

# The anaphase-promoting complex (APC): the sum of its parts?

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## Abstract

The APC (anaphase-promoting complex) is a multisubunit E3 ubiquitin ligase that targets cell-cycle-related proteins for degradation by the 26 S proteasome. The APC contains at least 13 subunits and is regulated by the binding of co-activator proteins and by phosphorylation. It is not known why the APC contains 13 subunits when many other ubiquitin ligases are small single-subunit enzymes. In the present study, the structures and functions of individual APC subunits are discussed. By dissecting the roles of its parts, we hope to gain insight into the mechanism of the intact APC.

## Introduction

Most cellular proteins form a complex network of interactions with other proteins and many are components of large multiprotein complexes [1–3]. In fact, it is estimated that every yeast protein has an average of nine interacting partners, whereas higher eukaryotes may have significantly more [3]. Some protein–protein interactions are dynamic and transient. These interactions are usually involved in propagating signals within cells, e.g. in signal-transduction pathways. However, many other proteins form stable interactions with their binding partners and probably never exist as monomers. The APC (anaphase-promoting complex) or cyclosome is a large multisubunit protein complex. It has 13 core components (with unknown stoichiometries) that remain stably associated. Why do large stable multiprotein complexes exist? The reason is not clear, but their complexity is probably required for their strict regulation. For example, the 26 S proteasome must be regulated so that only target proteins are recognized and degraded. The large complexes involved in transcription (polymerases and transcription factors) and translation (ribosomes) must be tightly regulated to allow the control of gene expression.

The APC is an ubiquitin ligase (E3) that catalyses the conjugation of Lys-48-linked polyubiquitin chains on to substrate proteins, thereby targeting them for degradation by the 26 S proteasome [4,5]. The proteolytic events triggered by the APC are required to release sister chromatid cohesion during anaphase, to control the exit from mitosis and to prevent premature entry into S-phase. This ubiquitin-mediated proteolysis at critical points of the cell cycle provides a mechanism for rapid and irreversible cell-cycle transitions [6].

Although the activity and specificity of E3s such as the APC are crucial, most E3s exist as much smaller, often

single-subunit, enzymes. Structural and biochemical studies of E3s have been extremely informative and have provided clues to the mechanisms of E3s [7,8]. They have not yet explained the complexity of multisubunit E3s, such as the APC. In this review, I discuss the composition of the APC and the functions of its subunits with the goal of gaining insight into the mechanism of the APC as a whole. For simplicity, I concentrate on proteins from the budding yeast, *Saccharomyces cerevisiae*, although equivalent proteins exist in most eukaryotes.

## The mechanisms of E3s

Ubiquitin is attached to target proteins by a three-step mechanism involving the E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme) and E3 (Figure 1). This pathway has been reviewed extensively elsewhere [8–11]. Importantly, the E3 helps to transfer ubiquitin from an E2 on to a lysine residue of the substrate protein to form mono- or polyubiquitin chains. The E3 provides substrate specificity by recognizing and binding to specific substrate sequences. Structural work on E3s suggests that they function to provide a platform that positions charged E2 in close proximity to the substrate [8,12]. The mechanism of catalysis is still not clear, but ubiquitin transfer may occur spontaneously when the highly labile E2–ubiquitin thioester bond is presented to a substrate lysine in a favourable conformation.

## The APC

The *S. cerevisiae* APC contains at least 13 subunits, eight of which are essential for viability (Table 1) [4,5,13–15]. Most of the APC subunits are conserved in all eukaryotes and remain tightly associated throughout the cell cycle. The activity and substrate specificity of the APC are dictated by phosphorylation and by the regulated binding of a co-activator protein: either Cdc20/Fzy/p55<sup>CDC</sup>, Cdh1/Hct1/Fzr or Ama1.

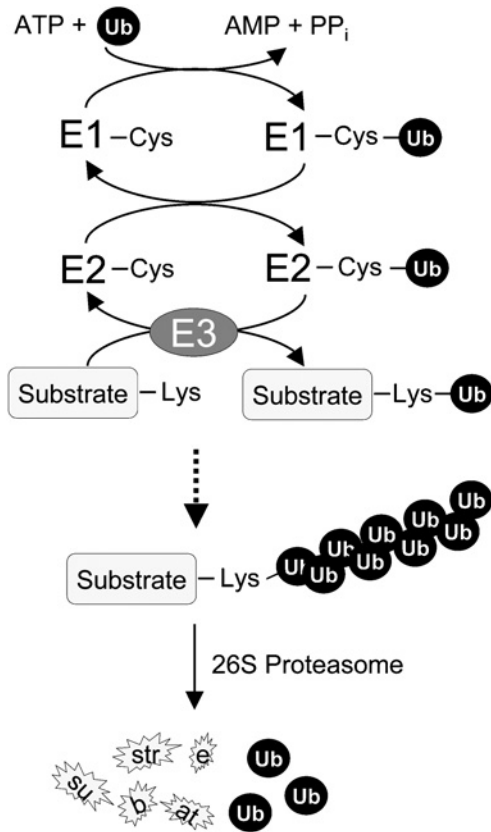
**Key words:** anaphase-promoting complex (APC), cell cycle, cyclosome, E3 ligase, ubiquitin.

**Abbreviations used:** APC, anaphase-promoting complex; RING, really interesting new gene; SCF, Skp1-Cdc53/Cul1-F-box protein; TPR, tetratricopeptide repeat.

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**Figure 1 | The ubiquitin–proteasome pathway**

Ubiquitin (Ub) is covalently attached to substrate proteins through a three-step mechanism involving the sequential actions of the E1, E2 and E3 enzymes. The attachment of multiple ubiquitin moieties by E3 results in the formation of a Lys-48-linked polyubiquitin chain that is recognized by the 26 S proteasome. The 26 S proteasome degrades the substrate protein and recycles ubiquitin.



## Roles of cullin and RING (really interesting new gene) finger proteins

E3s can be grouped into three classes: HECT (homology to E6-AP C-terminus), single-subunit RING and multisubunit E3s. Multisubunit E3s, including the APC, the SCF (Skp1-Cdc53/Cul1-F-box protein) and several SCF-like complexes, contain a RING subunit, a cullin subunit and other subunits (reviewed in [4,5,16,17]). Since all multisubunit RING E3s contain cullin and RING finger proteins (Apc2 and Apc11 respectively in the APC), these domains were expected to play a major role in ubiquitylation. Indeed, *in vitro* experiments suggested that Apc11 alone or an Apc2–Apc11 complex can assemble polyubiquitin chains [18–20]. However, on its own, this cullin–RING complex does not provide substrate specificity. Apc2 and Apc11 are also required for the activity of the holo-APC [21]. Structural studies have further defined the role of the cullin and RING subunits. The RING domain uses a hydrophobic groove to bind directly to E2 [22]. In an SCF crystal structure, the cullin (Cul1) and RING finger (Rbx1) proteins are intimately associated [12]. Since the

**Table 1 | Subunits of the *S. cerevisiae* APC**

<i>S. cerevisiae</i> subunit	Essential for viability?	Mass (kDa)	Motifs
Core subunits			
Apc1	Yes	196	Rpn1/2 homology (PC repeat)
Apc2	Yes	96	Cullin homology domain
Cdc27/Apc3	Yes	85	TPR motifs
Apc4	Yes	73	
Apc5	Yes	77	HEAT repeats
Cdc16/Apc6	Yes	94	TPR motifs
Cdc23/Apc8	Yes	70	TPR motifs
Apc9	No	30	
Doc1/Apc10	No*	26	Doc domain
Apc11	Yes	19	RING finger
Cdc26	No†	14	
Apc13/Swm1	No‡	19	
Mnd2	No‡	43	
Co-activators			
Cdc20	Yes	68	WD40 repeats
Cdh1/Hct1	No	64	WD40 repeats
Ama1	No‡	66	WD40 repeats

\* Doc1p is not essential for viability, but deletion of *DOC1* results in severe growth defects and temperature sensitivity.

† Required only at a high temperature.

‡ Required for meiosis.

function of an E3 may be merely to bring together ubiquitin-charged E2 and substrate, the ability of the cullin–RING complex to recruit and precisely position the E2 is probably critical for the APC to form Lys-48-linked polyubiquitin chains on specific substrates.

The structure of the SCF suggests that it is a rigid Cul1-based scaffold that binds E2 and substrate on opposite ends [12,22,23]. The APC may have a significantly different architecture. There is no sequence similarity between Apc2 and Cul1 outside of the C-terminal cullin homology domain. Therefore Apc2 may not contain the helical scaffold found in the N-terminal domain of Cul1. Instead, other subunits could be responsible for forming a rigid scaffold between the substrate- and E2-binding sites.

## The Doc domain

One APC subunit, Doc1/Apc10, contains a Doc domain. This domain is present in several other proteins containing ubiquitylation-linked domains such as cullin domains, RING fingers and HECT domains [24–27]. Doc1/Apc10 is highly conserved from humans to the microsporidia, *Encephalitozoon cuniculi*, and is essential for normal growth in yeast [24,28]. Deletion of *DOC1* inactivates APC function without disrupting APC complex formation [15,25,29].

The crystal structure of Doc1/Apc10 revealed a  $\beta$ -jelly roll fold with structural homology to diverse ligand-binding

proteins such as galactose oxidase, the C2 domain of coagulation factor and XRCC1 [30,31]. Most of the conserved residues in the Doc domain are located on the surface responsible for ligand interactions in structurally homologous proteins, suggesting that the Doc domain may have a role in ubiquitylation involving biomolecular interactions. Recent reports have implied that Doc1 is involved in the processivity of the APC [29] and substrate recognition [15]. However, direct substrate–Doc1 interactions have not yet been detected. Since the Doc domain is found in other E3s unrelated to the APC, it probably plays a ‘general’ role in ubiquitylation. It may be involved in binding to ubiquitin or inducing a conformational change in E2, or it could play a catalytic role. Apc2 is the only cullin protein that is not neddylated, a modification required for the activity of other cullin-based E3s [32]. Thus an intriguing idea is that the Doc domain activates the E3 activity of the APC, playing an analogous role to Nedd8 modification. Further studies will be required to elucidate the function of the Doc domain.

### Subunits containing TPRs (tetratricopeptide repeats)

The Cdc16, Cdc23 and Cdc27 subunits of the APC each contain 8–10 copies of the 34-amino-acid TPR motif [33,34]. A fourth TPR-containing subunit, APC7, has so far been identified only in vertebrate APC [35]. Each TPR motif consists of a pair of antiparallel  $\alpha$ -helices, and a series of tandem TPR motifs would pack together to form a right-handed superhelix [36–38]. Tandem TPR motifs appear to act as versatile protein–protein interaction domains and it has been hypothesized that the three TPR-containing proteins in the APC form a scaffold on to which the other subunits assemble. In favour of this proposal, mutations in TPR-containing subunits that would disrupt the packing of neighbouring  $\alpha$ -helices cause loss of function and cell-cycle arrest at the metaphase-to-anaphase transition [36,39–41]. In addition, Cdc27 (and APC7 in vertebrates) mediates the binding of several proteins containing a C-terminal Ile–Arg motif [15,21]. This motif is found in the co-activator proteins as well as Doc1. Therefore the TPR subunits may function as receptors for the substrate-binding components of the APC, supporting the idea that they provide an assembly scaffold for functional APC.

### Apc1

With 1748 amino acids, Apc1 is the largest subunit of the APC and, intriguingly, it has no known function. It contains repeat regions homologous with the Rpn1 and Rpn2 subunits of the 19 S regulatory complex of the 26 S proteasome [42]. These PC (proteasome/cyclosome) repeats are related to TPRs and are predicted to form an  $\alpha$ -helical toroid [43]. The toroid would contain a central pore that is large enough to accommodate the passage of an unfolded protein; hence, Rpn1 and Rpn2 are predicted to assist in protein unfolding at the proteasome. This raises the interesting possibility that Apc1 may also contribute to 26 S proteasome function.

### Subunits with unknown functions

Functions for the remaining APC subunits are less clear. At least one other APC subunit, Apc5, is predicted to be an  $\alpha$ -helical repeat protein, containing HEAT motifs [38] (D. Barford, personal communication). However, Apc4, Apc9, Cdc26, Swm1 and Mnd2 do not share any significant homology with proteins of known function and they are not highly conserved between species. Cdc26 may stabilize the association of other APC subunits since deletion of *CDC26* in budding yeast results in decreased association of Apc9, Cdc16 and Cdc27 [44]. Consistent with its role in APC assembly/stabilization, overexpression of *hcn1*, the fission yeast homologue of Cdc26, suppresses the temperature-sensitive phenotype of a *cut9* (Cdc16 homologue) mutant [45]. Similarly, other subunits may play roles in stabilizing the complex, since deletion of *APC9* results in decreased association of Cdc27 [15,44], whereas Cdc16, Cdc27, Apc9 and Cdc26 are lost from the APC after the deletion of *SWM1* [46].

### The sum of its parts?

One of the major questions regarding APC structure and function is the arrangement of its subunits. Since most of the subunits are intimately associated with other core APC subunits, they have not been studied in isolation and little is known about their interactions and functions within the complex. As described above, recent studies have begun to map out protein–protein interactions within the APC [14,15,20,21,30,44,46], but the role of many subunits and the reason for the unusual complexity of the APC remains a mystery. So far, cryo-electron microscopy of human and yeast APC has indicated that the APC has a complex architecture with an outer protein wall that adopts a cage-like shape [47] (L.A. Passmore, C.R. Booth, S.J. Ludtke, W. Chiu and D. Barford, unpublished work). The cage-like shape is reminiscent of other large multiprotein complexes such as the 26 S proteasome and chaperonins including GroEL and CCT. These complexes process their substrates within an inner cavity, thereby limiting access to their catalytic sites. In this respect, the use of a ‘cage’ could make biological sense for the APC, since it could limit access to the ubiquitin-charged E2, preventing the highly labile thioester-linked ubiquitin from reacting non-specifically with other cellular proteins. Indeed, the ability of an Apc2–Apc11 complex to freely ubiquitylate proteins is restrained within the holo-APC [21]. However, the APC is an exceptionally complex E3: other E3s are much smaller in size and, therefore, probably do not use a cage mechanism.

It is probable that the complexity of the APC is related to its intricate regulation. Whereas the SCF is constitutively active with its substrates being regulated by phosphorylation, APC activity is regulated directly in a cell-cycle-specific manner, both spatially and temporally. This regulation of APC E3 activity occurs through post-translational modifications as well as binding of regulatory proteins, and may explain the large number of subunits. Regulated binding

of alternate APC co-activators dictates the timing of APC activation as well as the substrate specificity. However, there is some debate as to the precise function of the co-activators. For example, they could be substrate receptors, they could allosterically activate the APC or they could recruit other activators (e.g. kinases) to the APC [48]. It will be a challenge to elucidate their mechanisms, since they bind to the APC transiently and at substoichiometric levels. Recent experiments suggest that the core APC complex may be directly involved in substrate binding [15,48–50]. Certainly, the complex architecture of the APC is indicative of numerous protein–protein interaction surfaces that could be used to interact with substrates, E2s, co-activators or other regulators, the 26 S proteasome, ubiquitin and even E1.

In summary, it appears that the APC has evolved into a large multiprotein complex to allow its complicated regulation: both to protect non-substrate proteins from promiscuous ubiquitylation and to permit the intricate spatial and temporal regulation of its activity. Based on what we know about its individual subunits, the APC could be viewed as a large, elaborate scaffold built around a cullin–RING complex. Knowledge of the architecture of the APC, including the specific locations of each subunit, will provide us with further insight into the function of each subunit as well as the catalytic and regulatory mechanisms.

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