complex, resulting in their proteasomal degradation. Although several mechanisms contribute to APC/C regulation during mitosis, the APC/C is able to discriminate between its many substrates by exploiting differences in the processivity of ubiquitin chain assembly. Here, we discuss how the APC/C achieves processive ubiquitin chain formation to trigger the sequential degradation of cell cycle regulators during mitosis.

Keywords

ubiquitin; anaphase-promoting complex; APC/C; Ube2C; Ube2S; processivity

Introduction

During mitosis, eukaryotic cells undergo dramatic structural reorganizations that allow them to evenly distribute the genetic material between their daughter cells. To accomplish this with high precision, the dividing cells need to adhere to a carefully scripted and highly conserved program that starts with nuclear envelope breakdown and chromosome condensation and proceeds through spindle formation, sister chromatid separation and cytokinesis. How the sequence of mitotic reactions is established is an area of intense research.

Among the many pathways controlling mitosis, protein degradation has emerged as a key player in orchestrating the series of events that leads to successful cell division. In eukaryotes, most proteins are degraded by the 26S proteasome [1]. This tightly regulated, compartmentalized protease recognizes its substrates after these have been covalently modified with ubiquitin chains connected through Lys11 or Lys48 of one ubiquitin molecule and the C-terminus of the next ubiquitin [2-4]. Underscoring the importance of protein degradation for mitosis, the inhibition of the 26S proteasome or loss of K11- or K48-linked ubiquitin chain formation interfere with cell division in multiple organisms [4, 5].

The attachment of proteolytic ubiquitin chains to cell cycle regulators requires the collaborative effort of at least three enzymatic activities, referred to as E1, E2, and E3. An

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E1 activates ubiquitin in an ATP-dependent reaction by forming a thioester bond between a Cys residue at its active site and the C-terminus of ubiquitin [6]. Following activation, ubiquitin is transferred to a Cys residue in an E2 active site [7]. Ubiquitin-charged E2 enzymes are then recognized by E3s, the majority of which contain a signature RING-domain [8, 9]. These RING-E3s bind a charged E2 and a specific substrate at the same time, thereby promoting the transfer of ubiquitin from the E2 active site to a substrate lysine. When these reactions are repeated, but Lys residues in substrate-attached ubiquitin molecules are modified, polymeric ubiquitin chains are being generated.

Although several of the ~600 human E3s have been ascribed critical roles during mitosis [10], it is one RING-E3, the anaphase-promoting complex (APC/C), that is essential for establishing the correct temporal order of mitotic events [11]. Rather than degrading all its substrates at the same time, the APC/C ubiquitinates its targets only after they have fulfilled their mitotic functions. This creates a sequence of APC/C-dependent degradation reactions: inhibitors of sister chromatid separation are degraded prior to proteins required for spindle elongation, and those are ubiquitinated before components of the cytokinetic machinery (Figure 1; [12-14]). At the end of this proteolytic sequence, the APC/C promotes the degradation of its own E2 enzymes to shut off its mitotic activity [15, 16]. By degrading key regulators at specific times during cell division, the APC/C orchestrates progression of cells through mitosis.

Given its many substrates, determining the proper time of substrate degradation is no easy task for the APC/C. Based on the current literature and unpublished screens performed in this laboratory, the human APC/C can be estimated to have more than 100 substrates (Table S1), and indeed, activation of this global mitotic player leads to a dramatic increase in the abundance of K11-linked ubiquitin chains, the product of human APC/C-activity [17]. Due to its importance for accurate cell division, the ability of the APC/C to discriminate between its substrates is controlled by multiple mechanisms. Early in mitosis, the spindle assembly checkpoint inhibits the APC/C to prevent chromosome missegregation [18]. However, few APC/C-substrates, such as cyclin A or Nek2A, are degraded under these conditions, thereby moving these proteins to the top of the proteolytic line [19, 20]. Once the spindle checkpoint has been satisfied, the APC/C ubiquitinates a small number of proteins, including cyclin B1, that are delivered by a WD40-repeat protein, Cdc20 [21, 22]. This burst in APC/C-activity results in a drop in cyclin B1-levels and leads to the activation of a different APC/C-adaptor with much broader substrate spectrum, Cdh1 [23-25]. Thus, the switch from Cdc20 to Cdh1 provides another layer of ordering mitotic degradation events. Finally, fully active APC/C^{Cdh1} degrades cell cycle regulators in a defined sequence, which is required for coordinating spindle elongation, cytokinesis, and APC/C-inactivation in cells. APC/C^{Cdh1} achieves this feat by exploiting differences in the processivity of ubiquitin chain formation between substrates [26].

To comprehend the complexity and regulation of mitotic degradation reactions, the mechanism of ubiquitin chain formation by the APC/C has to be fully understood. Here, we discuss how the APC/C catalyzes processive ubiquitin chain formation and how differences in substrate recognition and ubiquitin transfer can be translated into sequential substrate degradation during mitosis. Integrating intrinsic means of substrate discrimination by the APC/C with pathways of APC/C-regulation allows us to propose a comprehensive model of temporal APC/C-control during mitosis.

The APC/C requires multiple E2 enzymes to catalyze ubiquitin chain formation

The RING-E3 APC/C catalyzes ubiquitin chain formation by simultaneously recruiting substrates and cognate E2 enzymes [7]. In both yeast and human cells, distinct E2s collaborate with the APC/C to initiate and elongate ubiquitin chains, respectively. In *S. cerevisiae*, Ubc4, Ubc5 or Ubc11 are able to catalyze the transfer of the first ubiquitin to substrate Lys residues to initiate chain formation [5]. Ubc4 is expressed in proliferating cells, while Ubc5 and Ubc11 are upregulated during meiosis [27-29]. Following initiation, the E2 Ubc1 promotes the elongation of K48-specific ubiquitin chains [5, 30]. Similar to the well-characterized E2 Cdc34 [31], Ubc1 is capable of modifying substrate Lys residues, but chain elongation is kinetically preferred over initiation. Both Ubc4 and Ubc1 need to bind the RING-domain of APC11 for activation, and thus cannot be active simultaneously on the same APC/C.

In fission yeast, the APC/C also promotes ubiquitin chain formation by employing two distinct E2 enzymes, UbcP1 and UbcP4 [32, 33]. While it has not been reconstituted *in vitro*, several observations suggest that UbcP4 and UbcP1 act during chain initiation and elongation, respectively. Deletion of UbcP1 or UbcP4 both stabilize the *S. pombe* cyclin B1-homolog, and the resulting cell cycle arrest is not rescued by overexpression of the respective remaining E2 [32, 33]. Furthermore, while no chains were detected in strains lacking UbcP4, the deletion of UbcP1 led to the accumulation of short chains on APC/C-substrates [33]. These phenotypes are consistent with UbcP4 modifying Lys residues in substrates, while UbcP1 might extend ubiquitin chains. Both UbcP1 and UbcP4 are “classical” E2 enzymes that contain all elements required for recognition by RING-E3s [7], suggesting that these E2s also act sequentially to catalyze ubiquitin chain formation by the *S. pombe* APC/C.

In higher eukaryotes, the APC/C appears to have evolved mechanisms that increase the efficiency of ubiquitin chain formation. At least in *Drosophila*, *Xenopus*, and human cells, the APC/C utilizes specialized E2 enzymes that so far have only been shown to function with the APC/C, but no other E3 [4, 15, 16, 34-38]. In addition, the two APC/C-specific E2s of higher eukaryotes do not compete for the same binding site on the APC/C, suggesting that they can truly cooperate during chain formation [16, 39].

The first of the APC/C-specific E2s, Ube2C (UbcH10, UbcX, Vihar), acts as the chain-initiating enzyme that links the first ubiquitin molecules to the substrate [4, 16]. To achieve this with high efficiency, Ube2C requires degenerate sequence motifs in APC/C-substrates, the TEK-boxes, which help to expose substrate Lys-residues to the active site of Ube2C [4]. Interestingly, ubiquitin contains a similar TEK-box surface close to Lys11, allowing Ube2C to assemble short chains that are preferentially linked through K11 of ubiquitin [4, 39]. Besides Ube2C, the Ube2D/UbcH5-family of E2s can initiate chain formation on APC/C-substrates *in vitro* [16, 37, 38]. However, these E2s interact with hundreds of E3s in human cells, suggesting that their available concentration for the APC/C is very low [40]. Furthermore, Ube2D does not display any specificity for ubiquitin Lys residues and thus generates linkages that are not readily susceptible to elongation by Ube2S [41]. Finally, the depletion of all four Ube2D-family members by siRNAs did not yield mitotic phenotypes [16, 42], in contrast to reduced levels of Ube2C [15, 32, 34-36, 43-45]. These observations suggest that the majority of initiation events for human APC/C depend on a specific E2, Ube2C.

Chain elongation by the APC/C of higher eukaryotes is specifically catalyzed by a second dedicated E2, Ube2S [16, 37, 38]. Ube2S binds to a different site on the APC/C than Ube2C,

suggesting that it acts independently of the RING-domain [16, 39]. Under normal conditions, Ube2S exclusively modifies Lys11 of ubiquitin, but no other residue [16, 38, 39]. To achieve linkage-specificity, Ube2S recognizes the TEK-box in the acceptor ubiquitin, which is fully exposed in K11-linked ubiquitin chains, but not necessarily in chains of other topologies [39]. The recognition of this acceptor ubiquitin surface around Lys11 brings Glu34 of ubiquitin in proximity to the catalytic cysteine of Ube2S, which is required to form a catalytically competent active site composed of residues of Ube2S and ubiquitin itself; thus, linkage-specific chain formation by Ube2S occurs through substrate-assisted catalysis [39]. The co-depletion of Ube2C and Ube2S ablates formation of K11-linked ubiquitin chains in mitotic cells, strongly stabilizes APC/C-substrates, and results in cell cycle arrest [16, 17, 39]. Similar to loss of Ube2C and Ube2S, the inhibition of K11-linked chain formation by injection of a ubiquitin mutant lacking Lys11 blocked cell division in *Xenopus* embryos [4]. These observations lend strong support to the notion that Ube2C and Ube2S form the physiological E2 module of the APC/C in higher eukaryotes. Together, the APC/C, Ube2C, and Ube2S catalyze the formation of K11-linked ubiquitin chains to orchestrate progression of cells through mitosis.

The APC/C catalyzes processive ubiquitin chain formation

Both yeast and human APC/C catalyze ubiquitin chain formation processively, in that they are able to transfer more than one ubiquitin molecule to a growing chain within a single substrate-binding event [26, 46]. The processivity of ubiquitin chain formation by the APC/C is determined by the ratio between the catalytic rates of ubiquitin chain initiation and elongation ($k_{\text{cat, initiation}} + k_{\text{cat, elongation}}$) and the rate for substrate dissociation (k_{off}) [47]. Differences in the rates of catalysis and dissociation can lead to distinct degrees of processivity for various substrates.

The most straightforward way to generate processivity in chain formation is to limit the dissociation of a substrate from the APC/C. This has been demonstrated for yeast Hsl1, which binds APC/C^{Cdh1} with high affinity and is ubiquitinated with high processivity [48-50]. The APC/C engages its substrates through a core subunit, APC10, and its adaptors Cdc20 or Cdh1 [23, 49, 51]. Together, APC10 and Cdc20/Cdh1 bind to sequence motifs, the D-boxes, which can be found in almost every APC/C-substrate [49, 51, 52]. Accordingly, the deletion of APC10, alterations in its conserved ligand binding loop, or mutations in the D-box sequence of a substrate abolish processive chain formation by the APC/C [26, 46, 48]. In addition to their association via a D-box, some human APC/C substrates, such as cyclin B or Nek2A, engage in additional interactions with core subunits of the APC/C, and the resulting multivalent binding to the APC/C results in an increase in their processivity of ubiquitin chain formation [20, 53].

Besides substrate dissociation, the catalytic rate of ubiquitin transfer is crucial to determining the processivity of chain formation. For yeast APC/C, the initiating E2 Ubc4 can modify several substrate Lys residues during a single substrate-binding event [46, 48]. Similar to Ubc4, the human initiating E2 Ube2C can transfer multiple ubiquitin molecules in a single substrate-binding event, which results in the modification of several substrate Lys residues or the formation of short K11-linked ubiquitin chains [4, 26, 46]. Recent experiments showed that the rate of chain initiation differs between APC/C-substrates, depending on the presence of TEK-box sequences that help expose substrate Lys residues to the active site of Ube2C ([4]; Williamson et al., in revision). In addition, the capacity of Ube2C to assemble short ubiquitin chains can vary between substrates, potentially due to differences in their mode of interaction with the APC/C [26]. Qualitative analyses suggested that initiation by Ube2C occurs much more slowly than chain elongation by Ube2S [16], an observation that is consistent with a recent detailed kinetic analysis of chain formation by a

related cell-cycle E3, the SCF [54]. Moreover, the addition of Ube2C to cell extracts can dramatically increase the degradation rate for several APC/C-substrates, while the loss of Ube2C during G1 coincides with the APC/C-substrate stabilization [4, 15]. Thus, initiation by Ube2C appears to be a rate-limiting step for chain formation by the APC/C. As a result, substrate motifs that promote ubiquitin chain initiation are likely to have strong effects on the processivity of ubiquitin chain formation by the APC/C.

Following initiation, the elongating E2s of yeast and human APC/C, Ubc1 and Ube2S, catalyze chain elongation with high processivity [5, 16, 39]. Ube2S, for example, is able to add up to 13 ubiquitin molecules to cyclin A in a single substrate-binding event [39]. The efficiency of Ube2S to extend ubiquitin chains is comparable to the SCF-E2 Cdc34, which can transfer up to 14 ubiquitin molecules to a growing chain within a single binding event of its substrate -catenin [54]. The high processivity of Ube2S and the human homolog of Cdc34, Ube2R1, depend on a non-covalent interaction between the donor ubiquitin molecule and the E2 to which this donor is connected via the thioester bond [39]. This interaction ensures that the donor ubiquitin molecule does not interfere with recognition of the acceptor ubiquitin, and it also places the donor ubiquitin into a perfect position for nucleophilic attack by the acceptor Lys11. NMR-based docking analyses suggested that Ubc1 engages in a similar non-covalent interaction with the donor ubiquitin [55]. Ubc1 also utilizes a second non-covalent ubiquitin-binding site, its UBA-domain, to increase the processivity of chain elongation [5], while comparable interactions with donor ubiquitin appear to be less important for the function of chain-initiating E2s [56]. Consistent with being a highly efficient enzyme, the addition of Ube2S to extracts does not further accelerate protein degradation [38], and reducing Ube2S levels in cells produces weaker phenotypes as observed upon Ube2C-depletion [16].

Finally, dimerization of the APC/C has been proposed to increase the processivity of chain formation [51, 57]. The dimerization of an E3 can provide multiple interaction sites for substrates that have more than one E3-binding motif. Even if one substrate motif disengages from the E3, the substrate remains bound to the dimer and might still be competent for ubiquitination, a feature known to contribute to the processivity of ubiquitin chain formation by the E3 SCF^{Cdc4} [58]. The dimerization of an E3 might also increase the local concentration of E2s that participate in chain formation. Consistent with these hypotheses, APC/C-dimers have been observed *in vitro* [51, 57], and many APC/C-substrates have multiple interaction motifs, such as D- and KEN-boxes [24, 50, 59-66] (Table S1). However, whether APC/C dimerizes in cells and whether this event plays a general role in determining the processivity of chain formation remains unclear.

The processivity of ubiquitin chain formation by the APC/C provides a blueprint for substrate degradation during mitosis

An *in vitro*-analysis of APC/C-dependent ubiquitination reactions found that differences in the processivity of chain formation between substrates correlate well with the timing of their degradation [26]. Processive substrates, such as cyclin B1 or securin, are rapidly degraded upon full APC/C-activation at the metaphase-anaphase transition, whereas distributive substrates, such as Aurora A or Plk1, are degraded much later in mitosis [21, 22, 67, 68]. The most distributive substrate of the APC/C, its E2 Ube2C, is degraded even later during G1 [15]. These findings suggest that the processivity of ubiquitin chain formation provides a blueprint for the sequence of APC/C-dependent degradation reactions during mitosis.

How can variations in the processivity of ubiquitin chain formation be translated into differences in the timing of degradation? The proteasome recognizes its substrates only after these have been modified with ubiquitin chains of at least four molecules [2, 3]. The

assembly of such a polymeric ubiquitin chain is slower for a distributive substrate, which has to undergo multiple association and dissociation events for chain buildup, as opposed to a processive substrate that needs to bind the APC/C only once. This situation is reminiscent to multisite-phosphorylation, which is completed much more rapidly on processive than on distributive substrates [69]. Thus, the requirement for a ubiquitin chain, rather than a single ubiquitin, to trigger degradation introduces a delay between the initial recognition of a distributive substrate by the APC/C and its turnover by the proteasome [67].

Distributive substrates, therefore, need to associate with the APC/C multiple times to obtain a proteolytic ubiquitin chain. The initial excess of substrates (estimated concentration of the >100 substrates: ~5-10 μ M) over active APC/C (concentration in human cells ~80nM; [70, 71]) generates a competitive environment that impedes the rebinding of a dissociated substrate during early stages of mitosis. Indeed, global interaction studies found evidence for the preferential binding of processive over distributive substrates to the APC/C [72], and even slight increases in the levels of a processive substrate, such as securin, result in a block of APC/C-substrate degradation and mitotic arrest [73]. This type of regulation can also be reconstituted *in vitro*: if identical levels of a processive and a distributive substrate are exposed to the APC/C at the same time, ubiquitin chains will almost exclusively be formed on the processive substrate [26]. As a result, the probability of degrading a distributive substrate increases at later stages of mitosis, when the levels of competing substrates have been reduced.

Reminiscent of phosphatases in multisite phosphorylation [74], deubiquitinating enzymes (DUBs) enhance differences in the rate of APC/C-binding between distributive substrates [75]. By contrast, processive substrates that only require a single APC/C-binding event to obtain a degradation-competent ubiquitin chain are less sensitive to DUB-activity. Several DUBs were found to play important roles in mitotic control [76-82]. Furthermore, DUBs were shown to associate with the APC/C, as seen in proteomic analyses [83] or as implicated by functional studies that found DUB-activity to co-purify with APC/C from human or *Xenopus* sources [5, 26]. However, DUBs that are specific for K11-linked ubiquitin chains and have an important role in mitosis, remain unknown, leaving the importance of this class of enzymes for the correct timing of APC/C-substrate degradation poorly understood.

In the presence of DUBs, the activity of an E3 has to be above a certain threshold to achieve the degradation of distributive substrates [47]. If the activity of an E3 is too low, chain formation is slow compared to deubiquitination and substrates are stabilized. During G1, the activity of the APC/C is strongly reduced by the degradation of its E2s Ube2C and Ube2S and its activator Cdh1 [15, 16, 84]. As a result, distributive substrates are stabilized under these conditions, while processive substrates can still be degraded [15] (Williamson et al., in revision). This type of regulation allows APC/C-substrates with roles in S phase entry or DNA replication, such as cyclin A, Cdc6 or Cdt1, to accumulate already during late G1 [15, 26, 85].

Together, variations in the processivity of ubiquitin chain formation, amplified by a limiting APC/C-concentration and counteracting DUBs, can lead to differences in the timing of APC/C-substrate degradation. This mechanism, akin to kinetic proofreading [86], thus provides a blueprint for substrate degradation during mitosis.

Substrate-specific mechanisms to regulate processive ubiquitin chain formation by the APC/C

To achieve precise control over the degradation time for specific APC/C-substrates, differences in the processivity of chain formation or the sensitivity towards DUBs can be further modulated [87]. Increasing the affinity of a substrate for the APC/C, for example, should improve the processivity of ubiquitin chain formation and accelerate degradation. A point in case is made by the APC/C-substrate cyclin B1, which is found in complexes with the kinase Cdk1 and the accessory factors Cks1 or Cks2 [88]. Cyclin B1 is recognized by the APC/C through a canonical degron, the D-box [89]. In addition, Cks1/2 contains a phosphorylation-binding motif that enables the cyclin B/Cdk1/Cks-complex to associate with APC/C-subunits that are phosphorylated during mitosis [72, 90-92]. In fact, the interaction between the fission yeast Cks1-homolog p13^{Suc1} and phosphorylated APC/C is sufficiently stable to allow purification of the APC/C [90]. The two independent APC/C-binding motifs in the cyclin B1/Cdk1/Cks-complex lead to a multivalent interaction that is required for the very rapid degradation of cyclin B1 in early anaphase [88].

Similar to cyclin B1, cyclin A also requires Cdk1- and Cks-binding for its correctly timed degradation during mitosis [53, 93]. Cyclin A can also be degraded during activation of the spindle checkpoint, which prevents the recognition of most competing substrates by the APC/C [19, 94]. Together, the increase in affinity upon Cks-binding to phosphorylated APC/C and the lack of competition due to checkpoint activation might allow cyclin A to be turned over among the very first APC/C-substrates. Cyclin A is already re-synthesized during late G1, when it binds Cdk2 to phosphorylate Cdh1 and inhibit the APC/C [15, 26, 95, 96]. At this time of the cell cycle, Cks has been degraded by the APC/C [97], and APC/C has been dephosphorylated [98-100]. The APC/C has also turned over its own E2s Ube2C and Ube2S, which reduces the catalytic rate of APC/C-dependent ubiquitination reactions [15, 16]. Finally, reports suggest that early synthesis of an S-phase APC/C-inhibitor, Emi1, during late G1 would further inactivate APC/C [101, 102]. Together, the decrease in APC/C-affinity of cyclin A and the reduction in general APC/C-activity turn cyclin A into a distributive substrate that is able to bind APC/C^{Cdh1} without being degraded [26]. Fine-tuning the processivity of chain formation can, therefore, affect the timing of degradation during mitosis as well as that of substrate accumulation during G1.

Rather than stimulating ubiquitin chain formation, reversible protein interactions are also able to decrease the affinity of substrates for the APC/C to prevent premature degradation. This regulatory mechanism has been described for the spindle assembly factors HURP and NuSAP, which are stabilized through their binding to importin- β [103]. Importin- β associates with motifs in HURP and NuSAP that overlap with D-, KEN-, and TEK-boxes, thereby blocking the recognition of these degrons by the APC/C. The small GTPase Ran, which accumulates in an active, GTP-charged state close to chromatin [104], can dissociate HURP or NuSAP from importin- β , thereby allowing for their ubiquitination by the APC/C. Similarly, modifications occurring in the proximity of D- or KEN-boxes can protect substrates from ubiquitination by the APC/C, as described for phosphorylation of securin during metaphase or of Cdc6 during G1 [85, 105]. These observations suggest that several mechanisms can impinge on the processivity blueprint for mitotic protein ubiquitination to fine-tune the timing of substrate degradation.

As Ube2C appears to be rate limiting for APC/C-dependent protein degradation, it can be predicted that regulating the efficiency of chain initiation should also affect the processivity of chain formation and timing of substrate degradation. Although this has yet to be demonstrated experimentally, it is possible that phosphorylation events or protein interactions that occur close to the TEK-boxes in APC/C-substrates impede recognition of

substrate Lys residues by Ube2C, thereby delaying ubiquitin chain formation and substrate degradation. Controlling ubiquitin chain formation in this manner would be highly reminiscent of the prevailing types of regulation for other processive reactions, such as transcription or translation [106].

Conclusions

Ubiquitin chain formation by the APC/C and subsequent protein degradation by the 26S proteasome are critical reactions regulating the progression of cells through mitosis. By targeting its many substrates at defined times during cell division, the APC/C establishes a crude order of events, thereby helping to coordinate many structural reorganizations that occur in a mitotic cell. Although its activity is regulated on many levels, the APC/C has the inherent capability to discriminate between substrates based on differences in the processivity of ubiquitin chain formation. These differences can result from variations in the affinity of substrates to the APC/C or the efficiency of ubiquitin chain initiation by the specific E2, Ube2C. By contrast, the chain-elongating E2, Ube2S, is highly processive, thus ensuring protein degradation once initiation has been accomplished. The intrinsic capacity of the APC/C to discriminate between substrates is overlaid by several mechanisms that ensure more precise temporal control of mitotic degradation: global mechanisms of APC/C-regulation, such as the spindle checkpoint or the availability of adaptors, E2 enzymes, and DUBs, determine the overall level of APC/C-activity, whereas substrate-specific mechanisms control APC/C-affinity or processivity of chain formation for small groups of important cell cycle regulators, including the cyclins or spindle assembly factors. Together, these tightly interwoven regulatory mechanisms allow the APC/C to establish the conserved sequence of degradation events that steers cells through the complex processes that make up mitosis.

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Figure 1. Sequential degradation of APC/C substrates during mitosis

The APC/C degrades its many substrates only after they have fulfilled their mitotic functions, thereby creating a sequence of degradation events. Processive APC/C-substrates are degraded earlier in mitosis than distributive substrates, and distributive substrates can re-accumulate more easily during late G1. In early mitosis, APC/C^{Cdc20} is inhibited by the spindle assembly checkpoint, but few substrates, such as cyclin A and Nek2 can be degraded under these conditions. Later in mitosis, the APC/C switches to a substrate adaptor with broader specificity, Cdh1. During G1, APC/C^{Cdh1} activity decreases through the degradation of the E2s Ube2C and Ube2S. P: prophase, PM: prometaphase, M: metaphase, A: anaphase, T: telophase.

Figure 2. Mechanism of processive ubiquitin chain formation by the APC/C

A. Yeast APC/C catalyzes the attachment of K48-linked ubiquitin chains to its substrates. The E2 Ubc4 first modifies substrates with ubiquitin molecules to initiate chain formation. The E2 Ubc1 then extends K48-linked ubiquitin chains with high processivity. Ubc1 requires a UBA-domain for processive chain formation. As both Ubc4 and Ubc1 have to interact with the RING domain in APC11 for activation, they need to act sequentially. **B.** Metazoan APC/C forms K11-linked ubiquitin chains on its substrates. Chain initiation is catalyzed by the E2 Ube2C, which interacts with the APC/C through the RING-domain in APC11. A second E2, Ube2S then elongates K11-linked ubiquitin chains with high processivity. Binding of Ube2S to the APC/C is most likely independently of the RING finger, and requires a positively charged C-terminal appendix. **C.** Mechanism of linkage-specific chain elongation by Ube2S. Activated ubiquitin (the “donor”) is transferred from the E1 to the catalytic site Cys residue of Ube2S. The donor ubiquitin engages in a non-covalent interaction with Ube2S, which is required for processive chain elongation. The “acceptor” ubiquitin, whose Lys11 will be modified, is then recognized by a different surface on Ube2S. Binding of the correct acceptor ubiquitin surface leads to formation of a catalytically competent active site, which is composed of residue of both Ube2S and the acceptor ubiquitin (E34). Thus, K11-linkage specific chain formation by Ube2S depends on substrate-assisted catalysis.

Table 1

Reported APC/C substrates

	Reference(s)
<i>Homo sapiens</i>	
Anillin	[1]
Aurora A	[2-5]
Aurora B	[6, 7]
B99	[8]
BARD1	[9]
Bub1	[10]
Cdc6	[11, 12]
Cdc20	[13]
Cdh1	[14]
CDR2	[15]
Centromere protein F	[16]
Cdc25	[17]
Cdc5	[18]
Cdt1	[19]
CKAP2	[9, 20]
Cks1	[21]
Clasp	[22]
Cyclin A	[23]
Cyclin B	[24]
DMYB	[25]
E2-C	[26]
E2F1	[27]
FoxM1	[28, 29]
Geminin	[30]
glutaminase 1	[31]
Hec1	[32]
Hmmr	[9]
HSF2	[33]
HURP	[9]
Id2	[34]
JNK	[35]
Kid	[7]
Liprin- α	[36]
Mcl-1	[37]
Mip130	[25]
Nek2a	[38]

	Reference(s)
NIMA	[39]
Nlp	[40]
NuSAP	[9, 41]
P21	[42]
PC-PLC	[43]
Pfkfb3	[44]
PIF	[45]
Plk1	[5]
Rcs1	[46]
Securin	[47]
Sgo1	[48, 49]
Skp2	[21]
SnoN	[50]
sororin	[51]
TK1	[52]
TMPK	[52]
Tome-1	[53]
TRB3	[54]
Tpx2	[55]
<i>Saccharomyces cerevisiae</i>	
Cik1	[56]
Cin8	[57]
Dbf4	[58]
Hsl1	[59]
Iqg1p	[60]
Kipl	[61]
Pdsl	[62, 63]
Ume6p	[64]
<i>Schizosaccharomyces pombe</i>	
Ase1	[65]
Mes1	[66]
Rep2	[67]
Rhp54	[68]
<i>Drosophila melanogaster</i>	
E2-C vihar	[69]
Miranda	[70]
PIM	[71, 72]
<i>Caenorhabditis elegans</i>	
EGG-3	[73]

Reference(s)	
<i>Xenopus laevis</i>	
Dvl	[74]

Reported APC/C substrates in the current literature. Conserved substrates are listed as their human homologue.