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State of the APC/C: Organization, function, and structure

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Abstract

The ubiquitin-proteasome protein degradation system is involved in many essential cellular processes including cell cycle regulation, cell differentiation, and the unfolded protein response.The anaphase-promoting complex/cyclosome (APC/C), an evolutionary conserved E3 ubiquitin ligase, was discovered 15 years ago because of its pivotal role in cyclin degradation and mitotic progression. Since then, we have learned that the APC/C is a very large, complex E3 ligase composed of 13 subunits, yielding a molecular machine of approximately 1 MDa. The intricate regulation of the APC/C is mediated by the Cdc20 family of activators, pseudosubstrate inhibitors, protein kinases and phosphatases and the spindle assembly checkpoint. The large size, complexity, and dynamic nature of the APC/C represent significant obstacles toward high-resolution structural techniques; however, over the last decade, there have been a number of lower resolution APC/C structures determined using single particle electron microscopy. These structures, when combined with data generated from numerous genetic and biochemical studies, have begun to shed light on how APC/C activity is regulated. Here, we discuss the most recent developments in the APC/C field concerning structure, substrate recognition, and catalysis.

Keywords

Ubiquitination; anaphase-promoting complex; spindle assembly checkpoint; mitosis; cyclosome; phosphorylation; electron microscopy

Introduction

The ubiquitin-proteasome system is a highly regulated signaling network that targets specific proteins for degradation. Ubiquitination, the covalent addition of a ubiquitin (Ub) or Ub chain to a substrate protein, is carried out through a cascade of enzymes. Ub-activating enzymes (E1) covalently bind ubiquitin through a thioester linkage and transfer it to a Ub-conjugating enzyme (E2), which ultimately cooperates with a Ub ligase (E3) to mark a lysine on a substrate protein (Hershko, 1997). After assembly of a ubiquitin chain on a substrate, the poly-Ub protein is shuttled to the 26S proteasome and degraded. The

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Declaration of interest

The authors report no declaration of interest.

ubiquitination process is counteracted by deubiquitinating enzymes that remove Ub and/or disassemble free Ub chains (Reyes-Turcu et al., 2009). Thorough reviews on the topics of proteasome structure, assembly, and mechanism of proteolysis are available (Gallastegui & Groll, 2010; Bedford et al., 2010; Tanaka, 2009; Kim et al., 2010b) and will not be discussed further here. Targets of the Ub-proteasome system include key cell cycle machinery, as well as improperly folded and/or damaged proteins (housekeeping protein turnover).

Proper cell cycle progression requires oscillation of regulatory protein levels in all eukaryotes. Two highly conserved E3 ubiquitin ligases, the anaphase-promoting complex/cyclosome (APC/C) and Skp1-Cul1-F-box protein (SCF), are differentially regulated over the course of the cell cycle. The APC/C is activated during M phase and remains active during G1 phase (reviewed in Peters, 2006; Thornton & Toczyski, 2006; van Leuken et al., 2008; Matyskiela et al., 2009), while the SCF is active throughout the cell cycle. Excellent review articles describing the details of SCF structure, regulation, cellular targets, and cross-regulation with the APC/C are available (Peters, 1998; Cardozo & Pagano, 2004; Vodermaier, 2004; Skaar & Pagano, 2009; Ang & Harper, 2005).

The APC/C is perhaps the most complex ubiquitin ligase known. It is composed of more than a dozen subunits (Table 1), yielding a molecular machine approximately 1 MDa in size. The APC/C can be divided into four modules: (1) catalytic (2) tetrico-peptide repeats (TPRs) (3) scaffolding, and (4) activator (Figure 1). The catalytic subunits of the APC/C include Apc11, a protein that contains a Zn^{+2}-binding RING (really interesting new gene) domain, and Apc2, a cullin-like protein; thus, the APC/C is classified as a RING-cullin E3 ligase (Gmachl et al., 2000; Tang et al., 2001b). The TPR repeat proteins modulate activator interactions (Vodermaier et al., 2003; Kraft et al., 2005; Thornton et al., 2006; Matyskiela & Morgan, 2009). The scaffolding proteins bridge the catalytic and TPR modules, perhaps to optimize spacing between these regions for efficient catalysis. Finally, APC/C activators are members of the Cdc20 family of tryptophan-aspartate (WD) repeat proteins and are necessary for efficient substrate binding and ubiquitination (reviewed in Visintin et al., 1997; Kramer et al., 1998; Yu, 2007). Multiple recent reviews on the APC/C are available, for example (Peters, 2006; Thornton & Toczyski, 2006; van Leuken et al., 2008; Matyskiela et al., 2009). Here, we have emphasized the newest developments in the APC/C literature, particularly APC/C structure and modes of activator substrate recognition. (Note that we will use mammalian protein names except where species-specific names are required; please refer to Table 1 for clarification of nomenclature.)

Functions of the APC/C

Mitosis and meiosis

The yeast APC/C has two essential mitotic targets—cyclin and securin (see Table 1 for species-specific names) (Thornton & Toczyski, 2003). The APC/C ubiquitinates A and B type cyclins to downregulate mitotic cyclin-dependent kinase (CDK) activity and is essential for mitotic exit. In fact, the APC/C was discovered in yeast, frog, and clam oocyte extracts as one factor required for cyclin ubiquitination (Irniger et al., 1995; King et al., 1995; Sudakin et al., 1995). Thus, initially, the APC/C was termed the ‘cyclosome’ because of its pivotal role in cell cycle regulation (Sudakin et al., 1995). During mitosis, the APC/C is

sequentially activated by two Cdc20 family members—first Cdc20 and then Cdh1 (see Table 1 for species-specific names and Regulation of the APC/C section for more detail) (reviewed in Pesin & Orr-Weaver, 2008). APC/C^{Cdc20} promotes the metaphase to anaphase transition by ubiquitinating securin, an inhibitor of separase, the enzyme responsible for cleavage of the chromosome cohesin complex, initiating separation of sister chromatids (Cohen-Fix et al., 1996; Funabiki et al., 1996) as well as cyclin B (Holloway et al., 1993; Gorr et al., 2005). After anaphase onset, Cdc20 is ubiquitinated by APC/C^{Cdh1} and degraded (Visintin et al., 1997; Fang et al., 1998b; Prinz et al., 1998; Shirayama et al., 1998). APC/C^{Cdh1} then targets cyclin B and other substrates for degradation promoting mitotic exit (Fang et al., 1998b; Kramer et al., 1998; Zachariae et al., 1998; Fang et al., 1999; Blanco et al., 2000; Listovsky et al., 2000). APC/C^{Cdh1} remains active through G1 phase to continue degradation of cyclins and perhaps limit expression of Cdc20 until mitosis (Huang et al., 2001). During mitosis, the cellular localization of the human APC/C is dynamic; it localizes to centrosomes and the mitotic spindle, but active (phosphorylated) APC/C is enriched on centrosomes (Tugendreich et al., 1995; Kraft et al., 2003). Unfortunately, APC/C localization remains difficult to study in yeasts.

Although there is less information available on the role of the APC/C during meiosis, it is clear that APC/C activity is carefully modulated to achieve the special chromosome cohesion requirements of meiotic progression. That is sister chromatid cohesion must be maintained through meiosis I, but dissolved during meiosis II. The APC/C regulates cyclin and securin levels as in mitosis, but its activity is modified during meiosis by specific activators and inhibitors (see Regulation of the APC/C section) (Table 2) to prevent complete cyclin degradation between meiosis I and meiosis II, premature chromosome segregation and meiotic exit (Cooper et al., 2000; Asakawa et al., 2001; Blanco et al., 2001; Diamond et al., 2009) (reviewed in Irniger, 2006). The APC/C is required for homologous chromosome separation (meiosis I) in *Saccharomyces cerevisiae*, and securin (separase inhibitor) is targeted for destruction by the APC/C during both meioses in yeasts and mammals (Jin et al., 2010; Salah & Nasmyth, 2000); however, shugoshin protects sister chromatid cohesin from separase cleavage during meiosis I (Katis et al., 2004; Kitajima et al., 2004; Marston et al., 2004; Rabitsch et al., 2004) and is then targeted by the APC/C for degradation (Salic et al., 2004; Penkner et al., 2005; Karamysheva et al., 2009) to promote efficient and timely chromosome segregation in meiosis II. In mammals, where female meiosis is especially protracted (oocytes are blocked in prophase I before birth and resume meiosis in sexually mature females, often years later), loss of cohesin and shugoshin in older females correlates with increased homolog separation errors during meiosis I, resulting in more trisomy births (Lister et al., 2010; Revenkova et al., 2010).

Neuronal differentiation

In postmitotic neurons, the APC/C regulates axon and dendrite morphogenesis depending on its localization and bound activator (Konishi et al., 2004). While APC/C^{Cdh1} localizes to the nucleus and inhibits axon growth, APC/C^{Cdc20} localizes to dendrites and promotes dendrite growth and arborization (Yang et al., 2010). For a detailed review on the role of the APC/C in neuronal development and patterning, we refer the reader to a recent article by Yang and co-workers (2010).

Cancer, viruses, and the APC/C

Given the crucial roles played by the APC/C in maintenance of genetic fidelity through cell division, it is not surprising that many cancers and viruses have deregulated or usurped the APC/C, its activators, or conjugate E2 enzymes to escape cell death and/or promote viral reproduction. Mutations in APC/C subunits have been found in colon cancer cell lines and primary human colon cancer cells (Wang et al., 2003), and Cdc20 is overexpressed in some cancers, perhaps to promote cell division even in the presence of spindle defects and genetic aberrations (Chen et al., 2006; Yuan et al., 2006; Thirthagiri et al., 2007). The human APC/C's cognate E2s (see Catalysis section), UbcH10, and Ube2S, are overexpressed in primary tumors (Okamoto et al., 2003; Tedesco et al., 2007; van Ree et al., 2010) and ectopic expression of either UbcH10 or Ube2S promotes cell transformation and tumor invasion (Okamoto et al., 2003; Jung et al., 2006). Ube2S expression inversely correlates with the stability of the tumor suppressor von Hippel-Lindau E3 ligase (Jung et al., 2006), providing one potential mechanism of cell transformation for this E2. However, both of these E2 enzymes likely affect cell transformation and proliferation via the APC/C.

The APC/C is reported to regulate a number of genes involved in tumor suppression. For example, the APC/C^{Cdc20} directly regulates p21, a CDK inhibitor, in prometaphase (Amador et al., 2007). APC/C^{Cdh1} interacts with the tumor suppressor Rb (retinoblastoma) protein, presumably to direct the APC/C to specific substrates (Binne et al., 2007). Both Cdc20- and Cdh1-bound APC/C may be inhibited by RASSF1A, a centrosomal tumor suppressor protein (Mathe, 2004; Song & Lim, 2004; Song et al., 2004; Liu et al., 2007; Whitehurst et al., 2008). Furthermore, APC/C^{Cdh1} is required for stable G1 phase and governs the switch between cell proliferation and differentiation (Garcia-Higuera et al., 2008; Skaar & Pagano, 2008) and misregulation of APC/C^{Cdh1} in differentiated cells can lead to cell cycle reentry, dedifferentiation, and potentially carcinogenesis (Wirth et al., 2004; Wasch et al., 2010).

Viruses also appear to highjack the APC/C or its regulators to promote viral replication and/or cell transformation and carcinogenesis. The Human T lymphoma virus type-1 (HTLV-1) protein Tax is reported to impact APC/C function by interacting with Mad1, Cdc20, and securin, effectively mitigating the spindle assembly checkpoint (SAC, an inhibitor of anaphase and the APC/C, see Regulation of the APC/C section) while activating the APC/C to allow mitosis to proceed unchecked (Grassmann et al., 2005). Tax binding to the APC/C causes premature ubiquitination and degradation of cyclin B and securin, promoting aneuploidy and cancer initiation (Grassmann et al., 2005; Liu et al., 2005). An adenoviral protein, E4orf4, also interacts with Cdc20 and may also promote carcinogenesis in a similar way (Mui et al., 2010). Apoptin, another viral protein, is reported to inhibit the APC/C to induce G2/M arrest and p53-independent apoptosis, facilitating viral egress and infection of new cells (Teodoro et al., 2004). Human cytomegalovirus infection triggers a G1/S arrest (and E2F-dependent transcription), and appears to induce degradation of core APC/C subunits Apc4 and Apc5 (Wiebusch et al., 2005; Tran et al., 2008; Tran et al., 2010), promoting viral infection and replication. Finally, a poxvirus protein, with homology to Apc11 (APC/cyclosome regulator or PACR) causes cell cycle deregulation and accumulation of APC/C substrates (Mo et al., 2009).

Substrates and their recognition

Destruction signals

The recognition and destruction of many APC/C subunits depends on the presence of short conserved sequence motifs referred to as degrons or destruction motifs. A combination of genetic and biochemical studies has led to the characterization of a number of these specific sequences. At least eight different APC/C targeting motifs in substrates have now been identified (see Table 3). Few of the destruction sequences contain ubiquitination sites; however, in the case of cyclin B, the number of lysines (site of ubiquitin attachment) is highly enriched in the region C-terminal to the destruction box (D box) (Glotzer et al., 1991). Mutations in degron sequences often stabilize APC/C substrates, while inserting a degron into a protein that is not an APC/C substrate promotes its ubiquitination (Glotzer et al., 1991). Interestingly, degrons are most often found in disordered regions of proteins, as was found for both securin and cyclin B (Cox et al., 2002). APC/C degrons are very short sequences that are relatively common in the proteome, making it clear that the mere presence of a degron consensus sequence does not signify a bona fide APC/C substrate. Structural studies examining APC/C-substrate interactions will be required to understand the specific criteria of APC/C degron recognition.

The most common degradation motifs found in APC/C substrates are the D box and the KEN box. The D box was first characterized in cyclin B (Glotzer et al., 1991) (RxxLxxxN) and contains at a minimum an arginine and a leucine separated by two residues. This destruction signal is found in both APC/C^{Cdc20} and APC/C^{Cdh1} substrates. The KEN box (KENxxxE/D/N) is recognized by APC/C^{Cdh1} and is often found in APC/C substrates targeted for destruction after Cdc20 dependent substrates (Visintin et al., 1997; Pfleger & Kirschner, 2000; Bashir & Pagano, 2004).

In addition to the D box and KEN box, six other APC/C destruction motifs have been characterized including the TEK box (Jin et al., 2008), the A box (Littlepage & Ruderman, 2002), the GXEN box (Castro et al., 2002), the CRY box (Reis et al., 2006), the O box (Araki et al., 2005) and a sequence specific to Spo13 (Sullivan & Morgan, 2007) (Table 3). The TEK box consensus (R/KxxTxKT) forms a charged patch around K11 in Ub and human APC/C target lysines, directing K11 Ub chain formation (Jin et al., 2008); however, this motif is not found in yeast APC/C substrates. The other motifs listed above are not as common in APC/C substrates and, in general, they are often found in substrates that contain additional degrons making it unclear how they contribute to APC/C-substrate recognition. For example, the mitotic kinase Aurora A contains an A box, a D box and a KEN box. Although it is not clear whether the A box directly interacts with APC/C^{Cdh1}, this sequence is required for activation of the D box in Aurora A (Crane et al., 2004). It has been postulated that dephosphorylation of a conserved serine in the A box during mitotic exit allows APC/C^{Cdh1} to recognize Aurora A's previously silent D box (and possibly the A box too) and precisely control the timing of Aurora A destruction (Littlepage & Ruderman, 2002; Crane et al., 2004; Kitajima et al., 2007). Thus, it is likely that important auxiliary sequences help the APC/C recognize its degrons.

Modification of APC/C substrates by phosphorylation near or in degron sequences has also been shown to modify APC/C-substrate interaction. For example, in certain cases phosphorylation in or near a degron protects a substrate from APC/C-dependent ubiquitination, as is the case for Aurora A (Littlepage et al., 2002; Crane et al., 2004; Kitajima et al., 2007) and securin (Holt et al., 2008). However, there are other examples where phosphorylation in or near degrons may be necessary for APC/C-mediated ubiquitination, such as for Mcl1-1 (Harley et al., 2010) and *S. cerevisiae* Cdc5 (Simpson-Lavy et al., 2009). Because degrons are often found in disordered regions of proteins (Cox et al., 2002), it is possible that changes in phosphorylation affect the overall structure of the degron region making the motif more or less accessible to the APC/C.

Activators mediate substrate recruitment

The activation of the APC/C is mediated in part by the binding of transiently available subunits typified by the founding member of the family, Cdc20 (reviewed in Harper et al., 2002; Peters, 2006; Thornton & Toczyski, 2006; Yu, 2007). Cdc20 binds the core APC/C in mitosis and meiosis and is critical for anaphase initiation. A second family member, Cdh1, plays distinct roles in mitotic exit and G1 phase. Meiotic, species-specific APC/C activators (Mfr1 and Ama1) have been characterized in yeasts (Cooper et al., 2000; Blanco et al., 2001) and *Drosophila* (Cortex) (Pesin & Orr-Weaver, 2007; Swan & Schupbach, 2007) and likely exist in higher eukaryotes.

APC/C activators could facilitate substrate ubiquitination through a number of mechanisms including simple tethering of substrates or E2 enzymes to the APC/C to modulation of APC/C E3 ligase activity through conformational changes. Indeed, the Cdc20 family of activators has been shown to bind APC/C substrates (Burton & Solomon, 2001; Hilioti et al., 2001; Pfleger et al., 2001; Schwab et al., 2001; Kraft et al., 2005; Kimata et al., 2008b; da Fonseca et al., 2010; Buschhorn et al., 2010), activate ubiquitination (Kimata et al., 2008a), and recruit one of its conjugate E2 enzymes, Ube2S (Williamson et al., 2009). Cdc20 proteins contain three domains important for their role in modulation of APC/C activity. The largest and most prominent are the C-terminal WD40 repeats that can be directly cross-linked to substrates (Kraft et al., 2005; Kimata et al., 2008b) making this region a substrate-binding platform. The C-terminus also contains a short IR-motif that interacts directly with the *S. cerevisiae* TPR-containing proteins Cdc23 (Apc8) and Cdc27 (Apc3) (Vodermaier et al., 2003; Matyskiela & Morgan, 2009), suggesting that this domain tethers Cdc20 and Cdh1 to the APC/C. However, the IR domain cannot represent the only and/or most important APC/C-activator interaction because this Cdc20 domain is nonessential (Yamada et al., 2000; Thornton et al., 2006). The N-terminus contains an eight amino acid motif called the C-box (Schwab et al., 2001) that has been shown to both bind the APC/C and modulate its E3 ligase activity (Thornton et al., 2006; Kimata et al., 2008a), making this conserved motif of the Cdc20 family the only region currently shown to actually increase APC/C activity toward substrates. Interestingly, two structural studies have shown that adding recombinant Cdh1 or Cdc20 to the apo-APC/C (APC/C core subunits) causes the APC/C to adopt a more ‘open’ conformation (Dube et al., 2005; Herzog et al., 2009). How this more ‘open’ structure affects APC/C activity is still not clear, but these results confirm that both Cdh1 and Cdc20 function as more than simple substrate tethers.

APC/C-substrate interaction

Although Cdc20 and Cdh1 are clearly important for facilitating APC/C-substrate interactions, there is accumulating evidence that substrates can also interact directly with core components, often in a degron-dependent manner (Yamano et al., 1998; Meyn et al., 2002; Passmore et al., 2003; Carroll et al., 2005; Eytan et al., 2006; Hayes et al., 2006; da Fonseca et al., 2010; Buschhorn et al., 2010). These findings suggest that the APC/C core contains degron binding and/or substrate recognition domains, in addition to those found on the activators. One prime candidate for mediating the interaction between the APC/C core and substrates is Doc1/Apc10, which is essential for APC/C interaction with substrates and processivity of the ubiquitination reaction (Carroll and Morgan, 2002; Passmore et al., 2003). Indeed, two recent cryo-EM structural studies of the *S. cerevisiae* APC/C have shown that the D box directly contacts both Cdh1 and Doc1, creating a bridge between these two components (da Fonseca et al., 2010; Buschhorn et al., 2010). In addition, results from a recent analysis of the *S. cerevisiae* APC/C showed that the TPR subunits facilitate activator and perhaps substrate binding, with the TPR proteins serving as a platform for substrates to bivalently interact with the activator and core APC/C subunits (Matyskiela & Morgan, 2009).

Thus, although the activators are important for initially recruiting substrates to the APC/C, binding affinity for the substrate and processivity of the ubiquitination reaction are likely dictated by interaction of substrates with APC/C core components *and* activators. Furthermore, APC/C core-substrate interactions may be strengthened or modified by structural changes to the APC/C core induced by activator binding (Kimata et al., 2008a; Herzog et al., 2009). The ability of some substrates to bind more tightly to the APC/C, perhaps by engaging in multivalent interactions with core subunits, could also affect the order of substrate recognition by the APC/C and processivity of ubiquitination as proposed by Rape and co-workers (Rape et al., 2006). Finally, it has been known for decades that the small phosphobinding protein of Cdc2-cyclin, termed Suc1/Cks1, is required for the onset of anaphase (Hayles et al., 1986; Kaiser et al., 1999) and that active APC/C can be purified using Suc1 (Sudakin et al., 1997). Several studies have shown that Cks1 is necessary for efficient cyclin recruitment and ubiquitination by the APC/C (Patra & Dunphy, 1998; Wolthuis et al., 2008; Di Fiore & Pines, 2010; van Zon et al., 2010). A recent study by Di Fiore and Pines showed that Cdc20-bound cyclin A is targeted to the APC/C by Csk1 in a CDK-independent manner when the SAC is activated (Di Fiore & Pines, 2010). Another recent study showed that cyclin B-CDK complexes can be targeted by Cks1 to the SAC-inhibited APC/C in prometaphase, such that the complex is poised to ubiquitinate cyclin B in metaphase once the SAC has been satisfied (van Zon et al., 2010). The exact mechanism by which Cks1 selectively facilitates cyclin destruction is not known but might involve the need to extract cyclin from its Cdk1 partner at some point in the ubiquitin-mediated degradation process. In summary, the complicated web of interactions required for efficient ubiquitination of a large number of structurally diverse substrates at variable cell cycle times may explain why the APC/C is so complex compared to other E3 ligases.

Regulation of the APC/C

Activator role

As discussed in previous sections, the APC/C is activated by the Cdc20 family of proteins (Table 2). During mitosis, the APC/C is temporally regulated by binding to the activators Cdc20 and Cdh1 (reviewed in Pesin & Orr-Weaver, 2008). During metaphase/anaphase, APC/C^{Cdc20} promotes cyclin B and securin degradation to trigger anaphase (Holloway et al., 1993; Cohen-Fix et al., 1996; Funabiki et al., 1996; Gorr et al., 2005). Degradation of cyclin B downregulates CDK activity, leading to the removal of inhibitory CDK phosphorylation sites on Cdh1 by Cdc14 phosphatases (Jaspersen et al., 1999; Kotani et al., 1999; Visintin et al., 1998), promoting association of Cdh1 with the APC/C. APC/C^{Cdh1} marks Cdc20, cyclin B, and other key substrates to facilitate mitotic exit and then remains active during G1 phase to maintain low mitotic cyclin levels (Fang et al., 1998b; Kramer et al., 1998; Zachariae et al., 1998; Fang et al., 1999; Blanco et al., 2000; Listovsky et al., 2000). Cdh1 is nonessential from yeast to man for mitotic exit, but loss of Cdh1 in human RNAi experiments and mouse knockout models resulted in increased incidence of tumors and genome instability (Engelbert et al., 2008; Garcia-Higuera et al., 2008; Li et al., 2008). Cdc20 is essential in yeasts (Hartwell et al., 1973; Kim et al., 2010a) and required for mitosis during mouse embryogenesis, confirming that Cdc20 is also essential in mammals (Li et al., 2007).

During meiosis, the APC/C is activated by Cdc20 and other Cdc20 family members to tailor ubiquitin-mediated degradation events to meiotic progression (see Functions of the APC/C and Table 2). In the fission yeast, *Schizosaccharomyces pombe*, a Cdc20 family member, Mfr1 (meiotic fizzy-related 1), is required for cyclin degradation at the end of meiosis II and coordinates nuclear division with spore formation (Blanco et al., 2001). In budding yeast, Ama1 (activator of meiotic APC 1), another Cdc20 family activator only expressed during meiosis, is required for cyclin degradation (Cooper et al., 2000), securin degradation (Oelschlaegel et al., 2005), and spore wall formation (Coluccio et al., 2004). Although it is clear that Ama1 plays a role in meiotic progression, there are conflicting reports of the *ama1*Δ phenotype; Cooper et al. report that *ama1*Δ cells are stalled in meiosis I, whereas Coluccio et al. find that *ama1*Δ cells complete meiosis but not spore formation (Cooper et al., 2000; Coluccio et al., 2004). A recent report suggests that the essential role of Ama1 is actually to coordinate meiotic exit and cytokinesis during spore formation (Diamond et al., 2009). *Drosophila melanogaster* utilizes a meiosis-specific Cdc20 family activator called Cortex (see Table 2) (Swan & Schupbach, 2007) and it is likely that higher eukaryotes also utilize meiosis-specific APC/C activators that have not yet been identified.

Phosphorylation

Another layer of APC/C regulation is provided by post-translational modification of the core subunits, especially by protein kinases and phosphatases. Multiple subunits of the APC/C are phosphorylated in mitosis when the APC/C is active (Kraft et al., 2003; Steen et al., 2008; Wilson-Grady et al., 2008; Beltrao et al., 2009; Holt et al., 2009) and phosphatase treatment of the APC/C inactivates it (King et al., 1995; Lahav-Baratz et al., 1995; Kramer et al., 2000). Phosphorylation of the core APC/C has been correlated with an increased affinity for Cdc20 (Fang et al., 1998b; Shteinberg et al., 1999; Kramer et al., 2000; Yamada

et al., 2000; Kraft et al., 2003), a change in APC/C localization (Huang et al., 2007; Torres et al., 2010), and binding to Cks1-Cdk-cyclin complexes (Di Fiore & Pines, 2010; van Zon et al., 2010).

Cdk1 is required for APC/C activation but is not the only protein kinase involved in APC/C regulation (Lahav-Baratz et al., 1995; Kotani et al., 1998). *In vitro*, purified Cdk1 phosphorylates and activates the conserved mammalian kinase, Plk1 (see Table 1), which in turn can lead to APC/C activation (Kotani et al., 1998). Both protein kinases can phosphorylate multiple subunits of the complex *in vitro* (Harper et al., 2002). Protein kinase A (PKA) can also phosphorylate several members of the complex but PKA phosphorylation events are inhibitory (Kotani et al., 1998), consistent with the observed genetic interactions between *S. pombe* mutants affecting PKA activity and APC/C function (Yamashita et al., 1996; Yamada et al., 1997). While several components of the purified complex are phosphoproteins (Peters et al., 1996; Yamada et al., 1997; Kotani et al., 1998; Rudner & Murray, 2000; Steen et al., 2008; Holt et al., 2009), it is not yet clear which phosphorylation events on which components are important for altering activity and/or protein-protein interactions.

One notable investigation of APC/C phosphorylation was that of Rudner and Murray (2000). In this study, all consensus Cdk1 phosphorylation sites in *S. cerevisiae* Cdc27 (Apc3), Cdc16 (Apc6), and Cdc23 (Apc8) were altered to alanines. There were some defects in mitotic exit, Cdc20 binding, and increased sensitivity to the spindle checkpoint when cells produced the APC/C complex missing these serine and threonine residues. Similarly, loss of the sole Cdk1 site on *S. pombe* Hcn1/Cdc26 led to a mild defect in APC/C function (Yoon et al., 2006). However, the lack of significant defects in the context of previous data indicating an essential role of APC/C phosphorylation argued that critical phosphorylation sites had not been eliminated in these studies.

One comprehensive investigation of human APC/C phosphorylation indicated that there are at least 43 sites of phosphorylation within the human APC/C, 34 of which are specific to mitosis (Kraft et al., 2003). The approach taken to identify sites was mass spectrometry; however, the entire APC/C was not represented in the data (Kraft et al., 2003) indicating that the phosphorylation landscape of the APC/C might be even more complex. Indeed, other large-scale mass spectrometry studies revealed more APC/C phosphorylation sites (Steen et al., 2008; Wilson-Grady et al., 2008; Beltrao et al., 2009; Holt et al., 2009; Mazanek et al., 2010). However, many valuable lessons were learned by the analysis of phosphosites by Kraft and colleagues (2003). Cdk1 was able to phosphorylate many of the identified sites, Plk1 was able to phosphorylate others and still others were not phosphorylated by either of these protein kinases. While Kraft and co-workers proposed that Cdk1 phosphorylation alone can activate the APC/C to some extent *in vitro*, this and previous studies indicated that Cdk1 and Plk1 most likely cooperate to activate the APC/C (Kraft et al., 2003). The fact that Cdk1 phosphorylation can provide a docking site for Plk1 (Elia et al., 2003) raises the possibility that APC/C phosphorylation events might be ordered (Cdk1 followed by Plk1) and thus, have different consequences. It is also evident from the work of Kraft et al. (2003) and other studies that additional protein kinases are involved in APC/C phosphorylation. It is of course possible that some phosphorylation events inhibit aspects of APC/C function.

Indeed mitotic phosphorylation of the APC/C core component and meiotic inhibitor, Mnd2, during mitosis has been implicated as a regulatory switch for its role in APC/C inhibition during meiosis (Torres & Borchers, 2007). Clearly, it will require a comprehensive analysis of the phosphorylation sites of several subunits in order to understand an apparently complex phospho-regulatory scheme.

Inhibition of the APC/C by the SAC

Prior to anaphase, the APC/C is kept inactive by the spindle assembly checkpoint (SAC) (reviewed in Malmanche et al., 2006; Chen, 2007; Ciliberto & Shah, 2009; Musacchio & Salmon, 2007). The SAC ensures that a proper mitotic spindle is formed and all chromosomes are attached and aligned at the metaphase plate prior to chromosome segregation, a key to maintenance of genetic fidelity and prevention of aneuploidy and carcinogenesis. The spindle checkpoint mediates APC/C activity by inhibiting Cdc20; however, the mechanism of inhibition remains an area of intense research. SAC proteins, Mad2 and BubR1 (Mad3), bind Cdc20 either independently or as a single inhibitory complex called the mitotic checkpoint complex (MCC) (Fang et al., 1998a; Sudakin et al., 2001; Tang et al., 2001a; Millband & Hardwick, 2002; Tang et al., 2004) and a checkpoint kinase, Bub1, phosphorylates and inhibits Cdc20 activity (activation of the APC/C) (Tang et al., 2004). The ability of Mad2 and BubR1 to form complexes with Cdc20 suggests a model wherein the checkpoint proteins bind and sequester Cdc20, preventing it from activating the APC/C. However, there is also evidence that the MCC interacts directly with the APC/C core (Morrow et al., 2005; Sczaniecka et al., 2008; Herzog et al., 2009), converting the APC/C's overall structure to a more 'closed' conformation and inhibiting catalysis (Herzog et al., 2009). In addition, Mad3 (BubR1) contains a KEN box and is proposed to act as a pseudosubstrate, blocking interaction of the APC/C with bona fide substrates (Burton & Solomon, 2007; King et al., 2007; Sczaniecka et al., 2008). In fact, recent structural studies showing that the location of D box binding to the APC/C overlaps with the binding position of the MCC provide even further evidence supporting this model (Buschhorn et al., 2010; da Fonseca et al., 2010). Yet, another proposed model suggests that MCC interaction with the APC/C leads to Cdc20 degradation, preventing APC/C activity toward substrates such as cyclin B and securin (Pan & Chen, 2004; Nilsson et al., 2008). Finally, SAC activation and maintenance is affected by a class of CDK activators (RINGO/Speedy) distinct from cyclins that act in recruitment of SAC components and Aurora kinase to unattached kinetochores (Mouron et al., 2010).

From the above discussion, it is clear that there are substrates, such as Cdc20, cyclin A, Nek2A, and HOXC10 that are ubiquitinated and degraded even when the SAC is active (den Elzen & Pines, 2001; Geley et al., 2001; Hames et al., 2001; Fry, 2002; Gabellini et al., 2003; Nilsson et al., 2008; Wolthuis et al., 2008). How these substrates are distinguished from substrates such as cyclin B is still actively being investigated; however, there are a number of hypotheses that could explain this phenomenon. For example, it is possible that checkpoint independent substrates have distinct features that allow them to interact and be ubiquitinated by SAC-inhibited APC/C. This explanation is not completely satisfactory, because SAC-independent substrates, such as Nek2A and Cdc20, have degron sequences that are required for ubiquitination and degradation (Fry & Yamano, 2006; Hayes et al.,

2006). Another theory is that checkpoint-independent APC/C substrates may be more efficiently targeted to APC/C^{Cdc20} particles that have yet to be inhibited by the SAC. Further analysis of APC/C structure and cellular localization patterns is required to improve our understanding of how SAC signals selectively inhibit APC/C activity toward some but not all substrates.

Release of the APC/C from SAC inhibition is also not completely understood. One model of APC/C inhibition by the SAC describes an anaphase switch in which the human APC/C ubiquitinates Cdc20 to dissociate the inhibitory MCC and allow activation of the APC/C, while the deubiquitinating enzyme USP44 reverses this modification to strengthen APC/C inhibition by stabilizing the Cdc20-MCC interaction (Reddy et al., 2007; Stegmeier et al., 2007). It is not yet clear whether this is a general mechanism because deubiquitinating enzymes have not been implicated in SAC release in other organisms. A potentially parallel mechanism of SAC inactivation is catalyzed by competitive binding of p31^{comet} to Mad1- or Cdc20- associated Mad2, which results in dissociation of the MCC and activation of the APC/C (Xia et al., 2004; Mapelli et al., 2006; Yang et al., 2007). Other groups have confirmed that polyUb by the APC/C is required for checkpoint release in human cells and in particular that the E2, Ube2S, is required for release from prolonged SAC inhibition (Garnett et al., 2009; Williamson et al., 2009; Miniowitz-Shemtov et al., 2010). Miniowitz-Shemto and co-workers (2010) also showed that an unknown ATP-dependent process involving a β-γ cleavage of ATP is necessary for SAC release. Finally, the protein phosphatase, PP1 (Dis2), has been shown to effectively release the SAC in two divergent yeast by antagonizing Aurora kinase, an essential SAC component (Pinsky et al., 2009; Vanoosthuyse and Hardwick, 2009). A cohesive model of SAC release from yeast to humans has yet to be elucidated and will require better understanding of APC/C localization, binding partners and modification status before, during, and after checkpoint release.

During meiosis, APC/C activity must be temporally restricted by inhibitory proteins to prevent (1) complete degradation of cyclin B between meiosis I and meiosis II and (2) premature chromosome segregation (Shonn et al., 2000; Bernard et al., 2001; Izawa et al., 2005; Oelschlaegel et al., 2005; Penkner et al., 2005; Kimata et al., 2008b) (reviewed in Irniger, 2006). Meiotic inhibition of the APC/C is mediated by the SAC (Shonn et al., 2000; Bernard et al., 2001) and other meiosis specific factors (see section below). SAC components are essential for timing meiotic progression from yeast to mammals; loss of SAC function during meiosis accelerates meiotic progression and/or chromosome missegregation (Shonn et al., 2000; Tsurumi et al., 2004; Homer et al., 2005a; Homer et al., 2005b; Wei et al., 2010) and overexpression induces a meiosis I arrest (He et al., 1997; Sironi et al., 2001; Wassmann et al., 2003a; Wassmann et al., 2003b; Niault et al., 2007; Li et al., 2009; Wei et al., 2010).

Other APC/C inhibitors

As mentioned above, APC/C activity is restrained during meiosis by inhibitory proteins to permit efficient and faithful meiotic progression. In the fission yeast *S. pombe*, APC/C^{Cdc20} is partially inhibited by Mes1 (meiosis II protein), preventing the complete degradation of cyclin during meiosis I and making the cell competent for meiosis II (Izawa et al., 2005;

Kimata et al., 2008b). Mes1 restrains APC/C^{Cdc20} by competing with other substrates (e.g., cyclin) for Cdc20 binding and this inhibition is released by ubiquitin-mediated degradation of Mes1 (Kimata et al., 2008b). In budding yeast, the activity of APC/C^{Ama1} is inhibited during meiosis by an APC/C subunit called Mnd2 (meiotic nuclear divisions 2) to prevent premature chromosome segregation prior to meiosis II (Oelschlaegel et al., 2005; Penkner et al., 2005). The APC/C is inhibited in similar ways in higher eukaryotes. For instance, Emi2 (XErp1), an inhibitor of the APC/C, is an essential regulator of meiosis (Reimann and Jackson, 2002; Ohsumi et al., 2004; Tung and Jackson, 2005) and, as an essential component of cytostatic factor, blocks unfertilized eggs in metaphase II (Rauh et al., 2005; Schmidt et al., 2005; Tung et al., 2005; Schmidt et al., 2006).

Yeast and higher eukaryotes utilize mitotic inhibitors to restrain APC/C activity until the appropriate time(s) in mitosis (Reimann et al., 2001; Martinez et al., 2006; Dial et al., 2007; Enquist-Newman et al., 2008). Acm1 inhibits APC^{Cdh1} in interphase and is then degraded in late mitosis (Martinez et al., 2006; Dial et al., 2007; Enquist-Newman et al., 2008; Hall et al., 2008). In higher eukaryotes, Emi1 (Rca1), initially discovered in *Drosophila* is essential for regulation of G1 phase during eye development and asymmetric cell division in the central nervous system (Dong et al., 1997; Lear et al., 1999; Wai et al., 1999). Emi1 couples mitosis and DNA replication by inhibiting APC/C^{Cdh1} to stabilize cyclin and geminin and promote mitosis (Grosskortenhaus & Sprenger, 2002; Hsu et al., 2002; Di Fiore & Pines, 2007) and prevents re-replication (Machida and Dutta, 2007; Zielke et al., 2008). The Emi proteins (Emi1 and Emi2) may both mediate APC/C inhibition via competitive pseudosubstrate binding (Miller et al., 2006) and/or interaction of their C-termini with the APC/C core (Ohe et al., 2010; Tang et al., 2010) preventing substrate binding or APC/C activation, respectively.

Catalysis

E2 interaction and role of priming and elongation

E2-E3 interactions are tailored to provide substrate and ubiquitin chain specificity (see Ub chain topology section below) (Kirkpatrick et al., 2006; Jin et al., 2008; Matsumoto et al., 2010). The APC/C subunit Apc11 binds E2 ubiquitin-conjugating enzymes via its RING domain and mediates specific ubiquitination events (Leverson et al., 2000; Ye and Rape, 2009). In yeasts and vertebrates, the APC/C interacts with more than one E2 to accomplish substrate and site-specific processive ubiquitination (Osaka et al., 1997; Seino et al., 2003; Rodrigo-Brenni & Morgan, 2007; Garnett et al., 2009; Williamson et al., 2009). Evidence in yeast and human systems suggests that the roles of the E2s are complementary—one for initial substrate modification (priming) and another for ubiquitin chain elongation (Seino et al., 2003; Rodrigo-Brenni & Morgan, 2007; Garnett et al., 2009; Williamson et al., 2009) (Figure 1).

The first evidence for the priming and elongation model was reported by Seino and co-workers (2003) in *S. pombe*. Ubc4 and Ubc11, the *S. pombe* homologs of human UbcH5 (84% identity) and UbcH10 (61% identity), respectively, are required for mitotic progression in *S. pombe* and depletion of either E2 results in mitotic arrest and cyclin accumulation (Seino et al., 2003). Furthermore, in the absence of Ubc4, Ubc11 forms short ubiquitin

chains on APC/C substrates. Conversely, Ubc4 cannot ubiquitinate APC/C substrates in the absence of Ubc11, suggesting that Ubc11 is required for initial substrate ubiquitination. Similar models have been reported in budding yeast (Ubc1 and Ubc4) (Rodrigo-Brenni & Morgan, 2007) and human (UbcH10 and Ube2S) systems (Garnett et al., 2009; Williamson et al., 2009). It has long been thought that human UbcH5, a promiscuous E2 that can ubiquitinate APC/C substrates *in vitro* (Yu et al., 1996; Summers et al., 2008), is the chain elongation APC/C cognate, but recent evidence suggests that the true human APC/C cognate E2s are UbcH10 (priming) and Ube2S (elongation) (Garnett et al., 2009; Williamson et al., 2009).

UbcH10 was the first E2 enzyme identified as an important player in cyclin degradation (Townsley et al., 1997) and knockdown of UbcH10 phenocopies loss of APC/C function (Townsley et al., 1997; Bastians et al., 1999). UbcH10 is degraded in an APC/-dependent manner at the end of mitosis, effectively de-activating the APC/C as other APC/C substrates become limiting (Rape & Kirschner, 2004; Walker et al., 2008; Williamson et al., 2009). However, this mechanism of APC/C regulation does not appear to be conserved in yeasts because Ubc11 (UbcH10 homolog) protein expression is not cell cycle regulated (data not shown (Osaka et al., 1997)).

Interestingly, UbcH10 has a distinctive N-terminal extension (ca. 30 amino acids), which affects its ability to be charged with ubiquitin and in turn alters the regulation and substrate specificity of the APC/C (Huang et al., 2008; Summers et al., 2008). The UbcH10 N-terminal extension is conserved from *S. pombe* to humans, but is not present in *S. cerevisiae*, and it might contact APC/C subunits other than Apc11 (RING finger protein) (Tang et al., 2001b; Summers et al., 2008).

Ub chain topology

Ub chains are assembled by a condensation reaction catalyzed by an E2-E3 pair that forms an isopeptide bond between the C-terminal glycine of one Ub molecule to lysine residues of subsequent Ub molecules. Ub contains seven lysine residues (K6, K11, K27, K29, K33, K48, and K63) and chains formed via specific lysine linkages elicit unique cellular responses (Pickart & Fushman, 2004). It has long been known that K48 chains of at least four Ub molecules target substrates to the 26S proteasome for degradation (Thrower et al., 2000), but recent evidence strongly suggests that both K11 and K48 are capable of targeting proteins for degradation (Rodrigo-Brenni & Morgan, 2007; Jin et al., 2008; Xu et al., 2009). In fact, all polyUb chain structures can be targeted by the proteasome, albeit with different efficiencies (Xu et al., 2009). The topology of APC/C-catalyzed Ub chains differs between budding yeast (K48) and human (K11) (Kirkpatrick et al., 2006; Rodrigo-Brenni & Morgan, 2007; Jin et al., 2008; Garnett et al., 2009; Williamson et al., 2009; Bremm et al., 2010; Wu et al., 2010). This switch in topology is likely due to the absence of K11 specificity factors (TEK box and Ube2S) in budding yeast (Jin et al., 2008).

Structure

As discussed in previous sections, many questions remain regarding the mechanism of APC/C action and regulation, including how the core APC/C interacts with substrates, how

processive transfer of Ub to substrates is accomplished, and how APC/C regulators stimulate or inhibit E3 ligase activity. Just as structural studies of other large macromolecular machines, such as the proteasome, RNA polymerase and the ribosome, have proven instrumental in unraveling the mechanisms of protein degradation, transcription, and protein synthesis, respectively, generating a detailed three-dimensional (3D) map of the APC/C will be crucial in developing a mechanistic model for its function.

Efforts to structurally characterize the holo-APC/C using X-ray crystallography have been difficult due to cell cycle specific changes in composition and posttranslational modifications of the APC/C, as well as its low cellular abundance. In addition, the large size of the complex has made reconstituting the APC/C using purified components a daunting task. For these reasons, atomic-resolution structural studies have been limited to only a few individual components. These include the winged-helix motif found in the Apc2 cullin domain (Zheng et al., 2002), Apc10/Doc1 (Wendt et al., 2001; Au et al., 2002), the N-terminal domain of human Apc7 (Han et al., 2009), a subcomplex between the TPR repeats in Apc6 and a short N-terminal region of Cdc26 (Wang et al., 2009), the N-terminal TPR domains of Cdc27 (Apc3) (Zhang et al., 2010b), and a subcomplex of *S. pombe* Cut9 (Apc6) and Hcn1 (Cdc26) (Zhang et al., 2010a; Zhang et al., 2010b). These structures have provided some information about how distinct subcomplexes within the APC/C, especially the TPR repeats, may assemble and perhaps interact with substrates and/or activators. However, these small snapshots of individual domains have not provided direct answers to the fundamental questions about how the APC/C mechanistically functions.

Single particle cryo-electron microscopy (EM) has proven to be a viable approach for gaining insights into the overall shape and organization of the holo-complex. Cryo-EM studies of *S. pombe, S. cerevisiae, Xenopus*, and human APC/C have now been reported (Gieffers et al., 2001; Dube et al., 2005; Passmore et al., 2005; Ohi et al., 2007; Herzog et al., 2009; da Fonseca et al., 2010; Buschhorn et al., 2010). In addition, there are also 3D structures of the APC/C bound to the activators Cdh1 and Cdc20 (Dube et al., 2005; Ohi et al., 2007; Herzog et al., 2009), to the MCC inhibitory complex (Herzog et al., 2009), as well as substrates (da Fonseca et al., 2010; Buschhorn et al., 2010). These cryo-EM 3D maps have provided an essential starting point for understanding how the structural organization of the APC/C translates into function. In the following paragraphs, we describe each 3D model, taking note of how each complex was purified and visualized by EM, as well as discuss some of the similarities and differences found between the different APC/C 3D models.

There are currently three published 3D structures of the S. cerevisiae APC/C. The first ~20 Å structure was determined using particles purified from asynchronously growing cells and imaged in unstained vitrified ice (Passmore et al., 2005) (Figure 2A). Very recently, the same group published a second structure of the S. cerevisiae APC/C in vitrified ice bound to Cdh1 and a D box peptide (da Fonseca et al., 2010). This map reached the much higher resolution of ~10 Å allowing the visualization of a triangular shaped structure that contains a central cavity lined with a lattice-like scaffold. Importantly, the authors localized the position of a D box peptide bound in the central cavity and determined that the D box peptide contacts both Cdh1 and Doc1, a core APC/C subunit previously shown to be required for Ub processivity (Carroll and Morgan, 2002; Passmore et al., 2003). This work provides the first structural

model for understanding how the APC/C interacts with substrates and further strengthens the hypothesis that APC/C-substrate binding results from multivalent interactions with both activators and core APC/C components. This work was closely followed by a third ~25 Å cryo-negative stain structure determined from particles bound to Cdh1 and a D box peptide (Buschhorn et al., 2010). As with the structure from da Fonseca et al., Buschhorn et al. showed that the D box binds in the central cavity of the APC/C and contacts both the activator and Doc1. Unfortunately, the APC/C density maps from both of the above studies were not publically released at the time of this review and, thus, are not included in Figure 1. There are a number of conclusions that can be drawn from these two new *S. cerevisiae* APC/C structures. First, both Passmore et al., (2005) and Buschhorn et al., (2010) found evidence that the S. cerevisiae APC/C forms dimers; however, the presence of dimerized APC/C has thus far only been observed in budding yeast purifications making it unclear whether this is truly a physiologically relevant phenomenon. Second, the conformational changes seen in both the *Xenopus* and human APC/C upon activator binding (Herzog et al., 2009) were not observed in the 10 Å S. cerevisiae structure (da Fonseca et al., 2010) making it unclear whether this is a conserved mechanism of APC/C regulation. Third, the new *S. cerevisiae* APC/C structures (da Fonseca et al., 2010; Buschhorn, et al., 2010) look very similar in shape and size to structures of the APC/C determined from *S. pombe* and vertebrates (Ohi et al., 2007; Herzog et al., 2009), clearly showing that this complex has been conserved during evolution. Fourth, both *S. cerevisiae* structures clearly show that the D box peptide interacts with the APC/C by binding to both the activator and to Doc1, a core APC/C component (da Fonseca et al., 2010; Buschhorn et al., 2010), providing the first structural models of APC/C substrate recognition and binding modes.

The 27Å structure of the *S. pombe* APC/C^{Cdc20} was determined using particles purified from mitotically blocked cells and visualized in unstained vitrified ice (Ohi et al., 2007). The *S. pombe* APC/C^{Cdc20} adopts an asymmetric, tricorn-shaped structure, ~ 19 × 17 × 15 nm in size, with a deep internal cavity and a prominent horn-like protrusion emanating from the bottom of the cavity lip (Figure 2B). The size of the central cavity is ~11.5 × 9.5 × 6.5 nm and is large enough to hold both an E2-Ub conjugated complex and an APC/C substrate, such as the CDK-cyclin complex. Using antibody labeling and mutant analysis, this study localized the C-terminus of 12 of the 13 core APC/C components, as well the position of the activator Cdc20, generating the most comprehensive map of APC/C organization to date. From the position of the RING component Apc11 and the activator Cdc20, the authors proposed a model where Cdc20 could initially recruit the substrate to the complex for ubiquitination, but the processivity of the reaction would be determined by interactions between the substrate and core components lining the central cavity (Ohi et al., 2007).

There have been a number of cryo-negative stain structures reported of vertebrate APC/C (Figure 2C–F). These include *Xenopus* APC/C bound and unbound to recombinant Cdh1 (APC/C^{Cdh1} and apo-APC/C) (Dube et al., 2005; Herzog et al., 2009), mammalian apo-APC/C purified either from asynchronous lysates (Gieffers et al., 2001; Dube et al., 2005) or from spindle checkpoint active lysates (Herzog et al., 2009), mammalian APC/C bound to either recombinant Cdh1 or Cdc20 (hAPC/C^{Cdh1} and hAPC/C^{Cdc20}) (Herzog et al., 2009), and mammalian APC/C bound to the MCC purified from human cells arrested with an active SAC (hAPC/C^{MCC}) (Herzog et al., 2009). As the same group has determined all of these

structures, following the progression of their APC/C density maps, starting with the first reported structure in 2001 (Gieffers et al., 2001) highlights the steady improvements made in single particle EM methodologies over the last decade.

As with the *S. cerevisiae* and *S. pombe* APC/C (Passmore et al., 2005; Ohi et al., 2007; da Fonseca et al., 2010; Buschhorn et al., 2010) (Figure 2A and B), the vertebrate structures (~24–20Å resolution, Figure 2C–E) reveal a triangular-shaped complex. Unlike the original *S. cerevisiae* structure (Passmore et al., 2005), but similar to the *S. pombe* APC/C (Ohi et al., 2007) and new *S. cerevisiae* structures (da Fonesca et al., 2010; Buschhorn et al., 2010), the vertebrate APC/C contains a central cavity surrounded by what the authors label the 'arc-lamp'- and platform-like domains (Dube et al., 2005; Herzog et al., 2009) (Figure 2C–E). Antibody labeling studies were used to localize the position of Apc1, Apc2, Apc3 (Cdc27), Apc4, Apc5, Apc6, Apc7 (Dube et al., 2005; Herzog et al., 2009) and found that the 'arc-lamp'-like domain is composed of the TPR subunits Apc6 and Apc7, while Apc1, Apc2, Apc4, and Apc5 are located in or near the 'platform'-like domain. This subunit organization closely resembles that of *S. pombe* APC/C (Ohi et al., 2007) (Figure 2B and E). In fact, other than the mirrored handedness of the *S. pombe* with the *S. cerevisiae* and vertebrate structures, the overall structural organization of the APC/C appears very similar (Figure 2B, C, E, and data not shown), as would be predicted by the functional conservation of APC/C subunits across species (Table 1) (Thornton & Toczyski, 2006).

One apparent difference between the *S. pombe* and other structures is where Cdc20 and Cdh1 bind to the core complex (Figure 2B, C, and E). Structural analysis of the *S. pombe* APC/C purified from a mutant strain that precludes Cdc20 binding at the restrictive temperature, as well as antibody labeling studies of an endogenously tagged Cdc20 bound to the APC/C, localized the position of Cdc20 at the lip of the APC/C cavity close to the C-terminus of Apc2 (Figure 2B) (Ohi et al.). This position was very similar to the original localization of recombinant Cdh1 in *Xenopus* APC/C (Dube et al., 2005). In contrast, the densities corresponding to recombinant Cdc20 or Cdh1 bound to mammalian apo-APC/C and *S. cerevisiae* APC/C were found in the central cavity close to the TPR subunit (or 'arc-lamp'-like) domain and Cdc27 (Apc3) (Herzog et al., 2009), a subunit involved in activator binding (Figure 2E) (Vodermaier et al., 2003; Matyskiela & Morgan, 2009; da Fonseca et al., 2010; Buschhorn et al., 2010). Since this localization was different from what was previously published for *Xenopus* APC/C^{Cdh1} (Dube et al., 2005), the authors re-examined *Xenopus* APC/C^{Cdh1} using improved sample preparation and EM imaging techniques. This new analysis found that addition of recombinant Cdh1 to the *Xenopus* structure generated two extra densities, one on the side of the structure and one overlapping with the activator binding position of mammalian APC/C (Figure 2C) (Herzog et al., 2009). This suggests that the APC/C may contain multiple activator-binding sites. Why two sites are only detected in the *Xenopus* structure and not the mammalian or *S. pombe* APC/C remains to be examined, although it should be noted that variance mapping of *S. pombe* APC/C showed one high difference peak in a central location similar to that found for mammalian activator(Ohi et al., 2007). In retrospect, this variance peak could represent a second binding event for Cdc20 the *S. pombe* structure.

Interestingly, three major conformations of mammalian apo-APC/C were observed that differ mainly in the position of the 'arc-lamp' and 'platform'-like domains relative to each other (Herzog et al., 2009). Thus, apo-APC/C appears to adopt a continuum of flexible states ranging from open to closed states. Although this range of structural flexibility was not apparent in the yeasts, *Xenopus*, or earlier mammalian structures (Gieffers et al., 2001; Dube et al., 2005; Passmore et al., 2005; Ohi et al., 2007; da Fonseca et al., 2010), it is still tempting to speculate that different conformations may directly correlate with APC/C E3 ligase activity and that one role of APC/C activators and inhibitors is to shift the structural equilibrium between these open and closed states. At least for mammalian APC/C, this model is supported by the comparison of the 3D density maps of apo-APC/C, APC/C^{Cdc20}, and APC/C^{MCC} (Herzog et al., 2009). In these structures, the binding of the inhibitory MCC 'locks' the APC/C into a closed or more compact conformation (Figure 2F), while the presence of Cdc20 alone shifts the equilibrium to a more open state (Herzog et al., 2009). Interestingly, the position of Cdc20 in the APC/C^{MCC} density map is shifted by ~2 nm as compared to its position in APC/C^{Cdc20} (Herzog et al., 2009). This suggests that MCC binding may also affect APC/C activity by modulating how Cdc20 interacts with the complex, perhaps adding another layer of structural regulation to APC/C activity. It remains to be determined what, if any, structural effects MCC binding has on APC/C-substrate interactions.

Although the above reported structures have provided an important first glimpse into overall APC/C structure and organization, it is clear that more in-depth structural analyses will be required before we reach a comprehensive understanding of how the APC/C works and is regulated. In particular, it is still unclear how the APC/C transfers Ub to substrates and regulates processivity. Structures of the APC/C bound to substrates and activators and higher resolution EM maps to allow precise docking of atomic resolution structures of individual APC/C components and/or subcomplexes, are required to begin to understand the mechanisms of APC/C catalysis and regulation.

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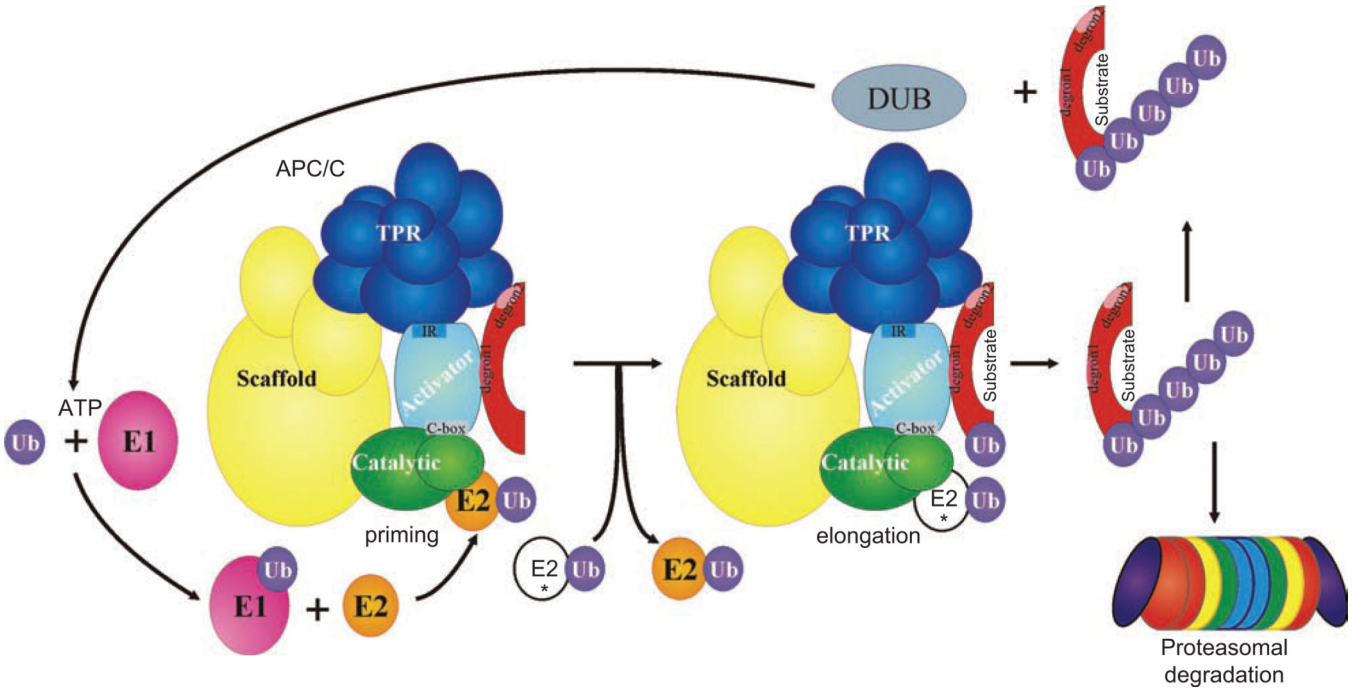

Figure 1.

Theoretical model of APC/C ubiquitination pathway. The four APC/C modules (Catalytic=Apc2, Apc11; Scaffold = Apc1, Apc4, Apc5; TPR=Apc3, Apc6, Apc8, Cdc26, Apc10, and species-specific subunits; and Activator=Cdc20 family member) are labeled. El = Ub-activating enzyme, E2 = Ub-conjugating enzyme, IR = IR motif in Cdc20 family members, C-box= conserved motif in Cdc20 family members, degrons=APC/C degradation motifs (e.g., D box, KEN box), DUB = deubiquitinating enzyme, * indicates elongation E2.

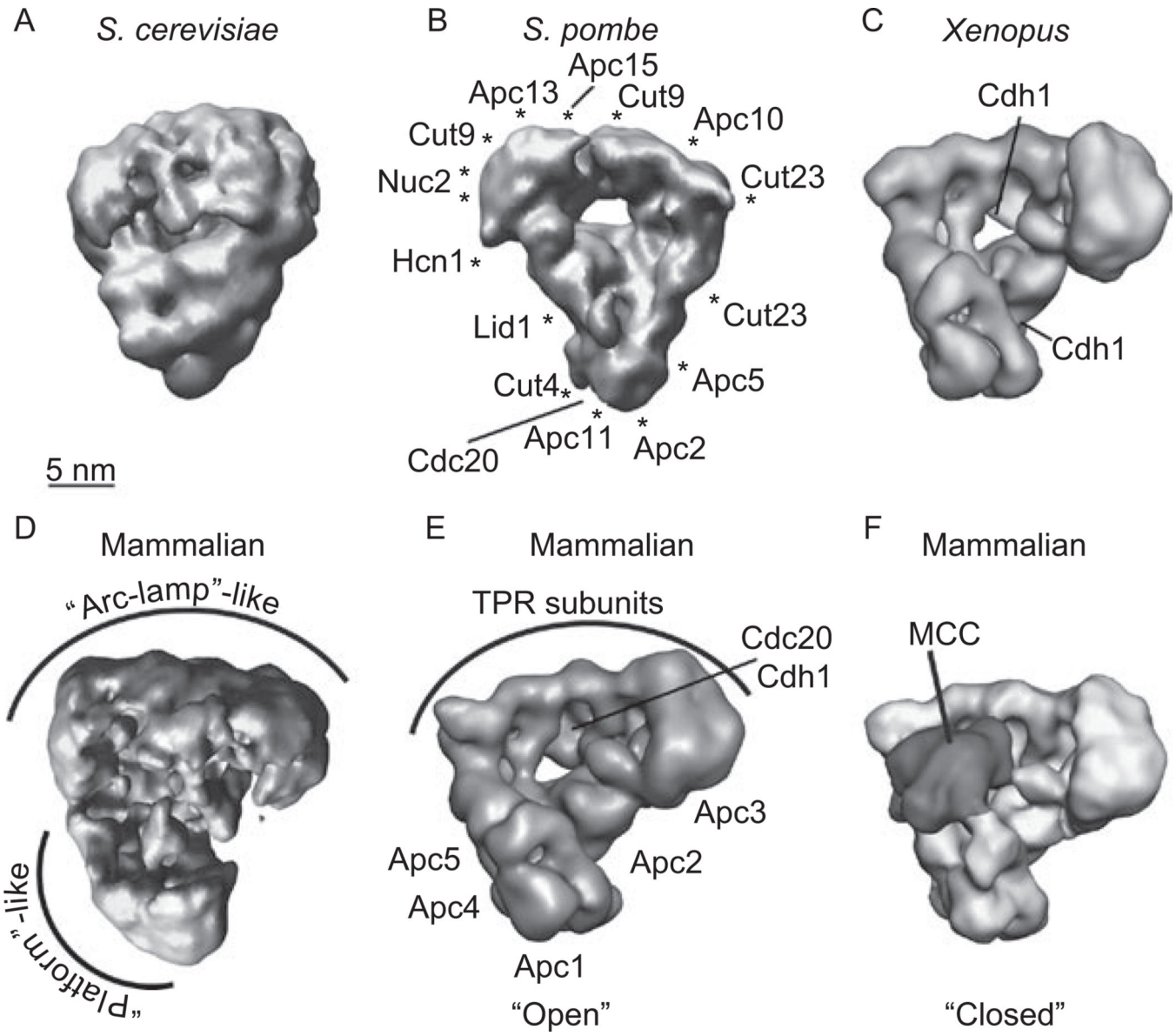

Figure 2.

Structural analysis of the APC/C spanning from yeasts to vertebrates. (A) ~20Å structure of the *Saccharomyces cerevisiae* APC/C purified from asynchronous cells (Passmore et al., 2005) (EMDB1174). (B) Single-particle cryo-EM analysis of the mitotic *Schizosaccharomyces pombe* APC/C^{Cdc20} (Ohi et al., 2007). Position of APC/C subunits and the activator Cdc20, found by antibody labeling and difference mapping, respectively, are labeled. Cut4 corresponds to human Apc1, Nuc2 corresponds to human Apc3, and Lid1 corresponds to human Apc4. (C) ~24Å structure of the *Xenopus* APC/C. Approximate locations of recombinant Cdh1 are labeled (Herzog et al., 2009). (D) Human APC/C (~26Å) purified from asynchronous cells (EMDB 1139) (Dube et al., 2005). (E) Human apo-APC/C (~20 Å) purified from checkpoint active lysates (EMDB 1592) (Herzog et al., 2009). Position of APC/C subunits as well as the activators Cdc20 and Cdh1, localized using antibody labeling and recombinant proteins, respectively, are noted. (F) ~20Å structure of

the human APC/C bound to the MCC inhibitory complex (APC/C^{MCC}, EMDB 1591) (Herzog et al., 2009). Position of MCC labeled in dark gray. Images for (D) and (F) were kindly provided by Franz Herzog (IMP-Vienna), Jan-Michael Peters (IMP-Vienna), and Holger Stark (Max Planck Institute). Scale bar for all panels, 5nm.

Table 1

Nomenclature of APC/C components and other proteins across species.

S. pombe	**kDa**	***S. cerevisiae***	**kDa**	***D. melanogaster***	**kDa**	***H. sapiens***	**kDa**	**Domain**
APC/C subunits								
Cut4	165	Apc1	196	Shattered A/B	227/56	Apc1	217	PC repeats
Apc2	81	Apc2	100	Morula	92	Apc2	94	Cullin-like
Nuc2*	53	Cdc27	79	Makos	101	Apc3	92	TPR repeats
Lid1	76	Apc4	85	Apc4	87	Apc4	92	
Apc5	83	Apc5	75	Ida A/B	89/81	Apc5	85	TPR repeats
Cut9*	76	Cdc16	95	Cdc16	82	Apc6	72	TPR repeats
—		—		Apc7A/B	60/70	Apc7	63	TPR repeats
Cut23*	60	Cdc23	79	Cdc23	78	Apc8	69	TPR repeats
—		Apc9	31	—		—		
Apc10	22	Doc1	33	Apc10	22	Apc10	21	
Apc11	11	Apc11	19	Lemming	10	Apc11	10	RING domain
Hcn1	9	Cdc26	14	—		Cdc26	10	
Apc13	16	Swm1	19	—		Apcl3	8	
Apc14	12	—		—		—		
Apc15	16	Mnd2	43	—		—		
—		—		—		Apc16	12	
Ubiquitin conjugating enzymes								
Ubc11	20	Ubc11	18	vihar/UbcH10	20	UbcH10/Ube2C	20	E2
—		—		Ube2S	23	Ube2S	24	E2
Ubc4	16	Ubc4/Ubc5	16	effete/UbcD1	17	UbcH5	17	E2
Ubc1	24	Ubc1	24	Ube2K	22	Ube2K/E2-25K	22	E2
Critical yeast substrates								
Cdc13	56	Clb2	56	cyclin B	60	cyclin B	48	cyclin box
Cut2	33	Pds1	42	Pim	58	securin	22	securin

*Denotes two copies of subunit present in each *Schizosaccharomyces pombe* APC/C complex (Ohi et al. 2007). — Denotes that subunit has not been identified in this species. Proteins listed in the same row indicate homologs.

Table 2

APC/C activators and inhibitors.

	S. pombe	*S. cerevisiae*	*D. melanogaster*	*X. laevis*	*H. sapiens*	Phase	
Activators							
*	Slp1	Cdc20	Fizzy	Cdc20/Fizzy	Cdc20/Fzy/p55^{CDC}	M/Me	
	Ste9/Srw1	Cdh1/Hct1	Fizzy-related	Fizzy-related	Cdh1/Fzr1	M/Me/G1	
	Mfr1	—	—	—	—	Me	
	—	Ama1	—	—	—	Me	
	—	—	Cortex	—	—	Me	
Inhibitors							Form inhibited
			Rca1	Emi1	Emi1	M/G1	APC/C^{Cdh1}
	—	—	—	Erp1	Emi2	Me	
	Mad2	Mad2	Mad2	Mad2	Mad2	M	Apc/c^{Cdc20}
	Mad3	Mad3	BubR1	BubR1	BubR1	M	
*	Mes1	—	—	—	—	Me	
*	—	Acm1	—	—	—	M/G1	Apc/C^{Cdh1}

*Also an APC/C substrate.

M = mitosis; Me = meiosis; G1 = G1 phase.

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Table 3

APC/C destruction motifs.

Destruction motif	Sequence	References
D box	RxxLxxxN	Glotzer et al., 1991
KEN box	KENxxxN	Pfleger Kirschner, 2000
A box or DAD (D box activating domain)	RxLxPSN	Castro et al., 2002; Littlepage Ruderman, 2002
CRY box	CRYxPS	Reis et al., 2006
GxEN	GxEN	Castro et al., 2003
O box	LxEKN	Araki et al., 2005
Spo13	LxExxxN	Sullivan Morgan, 2007
TEK box	R/KxxTxKT	Jin et al., 2008

“x” indicates any amino acid.