REVIEW

Whose end is destruction: cell division and the anaphase-promoting complex

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Cell proliferation depends on the duplication of chromosomes followed by the segregation of duplicates (sister chromatids) to opposite poles of the cell prior to cell division (cytokinesis). How cells ensure that chromosome duplication, chromosome segregation, and cell division occur in the correct order and form an immortal reproductive cycle is one of the most fundamental questions in cell biology. Without such coordination, cells would not maintain a constant chromosome number and sexual reproduction as we know and love it would not be possible.

A halfhearted cell cycle

The discovery of cyclin-dependent kinases (CDKs) went some way to answering this question. Successive waves of S- and M-phase-promoting CDKs first trigger chromosome duplication (S phase)—then the attachment of the replicated chromosomes to a bipolar spindle (M phase). In animal cells, S phase is induced by Cdk2 bound to S-phase cyclins (E- and A-type) whereas M phase is triggered by Cdk1 associated with mitotic cyclins (A- and B-type). In both fission yeast and budding yeast, S and M phase are induced by a single CDK (Cdk1) bound to S-phase- and M-phase-specific B-type cyclins, respectively. We now understand many of the regulatory mechanisms that activate S- and M-CDKs in the correct order. We also have a robust hypothesis for how cells ensure that no genomic sequence is duplicated more than once during the interval between the onset of S and M phases. Initiation of DNA replication requires two distinct steps: first, prereplicative complexes (pre-RCs) are assembled at future origins of replication, a process that can occur only in the absence of CDK activity. The second step, origin unwinding and the recruitment of replication enzymes, is triggered by CDK activation. Because pre-RC assembly is inhibited by CDK activity, chromosome re-replication requires a CDK cycle, a period of low CDK activity followed by a period of high CDK activity. Having activated S-CDKs in late G₁, cells maintain high

CDK activity until metaphase and this prevents refiring of replication origins.

However, several crucial elements were missing from this CDK-dominated view of the cell cycle. Missing was the impetus that causes sister chromatids to separate at the metaphase-to-anaphase transition; the machinery that destroys mitotic cyclins during anaphase; a mechanism for ensuring that sister chromatid separation normally precedes cytokinesis and chromosome reduplication; and an understanding of how events that trigger sister chromatid separation and exit from mitosis also create the conditions that cause the chromosome cycle to be repeated. Insight into all these questions has recently stemmed from the identification of the machinery responsible for degrading mitotic cyclins, a ubiquitin-protein ligase called the anaphase-promoting complex or cyclosome (APC/C). By destroying anaphase inhibitory proteins, the APC/C triggers the separation of sister chromatids; by destroying mitotic cyclins, it creates the low CDK state necessary for cytokinesis and for reforming the pre-RCs complexes needed for another round of genome replication. Since the discovery of the APC/C 4 years ago, there has been a veritable deluge of results on its roles and regulation, which this article attempts to summarize.

Cyclin degradation in vivo and in vitro

Much of our knowledge about cyclin degradation comes from experiments with eggs from the frog *Xenopus laevis* and from marine invertebrates such as sea urchins and the clam *Spisula solidissima*. Upon fertilization, these cells undergo a series of rapid and synchronous cell cycles that consist of alternating S and M phases. Mitotic cyclins A and B steadily accumulate during interphase (the time between two M phases) and are then suddenly degraded during mitosis (Evans et al. 1983). The development of cell-free extracts that reproduce many aspects of the cell cycle opened the way to a biochemical analysis of cyclin destruction (Luca and Ruderman 1989; Murray and Kirschner 1989). The amino terminus of cyclin B was found to be essential for degradation but dispensable for the formation of an active Cdk1-cyclin B kinase (Murray et al. 1989). An amino-terminal cyclin B fragment was sufficient to confer mitotic degradation to heterologous proteins, whereas a version of cyclin B lacking

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its amino terminus constitutively activated Cdk1, which prevented exit from mitosis. These data suggested that cyclin B degradation is essential for cell cycle progression. Cdk1 inactivation at the end of mitosis has subsequently been found to be essential for cell cycle progression in all eukaryotes. Degradation of mitotic cyclins appears to be a universal mechanism for Cdk1 inactivation even though other mechanisms also exist.

A closer inspection of the amino termini of mitotic cyclins revealed a degenerate nine amino acid motif called the destruction box, whose mutation renders cyclin B resistant to degradation (Glutzer et al. 1991). The observation that the destruction box was also required for the formation of cyclin-ubiquitin conjugates (Glutzer et al. 1991) and that cyclin degradation was sensitive to inhibitors of the ubiquitin system (Hershko et al. 1991) pointed to the protease responsible for cyclin degradation: the 26S proteasome, a multisubunit protease specific for multiubiquitinated substrates (Coux et al. 1996; Baumeister et al. 1998). In contrast to cyclin B ubiquitination, which occurs only in mitotic extracts, the proteasome is active throughout the cell cycle suggesting that cyclin ubiquitination rather than its degradation is cell cycle regulated (Mahaffey et al. 1993). Indeed, cyclin B-ubiquitin conjugates generated in mitotic extracts are efficiently degraded in interphase extracts (J.-M. Peters, pers. comm.).

Cyclin B was one of the first cellular substrates of physiological importance to be identified for the ubiquitin-proteasome pathway (see Fig. 1). During this process, ubiquitin is first activated by forming a high-energy thioester with a cysteine in a ubiquitin-activating enzyme known as E1. Ubiquitin is subsequently transferred to one of several ubiquitin-conjugating enzymes (called UBC or E2) to form a second thioester. Finally, an isopeptide bond is formed between ubiquitin's carboxyl terminus and a lysine residue of the substrate. More ubiquitin molecules are conjugated to those already attached to create a polyubiquitin chain that is recognized

by the 26S proteasome, leading to proteolysis of the substrate. The transfer of ubiquitin from E2 enzymes to substrates requires a third activity, called ubiquitin-protein ligase or E3. By interacting with both the substrate and the E2 enzyme, ubiquitin-protein ligases are thought to provide specificity. The E3, and not the E2 enzyme largely determines which proteins are ubiquitinated and subsequently degraded. Cells usually contain a single, conserved E1 enzyme and a family of related E2 enzymes. E3 enzymes appear, however, to be structurally diverse, ranging from single proteins to large multisubunit complexes such as the APC/C.

Cyclin A is degraded during metaphase and cyclin B slightly later at the metaphase-to-anaphase transition. The coincidence between cyclin degradation and entry into anaphase gave rise to the idea that cyclin degradation might be the signal that triggers sister chromatid separation. Expression of nondegradable cyclin variants does indeed block Cdk1 inactivation, spindle disassembly, and cytokinesis in many systems. However, sister chromatid separation is not inhibited (Holloway et al. 1993; Surana et al. 1993). Nevertheless, cyclin degradation and sister chromatid separation appeared to be connected. Inhibition of the cyclin degradation system by high concentrations of an amino-terminal cyclin B fragment also blocked sister chromatid separation in *Xenopus* egg extracts (Holloway et al. 1993). This observation led to the idea that sister chromatid separation might depend on destruction box-dependent degradation of a non-cyclin protein. Such anaphase inhibitors have indeed been identified in yeast and vertebrates (see below). However, recent experiments suggest that depletion of cellular ubiquitin contributes to the inhibitory effect of destruction box peptides (Yamano et al. 1998).

A needle worth the search: identification of the APC/C

The identity of the machinery responsible for cyclin B ubiquitination was revealed by a remarkable conver-

Figure 1. Degradation of cyclin B by the ubiquitin-proteasome pathway. Ubiquitin (Ub, gray circles) forms thioester intermediates first with the ubiquitin-activating enzyme E1 and then with an ubiquitin-conjugating enzyme E2. Finally, it forms an isopeptide bond with a lysine of the substrate—in this case, cyclin B. Transfer of ubiquitin from the E2 to the substrate is catalyzed by the ubiquitin-protein ligase (E3) activity of the APC/C. Multiubiquitinated cyclin B is recognized by the 26S proteasome (dark gray) and then proteolyzed to yield peptides and free, reusable ubiquitin.

gence of biochemical and genetic studies. Fractionation of extracts from clam oocytes and *Xenopus* eggs suggested that three components might be sufficient for cyclin ubiquitination: an E1 enzyme commonly required for all ubiquitin transfer reactions; a cyclin-specific E2; and a cyclin-specific ligase activity (Hershko et al. 1994; King et al. 1995; Sudakin et al. 1995) (Fig. 1). Unlike E1 and E2, the ligase activity was cell cycle regulated, being active when isolated from mitotic extracts but inactive when isolated from interphase extracts. Remarkably, the ligase activity sedimented as a large particle of 20S. Candidates for its components were suggested by a parallel study of cyclin B proteolysis in budding yeast: certain tetratricopeptide repeat (TPR) proteins known to be necessary for nuclear division were found to be essential for cyclin proteolysis in vivo (Irniger et al. 1995). Vertebrate homologs of two of these TPR proteins, Cdc16 and Cdc27, were detected in a 20S complex from human cells (Tugendreich et al. 1995) and were associated with the cyclin-ubiquitin ligase activity from *Xenopus* (King et al. 1995).

Key to isolating mutants specifically defective in B-type cyclin proteolysis was the recognition that this process is not confined to anaphase cells but continues during the subsequent G₁ period until cells enter S phase (Amon et al. 1994). By looking for mutants defective in degrading a cyclin- β -galactosidase fusion protein in G₁ cells, it was possible to exclude mutants that failed to degrade mitotic cyclins merely because they arrested in G₂ or metaphase, during which mitotic cyclins are stable (Irniger et al. 1995). Temperature-sensitive mutations in five essential genes were identified that cause a defect in cyclin degradation in anaphase and G₁. Furthermore, protein extracts from these mutants are defective in cyclin ubiquitination (Irniger et al. 1995; Zachariae and Nasmyth 1996; Zachariae et al. 1996). Whereas overexpression of a nondegradable mitotic cyclin arrests cells in late anaphase, the cyclin degradation mutants arrest earlier, in a metaphase-like state: sister chromatid separation, spindle elongation, Cdk1 inactivation, cytokinesis, and genome rereplication all fail to occur. Thus, all five genes are required for both anaphase onset and cyclin degradation. Furthermore, they all encode subunits of a 36S complex. The clam ubiquitin ligase particle was called the cyclosome, but when it became clear that the equivalent particle was required for the onset of anaphase in budding yeast, the yeast and *Xenopus* particles were called the anaphase-promoting complex or APC. Similar complexes have been found in fission yeast (Yamashita et al. 1996), *Aspergillus nidulans* (Lies et al. 1998), and in human cells (Tugendreich et al. 1995; Grossberger et al. 1999), suggesting that the APC/C is a conserved constituent of all eukaryotic cells.

Ubiquitin-conjugating enzymes of the APC/C pathway

Two classes of E2 enzymes are capable of collaborating with the APC/C. E2-C from clam and its homologs from *Xenopus* (UBCx), humans (UbcH10), and fission yeast (UbcP4) seem to function specifically in ubiquitination

mediated by the APC/C (Aristarkhov et al. 1996; Yu et al. 1996; Osaka et al. 1997; Townsley et al. 1997). E2-C is required for the ubiquitination of cyclins but not the bulk of other unstable proteins. Furthermore, expression of a catalytically inactive version of UbcH10 blocks anaphase onset and cyclin degradation in vivo. At least in vitro, cyclin ubiquitination by the APC/C from *Xenopus* and budding yeast is also supported by Ubc4, which belongs to a different class of E2 enzymes that have been implicated in other degradation pathways (Yu et al. 1996; Charles et al. 1998). However, in budding yeast, the APC/C does not seem to require any particular E2 enzyme. Yeast mutants lacking Ubc4, its close relative Ubc5, or the E2-C homolog Ubc11 do not have any major defect in cyclin ubiquitination/degradation (Zachariae and Nasmyth 1996; Townsley and Ruderman 1998). It has been reported that B-type cyclins are stabilized in budding yeast *ubc9* mutants (Seufert et al. 1995). However, the Ubc9 enzyme transfers the ubiquitin-like protein Smt3/Sumo1, not ubiquitin, and this modification does not induce proteolysis (Johnson and Blobel 1997; Schwarz et al. 1998). Ubc9 might support cyclin degradation only indirectly, as a result of its involvement in processes such as nuclear import (Lee et al. 1998).

The APC/C core particle: SCF's big brother?

The composition of APC/C has been investigated by immunopurification. The *Xenopus* and human particles contain ≥ 10 subunits, whereas the yeast particle contains ≥ 12 subunits (Table 1). Most, if not all, yeast subunits have counterparts in vertebrates, which suggests that the APC/C has a similar composition in all eukaryotes (Peters et al. 1996; Yu et al. 1998; Zachariae et al. 1998b; Grossberger et al. 1999). These subunits, the list of which might not yet be complete, remain tightly associated with each other throughout the cell cycle and therefore form the core of the particle (Peters et al. 1996; Grossberger et al. 1999). Additional regulatory subunits, such as the Trp-Asp repeat (WD) activator proteins Cdc20 and Cdh1 (see below), whose association with the APC/C core is both substoichiometric and cell cycle regulated, were initially not detected by biochemical studies.

It is possibly not surprising, considering the large number of APC/C subunits, to find that many subunits contain motifs thought to mediate protein-protein interactions. The largest subunit, Apc1, shares a motif of unknown function with Rpn1 and Rpn2, which are subunits of the 19S cap complex of the proteasome (Peters et al. 1996; Yamashita et al. 1996; Zachariae et al. 1996; Lupas et al. 1997). Cdc16, Cdc23, Cdc27, and Apc7, a Cdc27-like subunit found only in vertebrates, are all related proteins of 70–100 kD that contain 9–10 copies of the TPR motif (Lamb et al. 1994; Tugendreich et al. 1995; Yu et al. 1998). This degenerate 34-amino-acid motif is not, however, specific for the APC/C, as it is found in many other proteins with unrelated functions (Lamb et al. 1995). Each motif consists of a pair of antiparallel α -helices and multiple motifs are predicted to fold into a

Table 1. Subunits of the APC/C in yeast and mammalian cells

<i>S. cerevisiae</i>		<i>S. pombe</i>		Mammals ^a		Motif
<i>Core subunits</i>						
Apc1	1748	Cut4	1458	Tsg24 ^b	1944	Rpn1/2 repeats
Apc2	853			Apc2	821	Cullin domain
Cdc16	840	Cut9 ^b	671	Cdc16 ^b	619	10 TPR repeats
Cdc27	758	Nuc2	665	Cdc27 ^b	823	10 TPR repeats
—				Apc7	565	10 TPR repeats
Apc4	652	Lid1	719	Apc4	808	
Apc5	658			Apc5	755	
Cdc23	626	Cut23 ^c	517	Cdc23 ^b	591	9 TPR repeats
Apc9 ^d	265					
Doc1 ^e	283	Apc10	190	Apc10	185	Doc domain
Apc11	165	{Apc11} ^f	94	Apc11	84	RING-H2 domain
Cdc26 ^e	124	Hcn1	80	Cdc26 ^g	85	
<i>Activators</i>						
Cdc20	610	Slp1	488	Cdc20	499	7 WD repeats
Cdh1/Hct1 ^d	566	Srw1/Ste9 ^d	556	Cdh1	493	7 WD repeats
Ama1 ^h	593					7 WD repeats

The number of amino acids is given for each protein.

^aThe mouse Apc1-homolog is called Tsg24; all other entries refer to human proteins.

^bPhosphorylated in mitosis.

^cM. Yanagida (pers. comm.).

^dNot essential for proliferation.

^eEssential only at 37°C.

^fPresence in the *S. pombe* particle remains to be confirmed.

^gC. Gieffers and J.-M. Peters (pers. comm.).

^hExpressed only in meiosis (K.F. Cooper and R. Strich, pers. comm.)

superhelical structure with a continuous helical groove suitable for protein–protein interactions (Das et al. 1998). The small APC/C subunit Cdc26 seems to be important for assembly of the complex. Budding yeast Cdc26 is a heat shock protein that stabilizes the interaction of three core subunits (Cdc16, Cdc27, Apc9) with the rest of the particle (Zachariae et al. 1996; Zachariae et al. 1998b). Indeed, overexpression of Cdc26's fission yeast homolog, Hcn1, suppresses the assembly defect caused by a *cut9* (*cdc16*) mutation (Yamada et al. 1997).

Of yet greater interest are those subunits that contain motifs or domains found in other proteins implicated in ubiquitination. Doc1/Apc10, for example, is a 30-kD protein that contains a domain, called the Doc domain, found in several large mammalian proteins (Hwang and Murray 1997; Kominami et al. 1998; Grossberger et al. 1999). It is interesting that these proteins also contain either HECT (see below) or cullin domains, both of which are hallmarks of ubiquitin ligases. Nothing, however, is known about the function of these proteins.

Clearly, the most remarkable finding to emerge from these studies is that two of APC/C's subunits, Apc2 and Apc11, are related to subunits from SCF (for Skp1–cullin–F-box protein), another ubiquitin ligase complex with an important role in cell cycle regulation (Feldman et al. 1997; Skowyra et al. 1997). This suggests that APC/C and SCF might be derived from the same ancestral ubiquitin protein ligase. Apc2 is a member of the cullin family of proteins, which includes the SCF subunit Cdc53 from budding yeast (Kramer et al. 1998b; Yu et al. 1998; Zachariae et al. 1998b). Cdc53's carboxy-ter-

minal 'cullin' domain recruits the E2 enzyme Cdc34 to the SCF (Patton et al. 1998), whereas its amino-terminal region binds to Skp1, which in turn binds to proteins that associate with substrates (Bai et al. 1996). Cdc53 is therefore thought to have a key role in bringing substrates into contact with their ubiquitin-conjugating enzyme. Apc2 might fulfill a similar role within the APC/C. However, neither Skp1 nor related proteins have been implicated in APC/C function and the mechanism by which substrates are recruited to the APC/C remains obscure. The small subunit Apc11 (Zachariae et al. 1998b) is closely related to Hrt1 (also called Rbx1 or Roc1), which has recently been identified as the fourth essential subunit of the SCF (Kamura et al. 1999; Seol et al. 1999; Skowyra et al. 1999; Tan et al. 1999). Human Hrt1 is also a subunit of the VBC complex (for VHL protein–Elongin B–Elongin C), which contains the von Hippel–Lindau tumor suppressor protein (pVHL) and is implicated in ubiquitin-dependent protein degradation (Kamura et al. 1999; Maxwell et al. 1999). Interestingly, one of VBC's subunits was identified as the cullin CUL2 (Pause et al. 1997). Apc11 and Hrt1 share a RING–H2 finger domain that coordinates two zinc ions and is suspected to mediate protein–protein interactions (Borden and Freemont 1996). Hrt1 binds both to Cdc53's carboxyl terminus and to Cdc34 and is thought to tether the E2 enzyme to SCF. Apc11 likewise interacts with the carboxy-terminal region of its cullin, Apc2, and might therefore play a similar role in the APC/C (Ohta et al. 1999). However, a stable interaction between APC/C and its E2 enzyme has so far not been detected. Indeed,

such an interaction might be transient and only occur during the transfer of ubiquitin to substrate proteins. Taken together, these data suggest that APC/C, SCF, and VBC are members of a family of complex E3 enzymes that share a cullin and a RING finger subunit and might originate from the same ancestral ubiquitin protein ligase (Fig. 2A). It is remarkable that RING domains occur in several other proteins involved in ubiquitin-dependent proteolysis such as Ubr1, Hrd1, and Mdm2, which

function in the degradation of N-end rule substrates, endoplasmic reticulum (ER) proteins, and p53, respectively. Many RING domain proteins with no apparent role in proteolysis might function in other processes regulated by ubiquitination, such as internalization of cell surface proteins (Hicke 1999).

A key question concerns which APC/C subunits are intimately involved in APC/C's catalytic activity, ubiquitin-protein ligation. A good bet would be those sub-

Figure 2. Comparison of the APC/C with other ubiquitin-protein ligase complexes. (A) APC/C, SCF, and VBC belong to a family of E3 complexes containing a cullin (red) and a RING-finger (orange) subunit. Furthermore, these complexes use specialized subunits (yellow) for substrate recognition and recruitment. SCF collaborates with several different substrate recognition subunits; Cdc4 which binds to the substrate Sic1 is shown as one example. The von Hippel-Lindau protein (pVHL) is thought to play a similar role in the VBC complex by recruiting the hypoxia-inducible factor (HIF). pVHL binds to a Skp1-like protein called Elongin C via an F box-related domain called SOCS box. Arrows with question marks indicate suspected but not yet demonstrated interactions. (B) Different reaction mechanisms of HECT domain ligases (*top*) and cullin-RING finger ligases (*bottom*). Catalysis might involve neutralization of the oxyanion that is formed as ubiquitin's carboxyl terminus is transferred to the ϵ -amino group of a substrate lysine. HECT domain ligases transfer ubiquitin from the E2 to the substrate by forming an E3-ubiquitin thioester intermediate, which requires a conserved cysteine residue located in the HECT domain. Formation of the E3-ubiquitin thioester may occur before or after association with the substrate (Sub). The cullin-RING-finger ligase module is proposed to transfer ubiquitin directly, without forming an E3-ubiquitin thioester.

units, such as Apc2 and Apc11, that are found in other ligases. Insight into the role of these two proteins has recently come from studies on their counterparts in SCF. A Cdc53–Hrt1 subcomplex is sufficient to stimulate greatly the enzymatic activity of Cdc34 and therefore fulfills the definition of a minimal ubiquitin–protein ligase (Seol et al. 1999). Thus, APC/C, and SCF, and probably also VBC, might be members of a family of ubiquitin protein ligases whose catalytic centers consist of cullin-related and RING–H2 finger subunits (Fig. 2A). If so, what then might be the catalytic mechanism of ubiquitin transfer? The only ligases whose mechanism is remotely understood are the HECT (homology to E6-AP carboxyl terminus) domain proteins, which transfer ubiquitin from the E2 enzyme to the substrate via an E3 ubiquitin thioester intermediate (Scheffner et al. 1995) (Fig. 2B top). Recent experiments argue against such a mechanism for the SCF and, by implication for the APC/C (Seol et al. 1999) (Fig. 2B, bottom). The ligase activity of the Cdc53–Hrt1 subcomplex is resistant to alkylating agents known to block thioester-forming enzymes such as E1 and E2. Furthermore, the catalytic activity of Cdc34 can be activated by synthetic polycations lacking any sulfhydryl groups. It has been proposed that ubiquitin transfer might be catalyzed by stabilizing the oxyanion formed in the transition state as ubiquitin's carboxyl terminus is transferred to the ϵ -amino group of a substrate lysine (Seol et al. 1999). The cullin–RING–H2 finger ligase might provide a positive charge neutralizing the oxyanion or it might induce a conformational change in the E2 enzyme. The homology between APC/C and SCF subunits should help to test this hypothesis.

Despite their important and possibly fundamental similarities, the APC/C and SCF differ in several important respects. The APC/C appears to have many more stable core subunits, whereas the SCF appears, at the moment, to have many more ‘activator proteins,’ which are thought to recruit substrates to the core particle. It is interesting that the APC/C has thus far only been implicated in the degradation of proteins with functions in cell division, whereas SCF, possibly through its larger repertoire of substrate-recruiting proteins, mediates the degradation of proteins involved not only in cell division but also in cell signaling. A future challenge will be to explain the differences in APC/C and SCF in terms of the biology with which they are concerned.

WD activators: Cdc20 and Cdh1

Genetic studies in yeast and *Drosophila* have shown that APC/C-dependent degradation requires activator proteins, called Cdc20/Fizzy and Cdh1/Fizzy-related, which are evolutionarily conserved and play a fundamental role in regulating the activity and substrate specificity of the APC/C. The *Drosophila* fizzy gene encodes a protein composed of seven WD repeats, which is required for sister chromatid separation and degradation of cyclin A and B during mitosis (Dawson et al. 1995; Sigrist et al. 1995). A related WD protein called Fizzy-related is expressed later in development when cell

cycles contain a G₁ phase (Sigrist and Lehner 1997). In these somatic cycles, APC/C-dependent degradation during mitosis depends on Fizzy, whereas its maintenance during G₁ requires Fizzy-related. At least in *Drosophila*, the two WD proteins seem to function as cell cycle stage-specific activators of cyclin degradation.

A similar picture has also emerged from studies in budding yeast. The Fizzy homolog Cdc20 mediates degradation of the anaphase inhibitor Pds1 (Visintin et al. 1997; Shirayama et al. 1998), the S-phase B-type cyclin Clb5 (M. Shirayama and K. Nasmyth, unpubl.), and the mitotic cyclin Clb3 (Alexandru et al. 1999). Proteolysis of these Cdc20 substrates commences at the metaphase-to-anaphase transition. Degradation of another set of substrates including the mitotic cyclin Clb2, the polo kinase Cdc5, and the spindle protein Ase1, commences during anaphase and requires the Fizzy-related homolog Cdh1/Hct1 (Schwab et al. 1997; Visintin et al. 1997; Charles et al. 1998; Shirayama et al. 1998). Overexpression of Cdc20 and Cdh1 induces APC/C-dependent degradation of their substrates at all stages of the cell cycle. These data suggest, but do not prove, that these WD proteins confer substrate specificity and that their activity limits the rate of proteolysis mediated by the APC/C in vivo. In yeast and human cells, both Cdc20 and Cdh1 bind to the APC/C, but the timing and regulation of their association is regulated differently (Fang et al. 1998b; Kramer et al. 1998a; Zachariae et al. 1998a). The levels of Cdc20 protein rise and fall as cells enter and exit mitosis, with the result that Cdc20 is bound to the APC/C only during M phase (and possibly during late G₂). In contrast, the level of Cdh1 remains constant during the cell cycle, but it only binds the APC/C during G₁. For the rest of the cell cycle, the association of Cdh1 with the APC/C is inhibited due to its phosphorylation by Cdk1 (Zachariae et al. 1998a).

The APC/C appears to possess other activator proteins besides Cdc20 and Cdh1. Budding yeast, for example, contain a third *CDH1/CDC20*-related gene called *AMA1*, which is expressed and spliced only during meiosis (K.F. Cooper and R. Strich, pers. comm.). The Ama1 protein binds to the APC/C core and appears to be necessary for Clb1 proteolysis. Fission yeast contain a family of at least five related WD proteins, including a Cdc20 homolog (Slp1) and a Cdh1 homolog (Srw1/Ste9) (Matsumoto 1997; Yamaguchi et al. 1997; Kitamura et al. 1998; Kominami et al. 1998). Whether proteins with motifs other than WD repeats can also activate ubiquitination mediated by the APC/C is not known.

How the WD coactivators promote ubiquitination is currently unknown. One possibility is that they recruit substrates to the APC/C in a manner analogous to SCF's substrate recognition subunits. These proteins contain two domains: one required for substrate recognition and another one, the F box, which binds to the Skp1 subunit of the SCF complex (Patton et al. 1998). Interestingly, a subset of SCF's substrate recognition subunits (of which yeast Cdc4 is the founder member) bind their targets via WD-repeat domains. Cdc20 and Cdh1 might recruit specific substrates and present them to an E2 enzyme that

resides on the APC/C. A function as substrate recognition factors would be consistent with the stoichiometric binding of these proteins to the core APC/C particle and their dose-dependent stimulation of APC/C activity. However, this model predicts an interaction between the activator proteins and substrates, which has not yet been reported. Neither Cdc20 nor Cdh1 possess F boxes and presumably interact with the APC/C core particle via some other motif.

Cell cycle progression and the APC/C

Just as CDKs both promote the onset of DNA replication and inhibit the formation of pre-RCs needed for a new round, so does the APC/C both promote and hinder cell division progression depending on the state of the cell cycle. By destroying anaphase inhibitors such as Pds1 and Cut2, APC/C^{Cdc20} promotes sister chromatid separation at the metaphase-to-anaphase transition. By destroying B-type cyclins and geminin, APC/C^{Cdc20} promotes inactivation of mitotic CDKs (and thereby facilitates cytokinesis) and removes a block to chromosome rereplication at the same time as it promotes sister chromatid separation. However, by maintaining B-type cyclin degradation following exit from mitosis, APC/C^{Cdh1} delays both entry into S phase and preparations for a new round of mitosis. It is interesting that Cdh1 appears to be absent from embryonic cells whose cell cycles do not have a significant gap between M and S phases. The activity of APC/C^{Cdh1} is very possibly crucial for generating the G₁ phase of somatic cells. It seems likely that, as new APC/C substrates are discovered, the APC/C and its activators will be found to orchestrate a pattern of proteolysis, whose complexity rivals that of some transcriptional programs.

Control of sister chromatid separation

Sister chromatids are held together during G₂ by a multisubunit complex called cohesin. At least in budding yeast, sister chromatid separation at the metaphase-to-anaphase transition appears to be triggered by the sudden disappearance from chromosomes of Scc1, one of cohesin's subunits (Michaelis et al. 1997). Though the removal of Scc1 from chromosomes depends on APC/C^{Cdc20}, it is unlikely to be due to proteolysis mediated by the APC/C itself. Scc1 levels do decline during anaphase and Scc1's proteolysis during the subsequent G₁ period is at least partly dependent on APC/C. However, the key event for the removal of Scc1 from chromosomes appears to be its proteolytic cleavage, which is mediated by the separin protein Esp1 (Uhlmann et al. 1999). Esp1 is inhibited by Pds1, whose destruction is indeed mediated by APC/C^{Cdc20} and is essential for the activation of Esp1 (Cohen-Fix et al. 1996; Ciosk et al. 1998). Thus, APC/C^{Cdc20} mediates sister separation only indirectly, by mediating the proteolysis of a 'securin' protein, Pds1, which enables Esp1 to destroy cohesion directly (Fig. 3). As predicted by this hypothesis, inactivation of Pds1 allows cells to separate sister chromatids and remove Scc1 from chromosomes in the absence of APC/C^{Cdc20} activ-

Figure 3. APC/C^{Cdc20} mediates sister chromatid separation. APC/C^{Cdc20} ubiquitinates Pds1 and thereby marks it for proteolysis by the proteasome. This liberates the separin Esp1 from its inhibitor and allows it to mediate the proteolytic cleavage of the cohesin subunit Scc1 (green circles). Cleavage and disappearance from chromosomes of Scc1 results in the loss of cohesion between sister chromatids. It is not known whether the Esp1-Pds1 complex is dissociated upon ubiquitination or proteolysis of Pds1. It is currently unclear whether Esp1 itself cleaves Scc1 or whether it activates a protease. Lagging chromosomes and spindle damage block the activity of APC/C^{Cdc20}, which requires association of Mad2 with Cdc20.

ity. Surprisingly, Pds1 is neither essential for viability (Yamamoto et al. 1996a) nor for the correct timing of sister chromatid separation in budding yeast at low temperatures (Alexandru et al. 1999). Additional mechanisms must therefore participate in the control of sister chromatid separation. In an unperturbed cell cycle, Pds1 degradation is an essential precondition rather than the direct trigger for anaphase onset. Although Pds1 is not essential for controlling sister chromatid separation in cycling cells, it is necessary for blocking sister separation in the presence of lagging chromosomes or spindle damage (see below). Interestingly, Pds1 is essential for proliferation at high temperatures and may have some role in preparing Esp1 for its activity once Pds1 has been destroyed (Ciosk et al. 1998).

A slightly different picture of sister chromatid separation has emerged from studies of fission yeast, where anaphase onset is also regulated by a separin-securin complex, whose subunits are called Cut1 and Cut2, respectively. Though Cut2 shares many properties with Pds1, such as binding to Cut1 and being degraded by the APC/C shortly before entry into anaphase, it has no obvious sequence homology (Funabiki et al. 1996, 1997). The notion that Cut2 might be an anaphase inhibitor was complicated by the finding that loss of Cut2 function prevents sister chromatid separation, which is the same phenotype as that caused by a nondegradable Cut2

protein lacking its destruction boxes. Because the loading of Cut1 onto spindles depends on Cut2, it has been proposed that sister separation requires first, the loading of the Cut1–Cut2 complex onto spindles and second, the activation of Cut1 by APC/C-dependent degradation of its inhibitor Cut2 (Kumada et al. 1998). It has also been suggested that the Cut1 separin might promote sister separation by increasing the force exerted on sister chromatids by microtubules. Whether Rad21, the fission yeast Scc1 homolog, is a target of Cut1 is not yet known. It is conceivable that separins regulate anaphase spindle function at the same time as they induce dissolution of sister chromatid cohesion by changing the properties of Scc1/Rad21.

APC/C^{Cdc20} has also been implicated in promoting anaphase in animal cells, including humans (Dawson et al. 1995; Sigrist et al. 1995; Tugendreich et al. 1995; Kallio et al. 1998). Furthermore, degradation of a protein with similar properties to those of Pds1 and Cut2 coincides with proteolysis of B-type cyclins and is required for anaphase (Zou et al. 1999). Human Pds1 binds to an Esp1-like protein, suggesting that here too destruction of a securin by APC/C^{Cdc20} liberates a separin protein, which then proceeds to split sister chromatids. Whether securins are the sole targets of APC/C^{Cdc20} with regard to sister separation in animal cells is still unclear. Mutations in the *Drosophila pimples* gene cause a defect in sister chromatid separation at centromeric regions and the *pimples* gene product is degraded during mitosis (Stratmann and Lehner 1996). The properties of the Pimples protein therefore resemble those of the securin Cut2. It will be interesting to see whether degradation of Pimples is actually required for sister centromere separation and whether it is mediated by the APC/C.

Control of spindle function

Sister chromatid separation must be coordinated with spindle elongation and spindle disassembly with cytokinesis. It is conceivable that loss of sister chromatid cohesion is essential for proper spindle elongation at the onset of anaphase. Indeed, spindles fail to elongate when sister separation is inhibited by a noncleavable Scc1 variant (Uhlmann et al. 1999). Nevertheless, it would not be surprising were spindle dynamics altered by APC/C^{Cdc20} through mechanisms that were independent of separin activity. Attachment of spindles to kinetochores is thought to be stabilized by tension generated when sister kinetochores attach to spindles emanating from opposite spindle poles. This tension is clearly lost at the onset of anaphase and some other mechanism presumably ensures that chromosomes never disattach from microtubules during anaphase. Thus stabilization of spindle–kinetochore attachments, an increased tendency of kinetochores to move towards the poles, and increased repulsion between polar microtubules (which causes poles to migrate apart) might all directly or indirectly be promoted by APC/C-dependent proteolysis.

The kinesin-related motor proteins Kip1 (D.M. Roof, pers. comm.) and Cin8 (E. Hildebrandt and M.A. Hoyt,

pers. comm.), which are required for mitotic spindle function, are both degraded by APC/C^{Cdh1} during anaphase. These motors are thought to cross-link and slide microtubules from opposite poles to create an outward pushing force which counteracts the activity of another motor called Kar3 (Hoyt and Geiser 1996). CENP-E is a mammalian, kinesin-related protein that binds to kinetochores early in mitosis and then localizes to the spindle midzone (Brown et al. 1994). CENP-E is degraded in late mitosis, consistent with its degradation by the APC/C pathway. The budding yeast Ase1 protein localizes to the midzone of mitotic spindles and is important for anaphase spindle elongation (Pellman et al. 1995). Degradation of Ase1 at the end of anaphase and during G₁ depends on a destruction box-like sequence and is mediated by APC/C^{Cdh1} (Juang et al. 1997). Ipl1/Aurora is a conserved protein kinase that associates with spindles in yeast and animal cells. In yeast, Ipl1 regulates the interaction of kinetochores with microtubules by controlling, together with protein phosphatase 1, the phosphorylation status of the kinetochore component Ndc10 (Biggins et al. 1999; Sassoon et al. 1999). In yeast and animal cells Ipl1/aurora protein levels fluctuate during the cell cycle with kinetics consistent with degradation by the APC/C (Gopalan et al. 1997; Kimura et al. 1997; Roghi et al. 1998). However, it should be noted that degradation of none of these spindle-associated substrates is absolutely essential for spindle function in budding yeast. *pds1 apc* double mutants clearly manage to segregate chromosomes to opposite spindle poles, implying that Pds1 is the sole protein whose destruction is needed for sister chromatid segregation. Furthermore, *cdh1* mutants, which fail to degrade Ase1, are viable. In conclusion, the only proteins whose destruction by the APC/C is known to be crucial for chromosome segregation are securins like Pds1 and Cut2.

Exit from mitosis: inactivation of CDKs

Inactivation of mitotic Cdk1 kinases is essential for several events during exit from mitosis, including disassembly of the mitotic spindle, chromosome decondensation, cytokinesis, reformation of the nuclear envelope, reactivation of transcription, and rebuilding a Golgi apparatus (Murray et al. 1989; Luca et al. 1991; Gallant and Nigg 1992; Holloway et al. 1993; Surana et al. 1993; Sigrist et al. 1995; Gottesfeld and Forbes 1997; Nakamura et al. 1997). Destruction of mitotic cyclins has long been thought to have a key role in Cdk1 inactivation because expression of high levels of nondegradable cyclins blocks Cdk1 inactivation in a wide variety of systems. However, more recent work in budding yeast has questioned the validity of this conclusion. Expression of high levels of a nondegradable variant of the mitotic cyclin Clb2 does indeed block cells in late anaphase with high levels of Cdk1 kinase activity, but this arrest is not observed in cells that express more moderate levels of nondegradable Clb2 (Amon et al. 1994) or in *cdh1* mutants, which fail to degrade physiological levels of Clb2 (Schwab et al. 1997). These findings imply that neither degradation of Clb2

nor that of any other Cdh1-dependent substrate is essential for exit from mitosis in budding yeast. Exit from mitosis in the absence of Clb2 proteolysis is possible due to accumulation of the Cdk1 inhibitor Sic1 (Schwab et al. 1997).

These results do not, however, exclude an essential role for cyclin proteolysis in Cdk1 regulation. After all, at least six different B-type cyclins are present during mitosis in budding yeast. Cdk1 inactivation might depend on proteolysis of a B-type cyclin other than Clb2 by APC/C^{Cdc20}. Whereas deletion of *PDS1* allows *cdc20* or *apc* mutants to separate their sister chromatids, it does not permit them to inactivate Cdk1 and to exit from mitosis (Lim et al. 1998). The identity of the mysterious protein whose destruction by APC/C^{Cdc20} is necessary for Cdk1 down regulation has recently been revealed by looking for mutations that permit the proliferation of cells lacking both Cdc20 and Pds1. This showed, remarkably, that while APC/C^{Cdc20} mediates sister chromatid separation by destroying Pds1, it promotes Cdk1 inactivation by destroying the S-phase cyclin Clb5 (M. Shiyama and K. Nasmyth, unpubl.). This suggests that in budding yeast, as must be the case in embryonic animal cells, APC/C^{Cdc20} and not APC/C^{Cdh1} has the more crucial role in down regulating Cdk1 during anaphase. Curiously, destruction of Clb5 by APC/C^{Cdc20} is more important than that of other B-type cyclins. Why should this be the case? Cdk1 inactivation in budding yeast occurs through at least three mechanisms: (1) proteolysis mediated by APC/C^{Cdc20}, which destroys Clb3 and Clb5; (2) proteolysis mediated by APC/C^{Cdh1}, which destroys Clb3 and Clb2; and (3) accumulation of a CDK inhibitor (CKI) Sic1, which inactivates any surviving Cdk1–B-type cyclin complexes. By destroying Clb5, APC/C^{Cdc20} not only directly eliminates a B-type cyclin but also permits the Cdc14 phosphatase (see below) to dephosphorylate Sic1 and Cdh1, which both switches off the proteolysis of Sic1 by SCF and turns on APC/C^{Cdh1} (Fig. 4). Clb5 presumably has some unique ability to antagonize Cdc14, and Clb5 must therefore be destroyed by a mechanism that is independent of Cdc14 activity (i.e., by APC/C^{Cdc20}-mediated proteolysis). Having activated APC/C^{Cdh1} and the accumulation of CKIs, APC/C^{Cdc20} is no longer required to down regulate Cdk1. Thus, Cdc20, which must not reactivate the APC/C until sister chromatids produced during the next S phase are ready to be separated, is therefore destroyed as cells enter G₁. The destruction by APC/C^{Cdc20} of securins that block sister separation, and of B-type cyclins that block cytokinesis and the reformation of pre-RCs ensures that cells cannot attempt cell cleavage or chromosome reduplication until they have already separated sister chromatids during anaphase.

It is a reasonable working hypothesis that APC/C^{Cdc20} is responsible for the proteolysis of both cyclin A and cyclins B1 and B2 in animal cells. For example, neither protein is degraded in *fizzy* (*cdc20*) mutants in *Drosophila* (Sigrist et al. 1995). Furthermore, cyclins B1 and B2 disappear at the very onset of anaphase; that is, at the same time as the degradation of securins like Pds1. In

animal cells, APC/C^{Cdh1} is probably only required for maintaining mitotic cyclin proteolysis once cells have entered G₁. In yeast, however, also APC/C^{Cdh1} participates in the destruction of B-type cyclins as cells exit from mitosis. The degradation of Clb2 largely by APC/C^{Cdh1} helps to explain what has long been a curious difference between yeast and animal cells. Endogenous cyclin B molecules are unstable in animal cells arrested in late anaphase due to expression of nondegradable cyclin B variants, whereas Clb2 remains stable under similar circumstances. It turns out that Cdh1 is inactivated by Cdk1 and APC/C^{Cdh1} cannot therefore function (and degrade Clb2) until Cdk1 has been inactivated (see below).

Cytokinesis

In animal and budding yeast cells, the APC/C is essential for cytokinesis. The main role of APC/C in this regard is presumed to be cyclin proteolysis leading to Cdk1 inactivation. It is therefore curious that fission yeast *apc* mutants eventually arrest with a 'cut' phenotype (Yanagida 1998). The mutants fail to separate sister chromatids and to destroy mitotic cyclins but proceed with aspects of cytokinesis such as formation of a septum. Curiously, cytokinesis in fission yeast seems to require inactivation of Cdk1 as much as it does in other organisms (Yamano et al. 1996). One explanation for this paradox is that Cdk1 in fission yeast can be inactivated, albeit slowly, by an APC/C-independent mechanism such as phosphorylation on tyrosine 15.

NIMA

In *A. nidulans*, entry into mitosis requires Cdk1–cyclin B and a second kinase called NIMA (Osmani et al. 1991; Osmani and Ye 1996). NIMA protein levels fluctuate during the cell cycle with kinetics resembling that of cyclin B and NIMA is stabilized in *bimA^{CDC16}* and *bimE^{APC1}* mutants, suggesting that NIMA is an APC/C target (Ye et al. 1998). In contrast to most APC/C substrates, however, the degradation determinant of NIMA is located in the carboxyl terminus, which does not contain an obvious destruction box. Deletion of this domain does not affect NIMA's ability to promote entry into mitosis but blocks the mitotic degradation of NIMA and exit of cells from mitosis (Pu and Osmani 1995). At least in *A. nidulans*, mitotic degradation of NIMA is an essential function of the APC/C. The identification of NIMA as an APC/C substrate sheds new light on the previous proposal that BIME^{APC1} might be a negative regulator of entry into mitosis. This idea was based on the finding that *bimE^{APC1} nimA* double mutants do not arrest in G₂ as *nimA* single mutants do, but undergo aspects of mitosis such as chromosome condensation (Osmani et al. 1988). Recently, it was shown that cyclin B and the mutant NIMA protein accumulate in the double mutant, which superinduces Cdk1–cyclin B activity and produces sufficient NIMA kinase activity to induce mitosis (Ye et al. 1998). As predicted by this

Figure 4. Regulation of cell cycle progression by APC/C-dependent proteolysis. (A) Sister chromatid separation and Cdk1 inactivation both depend on APC/C^{Cdc20}. Activation of APC/C^{Cdc20} but not APC/C^{Cdh1} requires phosphorylation of core subunits by Cdk1-cyclin B. APC/C^{Cdc20} mediates sister chromatid separation and Cdk1 inactivation by degrading securins and B-type cyclins, respectively. APC/C^{Cdc20} also creates the conditions that keep Cdk1-cyclin B complexes inactive during the ensuing G₁ phase. Cdk1 inactivation by APC/C^{Cdc20} permits release from its inhibitor of the Cdc14 phosphatase which, by dephosphorylating Sic1 and Cdh1, causes accumulation of a CKI and activation of APC/C^{Cdh1}. Broken lines indicate proteolysis and P in a circle indicates phosphorylation (see text for details). (B) Changes in the abundance and activity of CDKs, the securin Pds1, the CKI Sic1, and both forms of the APC/C during the cell cycle.

model, *bimE^{APC1}* mutants cannot enter mitosis when the *nimA* gene is deleted, and the double-mutant cells arrest in G₂.

Control of DNA replication

Initiation of DNA replication depends on a period devoid of CDK activity in which pre-RCs are assembled followed by the activation of S-CDKs, which trigger origin

unwinding and the emergence of replication forks. Because of its essential role in mitotic Cdk1 inactivation, the APC/C is also thought to be essential for rereplication. Most, if not all, *apc* mutants arrest with replicated DNA and high Cdk1 activity but then fail to rereplicate their chromosomes. Furthermore, transient inactivation of Cdk1 kinases in yeast by expression of the Cdk1 inhibitor Sic1 causes rereplication in *apc* mutants shifted to their restrictive temperature (E.A. Noton and J.F.X.

Diffley, pers. comm.). This suggests that Cdk1 inactivation is the sole essential role of the yeast APC/C with respect to DNA replication. Recently, certain *apc* mutants were reported to rereplicate their chromosomes despite arresting with high Cdk1 activity (Heichman and Roberts 1996, 1998). It was proposed that the APC/C prevents rereplication by destroying an activator of DNA replication. This interesting but controversial claim fits awkwardly with the notion that cells naturally arrest the cell cycle (and prevent chromosome reduplication) by inhibiting the APC/C (see below). Another study of the same *apc* mutants detected replication only of mitochondrial DNA (Pichler et al. 1997). In vertebrate cells, DNA re-replication depends not only on degradation by the APC/C of mitotic cyclins but also of geminin (McGarry and Kirschner 1998). In human cells, geminin accumulates during S phase and is degraded during anaphase. Geminin prevents recruitment of MCM proteins into pre-RCs at origins. Initiation of DNA replication requires association of the conserved protein kinase Cdc7 with its regulatory subunit Dbf4 whose abundance fluctuates during the cell cycle. The Dbf4 protein appears as cells enter S phase and is degraded by APC/C^{Cdc20} at the metaphase-to-anaphase transition (M.G. Ferreira and J.F.X. Diffley, pers. comm.; Cheng et al. 1999). At least in budding yeast, Dbf4 proteolysis is not essential for proliferation but it might contribute to the high fidelity of DNA replication. The degradation of Dbf4 might prevent the firing of those origins that have acquired preRCs as Cdk1 activity drops during exit from mitosis.

Polo kinase and Cdc20: first activators, then substrates

Two proteins that regulate APC/C, its Cdc20 activator and the Polo/Cdc5 protein kinase, are themselves regulated by APC/C-mediated proteolysis. Both proteins remain constant during embryonic cell cycles in *Xenopus* eggs but they fluctuate during the cell cycle in budding yeast and human cells in a manner similar to cyclin B. Ubiquitination and degradation of Cdc5, the polo-like kinase of budding yeast, depend on APC/C^{Cdh1} and on two destruction box-like sequences (Charles et al. 1998; Shirayama et al. 1998). Expression of a nondegradable Cdc5 variant prevents the accumulation of mitotic cyclins and the formation of mitotic spindles suggesting that Cdc5 degradation is important for inactivating APC/C-dependent cyclin degradation as cells enter S phase. Plk1, the human polo-like kinase, is also ubiquitinated by the APC/C although it lacks an obvious destruction box (Fang et al. 1998b). Budding yeast Cdc20 contains two destruction boxes that are important for APC/C-dependent degradation during G₁ (Shirayama et al. 1998). In contrast to other substrates, Cdc20 is also degraded in an APC/C-dependent manner during other cell cycle stages, but this instability does not require the destruction boxes (Prinz et al. 1998). The fluctuation of Cdc20 results from constitutive degradation of the protein in combination with cell cycle-regulated transcrip-

tion. Cdc20 degradation does not require Cdh1; however, it remains to be determined whether Cdc20 has a role in promoting its own degradation or whether Cdc20 degradation requires another activator or no activator at all. In either case, the constitutive degradation of Cdc20 demonstrates that APC/C activity is not necessarily cell cycle-regulated and destruction box-dependent. This implies that there might be many more substrates of APC/C that have no role in cell cycle regulation.

Controlled destruction

At least five different mechanisms are known or suspected to contribute to APC/C regulation: (1) abundance and (2) phosphorylation of activator proteins; (3) phosphorylation of core APC/C subunits; (4) binding of inhibitory protein complexes; and (5) substrate phosphorylation. Furthermore, it is clear that different forms of the APC/C, such as APC/C^{Cdc20} and APC/C^{Cdh1}, can be regulated differently by the very same regulatory proteins. For example, Cdk1 kinases promote APC/C^{Cdc20} activity at the metaphase-to-anaphase transition whereas they inhibit APC/C^{Cdh1} activity from S phase until exit from mitosis. A major difference between embryonic and somatic cells is the absence of Cdh1 from early embryos (Sigrist and Lehner 1997; Lorca et al. 1998). This has the consequence that cyclins destroyed by APC/C^{Cdc20} at the end of mitosis start to reaccumulate almost as soon as they have been degraded. High levels of cyclin E presumably permits cells to inactivate CDK inhibitory proteins without an extensive transcriptional program. The appearance of Cdh1 later in development introduces a G₁ phase, a stable state in which cyclins remain unstable.

Regulation of APC/C^{Cdc20}

Activation of APC/C^{Cdc20} during metaphase promotes proteolysis of securins (Pds1/Cut2), which liberates separins (Esp1/Cut2) and thereby induces sister chromatid separation. It also triggers proteolysis of mitotic cyclins and thereby contributes to Cdk1 inactivation. It is through the activity of APC/C^{Cdc20} that eukaryotic cells link preparations for a new round of DNA replication with the prior separation of sister chromatids. Regulation of APC/C^{Cdc20} is therefore central to the eukaryotic cell cycle. In yeast, Cdc20 accumulates during G₂/M as a result of the transcriptional activation of the *CDC20* gene, which requires mitotic Cdk1 kinases (Prinz et al. 1998). Cdc20 fluctuates in abundance in a similar manner in human cells (Weinstein 1997). In yeast and human cells, Cdc20 binds to the APC/C and the abundance of APC/C^{Cdc20} complexes corresponds to the cellular amount of Cdc20 (Fang et al. 1998b; Kramer et al. 1998a). However, accumulation of Cdc20 alone is insufficient to trigger degradation of APC/C^{Cdc20} substrates like Pds1. Premature expression of *CDC20* in yeast causes Cdc20 to appear earlier during the cell cycle but has no dramatic effect on the timing of Pds1 degradation (Prinz et al. 1998).

Recent experiments with extracts from clam oocytes and with purified *Xenopus* and human APC/C suggest that activation of APC/C^{Cdc20} involves two steps (Shteinberg et al. 1999; E. Kramer and J.-M. Peters, pers. comm.). First, the APC/C core particle is phosphorylated by Cdk1–cyclin B kinase during mitosis. Subsequently, Cdc20 stimulates the activity of the phosphorylated APC/C core. This conclusion is based on the observation that cyclin B–ubiquitination activity of interphase APC/C is stimulated by Cdc20 only after phosphorylation by Cdk1–cyclin B kinase. Furthermore, phosphatase treatment of the purified mitotic APC/C prevents its activation by Cdc20, which is restored by Cdk1–cyclin B kinase activity. In *Xenopus* extracts, cyclin degradation was found to coincide with the phosphorylation of several APC/C subunits including Apcl and Cdc27 (King et al. 1995; Peters et al. 1996). Both reactions are defective in extracts induced to enter mitosis in the absence of the *Xenopus* Cks1 homologue (also called p9 or Suc1), which is a conserved subunit of Cdk–cyclin complexes (Patra and Dunphy 1996, 1998). Cks1/Suc1 stimulates phosphorylation of purified APC/C by Cdk1–cyclin B and a small fraction was found to be associated with the mitotic but not the interphase APC/C in *Xenopus* extracts. These data suggest that Cks1/Suc1 targets Cdk1–cyclin B to phosphorylate APC/C subunits. Also Cdc20 has been found to be phosphorylated in a cell cycle-regulated manner in *Xenopus* egg extracts and human cells (Weinstein 1997; Kramer et al. 1998a; Lorca et al. 1998). However, nonphosphorylatable forms of Cdc20 are still able to activate mitotic *Xenopus* and human APC/C indicating that phosphorylation of Cdc20 by Cdk1–cyclin B is not essential for APC/C activation (E. Kramer, N. Scheuringer, and J.-M. Peters, pers. comm.).

The notion that Cdk1–cyclin B is required for activation of APC/C^{Cdc20} explains how Cdk1 promotes its own destruction in extracts from embryonic cells (Felix et al. 1990). However, there must be a mechanism that delays the onset of cyclin proteolysis until after Cdk1–cyclin B kinases have triggered spindle formation, chromosome condensation, and nuclear envelope breakdown. Such a mechanism is particularly important for embryonic cells that supposedly lack surveillance mechanisms (checkpoints) that inhibit APC/C^{Cdc20} while there still exist lagging chromosomes (see below). Such a time lag could be generated by a signalling cascade which links activation of Cdk1–cyclin B to that of APC/C^{Cdc20}. Cyclin degradation upon release of *Xenopus* egg extracts from a meiotic metaphase arrest (CSF arrest) was shown to depend on the polo kinase Plx (Descombes and Nigg 1998). Activation of Plx by Cdk1–cyclin B appears to be indirect and requires at least one additional kinase called xPlkk, which was shown to directly phosphorylate and thereby activate Plx (Qian et al. 1998). Polo kinases have been implicated in regulating the APC/C core particle. Human Plk1, which is activated during mitosis in vivo, phosphorylates several APC core subunits in vitro and this stimulates the cyclin B ubiquitination activity (Kotani et al. 1998). Transient inhibition of APC/C^{Cdc20} could also account for the

time lag between activation and destruction of Cdk1–cyclin B. Several pieces of evidence suggest that APC/C is inhibited by protein kinase A (PKA). Yeast APC/C mutants are suppressed by mutations in the cAMP–PKA pathway that lower the kinase activity of PKA (Yamashita et al. 1996; Yamada et al. 1997). In vitro, PKA phosphorylates purified mammalian APC/C and inhibits its cyclin B ubiquitination activity (Kotani et al. 1998). Consistent with a role in APC/C regulation PKA activity was found to fluctuate during the cell cycle of mammalian cells in culture. PKA activity increased at the beginning of mitosis and fell at metaphase. Taken together, these data provide two mechanisms that might contribute to the timely activation of APC/C^{Cdc20} at the metaphase-to-anaphase transition: phosphorylation of core subunits by Plk1 and dephosphorylation of PKA phosphorylation sites by an as yet unknown phosphatase.

How is APC/C^{Cdc20} inactivated to allow reaccumulation during the next cell cycle of substrates such as B-type cyclins and Pds1? In yeast and human cells, the amount of Cdc20 drops dramatically as cells exit from mitosis. Considering the dose-dependent action of Cdc20, it is likely that this drop causes a considerable reduction in the activity of APC/C^{Cdc20}. However, APC/C^{Cdc20} might not be completely inactive in G₁ cells. Overexpression of *PDS1* was shown to cause accumulation of the Pds1 protein in G₁-arrested *cdc20* mutants but not in wild-type cells (Visintin et al. 1997).

Regulation of APC/C^{Cdh1}

In budding yeast, degradation of several APC/C substrates including Clb2, Cdc5, and Ase1 is solely dependent on Cdh1. Other substrates, like the mitotic cyclin Clb3, are degraded by APC/C^{Cdc20} during anaphase but by APC/C^{Cdh1} during telophase and G₁ (Alexandru et al. 1999). Many if not most APC/C substrates in animal cells behave like Clb3 in yeast. For example, most B-type cyclins are degraded by APC/C^{Cdc20} at the metaphase-to-anaphase transition and by APC/C^{Cdh1} during G₁ (Sigrist et al. 1995; Sigrist and Lehner 1997). Whereas APC/C^{Cdc20} is mainly active during mitosis, APC/C^{Cdh1} is only active from the end of mitosis until shortly before entry into the next S phase (Fig. 4B). APC/C^{Cdh1}'s activity is mirrored by the ability of Cdh1 to bind to the APC/C core. Although Cdh1 is present throughout the cell cycle, it only binds to the APC/C during late anaphase and G₁. In yeast, binding is inhibited from S phase until the end of mitosis by phosphorylation of Cdh1, which is mediated by Cdk1 (Zachariae et al. 1998a; Jaspersen et al. 1999). Ectopic inhibition of Cdk1 induces Cdh1 to bind to the APC/C irrespective of the cell cycle stage. Furthermore, a nonphosphorylatable Cdh1 variant binds and activates APC/C throughout the cell cycle, which prevents the accumulation of mitotic cyclins like Clb2 and Clb3. There is evidence that a similar phenomenon occurs in animal cells, where both Cdk1 and Cdk2 presumably conspire together to keep Cdh1 inactive (Knoblich et al. 1994; Lane et al. 1996). Indeed, phos-

phorylated human Cdh1 is unable to bind to and activate the APC/C, whereas nonphosphorylated Cdh1 can activate both interphase and mitotic APC/C (E. Kramer, N. Scheuringer, and J.-M. Peters, pers. comm.). Cdh1 and mitotic cyclins are therefore mortal enemies, each inhibiting the activity of the other. Supremacy in this battle switches from Cdh1 during G₁, where APC/C^{Cdh1} prevents the accumulation of mitotic cyclins, to cyclin during S, G₂, and M phases, where Cdk1 inhibits the binding of Cdh1 to the APC/C. The mechanism by which cells switch between these two self-sustaining states lies at the heart of the eukaryotic cell cycle.

Switching on APC/C^{Cdh1}

Activation of APC/C^{Cdh1} at the end of mitosis is part of a more general program for inactivating Cdk1 (Fig. 4). In yeast, this process coincides with the sudden accumulation of the Cdk1 inhibitor Sic1. During S, G₂, and M phases, Sic1 is phosphorylated by Cdk1 and then rapidly degraded by SCF^{Cdc4}. Meanwhile, Sic1 synthesis at the end of anaphase is activated by a transcription factor, Swi5 (Knapp et al. 1996), whose entry into the nucleus is inhibited by Cdk1 phosphorylation (Moll et al. 1991). Thus, the sudden accumulation of Sic1 at the end of mitosis depends on dephosphorylation of Swi5, which induces *SIC1* transcription, and dephosphorylation of Sic1 itself, which abolishes SCF^{Cdc4}-mediated proteolysis. Recent work has highlighted the role of a conserved phosphatase called Cdc14 in dephosphorylating simultaneously Swi5, Sic1, and Cdh1 at the end of mitosis (Visintin et al. 1998; Jaspersen et al. 1999). Activation of Cdc14 is therefore a key event in Cdk1 inactivation. During most of the cell cycle, a regulatory subunit called Net1/Cfi1 inhibits the catalytic activity of Cdc14 and sequesters Cdc14 into a large complex which is assembled in the nucleolus (Shou et al. 1999; Visintin et al. 1999). In anaphase, Cdc14 is released from its inhibitor and then spreads throughout the cell, where it dephosphorylates Cdh1, Swi5, and Sic1. This process requires the polo-like kinase Cdc5, the protein kinases Cdc15, Dbf2, and Dbf20, the Ras-like GTPase Tem1, and the GDP/GTP exchange factor Lte1. Of these Cdc14 activators, most is known about Cdc5, whose transcription is activated by Cdk1 during G₂. Cdc5 kinase activity appears somewhat later, suggesting that it is regulated by post-translational mechanisms such as phosphorylation (Cheng et al. 1998). Having promoted the activation of Cdc14 and thereby that of APC/C^{Cdh1}, Cdc5 is rapidly destroyed by APC/C^{Cdh1}. Proteins like Cdc5 and Cdc14 are vital for switching cells from the high Cdk1/low Sic1 state to a low Cdk1/high Sic1 state, but they are not required to maintain the low Cdk1 state during G₁.

It is not yet known what triggers the activation of Cdc14. This process does not require sister chromatid separation as the release of Cdc14 from the nucleolus and Cdk1 inactivation both occur in *esp1* mutants (Surana et al. 1993; Visintin et al. 1999). Instead, Cdc14 activation requires, like sister chromatid separation, proteolysis mediated by APC/C^{Cdc20}. Nondegradable Pds1

not only blocks anaphase onset but also Cdk1 inactivation (Cohen-Fix and Koshland 1999; Tinker-Kulberg and Morgan 1999), possibly by inhibiting the release of Cdc14 from the nucleolus. The dependence of Cdk1 inactivation on Pds1 proteolysis helps to ensure that sister chromatid separation precedes cytokinesis and chromosome rereplication. The S phase cyclin Clb5 has been identified as the second Cdc20 substrate whose degradation is essential for Cdk1 inactivation (M. Shirayama and K. Nasmyth, unpubl.). The Cdk1–Clb5 kinase might be especially potent in counteracting the phosphatase activity of Cdc14. In conclusion, APC/C^{Cdc20}-dependent activation of Cdc14 tilts the battle between Cdk1–cyclin B versus Cdh1 and Sic1 in favor of the latter and therefore has a key role in inactivating Cdk1 at the end of mitosis (Fig. 4). What roles the Cdc14 homologs in animal cells have, remains to be determined (Li et al. 1997). In animal cells, most if not all A- and B-type cyclins appear to be degraded by APC/C^{Cdc20}, whose activity does not depend on Cdc14. It is therefore unclear whether Cdc14's main role in animal cells will be to downregulate Cdk1, for example, by switching off proteolysis of CKIs like p27^{Kip1}, or to regulate other aspects of mitotic exit and cell cleavage.

Maintenance and termination of the low kinase state

How are Cdk1–Clb kinases kept inactive during G₁ and how are they reactivated as cells enter S phase? Once APC/C^{Cdh1} and Sic1 have overwhelmed Cdk1 activity with the help of APC/C^{Cdc20} and Cdc14, they promote their own activity by keeping mitotic cyclins unstable and Sic1 stable. The maintenance of the low kinase state and thereby the G₁ period depends on the suppression of any Cdk1 activity capable of phosphorylating Cdh1 and Sic1. In yeast, APC/C^{Cdh1} is important for G₁ cell cycle arrest in response to mating pheromones and nutrient starvation. *apc* and *cdh1* mutants are unable to halt DNA replication due to precocious accumulation of S phase-promoting B-type cyclins (Kumada et al. 1995; Irniger and Nasmyth 1997; Kominami et al. 1998). APC/C^{Cdh1} activity also persists in G₁ in human cells (Brandeis and Hunt 1996). Because differentiation usually requires arrest in G₁, it will be interesting to establish whether persistent APC/C^{Cdh1} activity is important in terminally differentiated cells.

Reactivation of Cdk1 activity must await the accumulation of a special class of cyclins whose activity is refractory to both APC/C^{Cdh1} and Sic1. In budding yeast, the G₁ cyclins Cln1 and Cln2 have this property. They suddenly appear in late G₁ as part of a transcriptional program that is activated as cells reach a critical size (Koch and Nasmyth 1994). The S-phase B-type cyclin Clb5 also accumulates at this stage due to the same transcriptional program. Though sensitive to Sic1, the Cdk1–Clb5 kinase is not destroyed by APC/C^{Cdh1}. The accumulation of Cdk1–Cln1 and Cdk1–Cln2 kinases in late G₁ leads to the phosphorylation of Sic1 and thereby to its proteolysis. This activates the Cdk1–Clb5 kinase, which by phosphorylating Cdh1 switches cells finally back to a

state in which mitotic cyclin B kinases can reaccumulate. The activation of Cdk1–Clb5 irreversibly switches the system to the high kinase state. Once established by Cdk1–Clb5, the high kinase state can be maintained by B-type cyclins such as Clb3 and later Clb2 which are susceptible to APC/C^{Cdh1}. In animals, Cdk2–cyclin E may have a role similar to that of Cdk1–Clb5 in yeast. In *Drosophila*, ectopic expression of cyclin E or a failure to express the Cdk2–cyclin E inhibitor Dacapo results in ectopic accumulation of mitotic cyclins (Knoblich et al. 1994; Lane et al. 1996). S phase-promoting kinases therefore have a more general role in organizing the cell cycle than previously anticipated. They trigger chromosome duplication and simultaneously prepare the stage for the separation of sister chromatids. It is therefore fascinating that their eventual destruction, for example, the proteolysis of Clb5 in yeast by APC/C^{Cdc20}, is a crucial trigger for exit from the high Cdk1 state. It is conceivable that destruction of cyclin A has a similar role in animal cells.

Stopping destruction

There are several occasions where cells block cell cycle progression by specifically inhibiting APC/C-dependent proteolysis. One example is the metaphase arrest of vertebrate eggs awaiting fertilization. Other examples are cell cycle delays induced by DNA damage, incomplete DNA replication or defects in the attachment of chromosomes on a bipolar spindle. These so-called checkpoint mechanisms are important for the integrity of chromosomes and the high fidelity with which they are transmitted during mitosis.

CSF arrest

As in most vertebrates, unfertilized *Xenopus* eggs are arrested in metaphase of the second meiotic division by an activity called cytotstatic factor or CSF. During this arrest, Cdk1 activity remains high and both sister chromatid separation and cyclin B degradation are blocked, suggesting that CSF prevents APC/C-dependent degradation. CSF arrest depends on the c-Mos protein kinase (Sagata et al. 1989) and on activation of the mitogen-activated protein (MAP) kinase cascade. How activation of MAP kinase blocks degradation of APC/C substrates is unknown (Haccard et al. 1993; Posada et al. 1993; Kosako et al. 1994). Active MAP kinase does not directly inhibit the APC/C but seems to activate an inhibitor that does not copurify with the APC/C. For example, APC/C purified from CSF extracts is as active in cyclin B ubiquitination as that from mitotic extracts (Vorlaufer and Peters 1998). Fertilization triggers a transient increase in the cytoplasmic calcium concentration that causes activation of APC/C-dependent cyclin A and B degradation and thereby release from the metaphase arrest. Release from the CSF arrest and cyclin degradation can also be induced by treatments that increase intracellular calcium or by adding calcium to CSF extracts. Calcium activates the degradation of c-Mos, but this is not the trigger for cyclin degradation because c-Mos is de-

graded considerably later than cyclin B (Watanabe et al. 1991). The calcium wave causes a transient activation of calmodulin-dependent protein kinase II (CaMKII), which is both necessary and sufficient for activation of cyclin degradation and release from the CSF arrest (Lorca et al. 1993). Unfortunately, the relevant substrate of CaMKII is unknown.

DNA damage checkpoint

DNA damage causes fission yeast and animal cells to block entry into mitosis by preventing the activation of Cdk1–cyclin B. In budding yeast, cell cycle progression is instead halted by blocking Pds1 degradation. DNA damage induces phosphorylation of Pds1, which depends on *MEC1* and *RAD9*, two genes known to be required for this checkpoint (Cohen-Fix and Koshland 1997). It has been suggested (but not yet proven) that Pds1's phosphorylation renders it resistant to attack by APC/C^{Cdc20}. The persistence of Pds1 in response to DNA damage not only blocks sister chromatid separation but also prevents inactivation of Cdk1 (Tinker-Kulberg and Morgan 1999).

The APC/C as a target of the spindle checkpoint

The segregation of sister chromatids to opposite spindle poles at the metaphase-to-anaphase transition occurs due to loss of the cohesion that holds sisters together. Because loss of cohesion between sister chromatids occurs simultaneously on all chromosomes, it is vital that this process be blocked by 'lagging' chromosomes whose sister kinetochores have not yet attached to microtubules emanating from opposite spindle poles. Lagging chromosomes not only block sister separation but also inactivation of Cdk1–cyclin B kinases; they therefore freeze cells in a metaphase-like state. The control mechanism responsible for this cell cycle arrest has been called the mitotic checkpoint. Although this checkpoint is not essential during an unperturbed cell cycle in yeast, it seems to be an integral part of cell cycle control in mammalian cells (Taylor and McKeon 1997; Gorbisky et al. 1998). It is a control mechanism without which mitosis cannot properly function. The same mechanism blocks cell cycle progression upon artificial destruction of the spindle with microtubule depolymerizing drugs. The APC/C has emerged as the key target of the mitotic checkpoint.

The isolation of mutants that die rapidly in the presence of microtubule-depolymerizing drugs has identified several genes involved in the spindle checkpoint: *MAD1*, *MAD2*, *MAD3*, *BUB1*, *BUB2*, *BUB3*, *MPS1*, and *PDS1* (Hoyt et al. 1991; Li and Murray 1991; Weiss and Winey 1996; Yamamoto et al. 1996b). Mutants in these genes are defective in halting various aspects of the cell cycle upon extended exposure to microtubule-depolymerizing drugs. Homologs of Mad1-3, Bub1, Bub3, and Pds1 have been identified in other organisms, including humans (Chen et al. 1996; Li and Benezra 1996; He et al. 1997; Taylor and McKeon 1997; Taylor et al. 1998; Zou et al. 1999).

In yeast and mammalian cells, Mad2 is found associated with Cdc20 and the APC/C (Fang et al. 1998a; Hwang et al. 1998; Kallio et al. 1998; Kim et al. 1998; Wassmann and Benezra 1998). At least in budding yeast, Mad1 and Mad3 also bind to Cdc20. There is compelling evidence that the Mad2–Cdc20 interaction is required to inhibit the ubiquitin ligase activity of APC/C^{Cdc20}, which then prevents the destruction of cyclin B and securins like Pds1. *CDC20* mutant alleles, which are defective in the binding of Mad2, have a phenotype similar to that of *mad* mutants (Hwang et al. 1998; Kim et al. 1998; Alexandru et al. 1999). These cells fail to block sister separation and cyclin B destruction when spindles are damaged or the mitotic checkpoint is ectopically activated. How Mad2 inhibits APC/C^{Cdc20} is unknown. It is interesting that tetramers of recombinant human Mad2 block cyclin B degradation in *Xenopus* egg extracts much more effectively than monomers do (Fang et al. 1998a). It is therefore possible that Mad1 and Mad3 catalyze the formation of active Mad2 tetramers in response to lagging chromosomes or spindle damage.

A key question is how cells sense that spindles are damaged or that kinetochores have not formed bivalent attachments to the mitotic spindle. Two lines of evidence suggest that kinetochores themselves have a key role. In vertebrate cells, Mad1, Mad2, Bub1, and Bub3 localize selectively to unattached kinetochores and disappear from them as they attach to the mitotic spindle (Chen et al. 1996, 1998; Li and Benezra 1996; Taylor and McKeon 1997; Gorbsky et al. 1998; Taylor et al. 1998; Waters et al. 1998). Meanwhile in yeast, components of the CBF3 complex that mediate the attachment of centromeres to microtubules are necessary for cell cycle arrest when spindles are damaged (Hyman and Sorger 1995). It has therefore been proposed that unattached kinetochores mediate the assembly or activation of a Mad2-containing complex that when released from the kinetochore inhibits APC/C^{Cdc20} throughout the cell (Chen et al. 1998; Gorbsky et al. 1998). Such complexes must be unstable and are regenerated as long as unattached kinetochores are present. When all kinetochores have been attached, the inhibitory activity declines, allowing APC/C^{Cdc20} to become active.

How do the Mad and Bub proteins block sister chromatid separation and Cdk1 inactivation that require, at least in budding yeast, different forms of the APC/C? Recent analysis of the kinetics of Pds1 degradation and Cdk1 inactivation in *mad* and *bub* mutants suggests that the cell cycle arrest induced by microtubule depolymerization involves two distinct pathways (Alexandru et al. 1999; Fesquet et al. 1999; Fraschini et al. 1999). One pathway involving Mad1, Mad2, Mad3, and Bub1 inhibits APC/C^{Cdc20}, which blocks degradation of Pds1 and thereby keeps Esp1 inactive. This mechanism blocks sister separation. The persistence of Pds1 also blocks Cdk1 inactivation by inhibiting release of Cdc14 from the nucleolus (M. Shirayama and K. Nasmyth, unpubl.). However, mutants lacking Mad2 or Pds1 or both still block exit from mitosis, suggesting that there is a second pathway blocking Cdk1 inactivation. This pathway de-

pends on Bub2 and Byr4. Only mutants lacking both pathways (e.g., *mad2 bub2* double mutants) inactivate Cdk1 and enter the next cell cycle as if nothing were amiss in the presence of microtubule depolymerizing drugs. Several pieces of evidence suggest that the Bub2 pathway blocks the release of Cdc14 from the nucleolus. The fission yeast homologs of Bub2 and Byr4 form a two-component GTPase-activating enzyme for a GTPase homologous to Tem1 (Furge et al. 1998). Bub2 and Byr4 might inactivate Tem1 by converting the GTP-bound form to the presumably inactive, GDP-bound form. Tem1–GTP might be required for activation of the Dbf2 kinase, which was also found to be blocked by the Bub2 pathway (Fesquet et al. 1999). The Bub2 pathway is unlikely to be activated by the kinetochore but by some other as yet unknown defect cause by microtubule depolymerization (Wang and Burke 1995). Unlike Mad proteins, Bub2 is associated with spindle poles (Fraschini et al. 1999) and might coordinate cytokinesis with the arrival of chromosomes at the poles in late anaphase.

Summary

The APC/C was found by searching for the apparatus responsible for destroying mitotic cyclins at the metaphase-to-anaphase transition. This apparatus is a key part of the regulatory network that generates oscillations in the activity of mitotic CDKs. Studies of how the various forms of APC/C are regulated promise to provide a deep understanding of how eukaryotic cells generate CDK waves. However, the APC/C turns out to have a much more fundamental role in the eukaryotic cell cycle than merely being a counterweight to cyclin B synthesis. It mediates the separation of sister chromatids and is the target of regulatory mechanisms whose role is to ensure that daughter cells inherit one (usually two) complete copies of the genome. The control of sister chromatid separation appears to have become more and more important as organisms became more complicated and as their genomes grew in size. Bacteria, for example, barely regulate sister chromatid separation. Indeed, they commence to separate sister chromatids almost as soon as replication has been initiated. At the other extreme are mammalian cells, in which a surveillance mechanism merely needed for high fidelity chromosome transmission in yeast has clearly become an integral part of mitosis.

It was the discovery of the APC/C (and SCF) and the key roles that they have in eukaryotic cell reproduction that established once and for all the importance of ubiquitin mediated proteolysis in eukaryotic cell biology. Once perceived as a system exclusively involved in removing damaged proteins from the cell, ubiquitination is now perceived as a universal regulatory mechanism whose importance approaches that of protein phosphorylation.

The irreversibility of proteolysis is utilized by cells to give the cell cycle directionality. Once CKIs have been destroyed by SCF, it is very difficult for cells to inactivate Cdk1. Their activity drives the onset of DNA repli-

cation and entry into mitosis. Likewise, the destruction of cyclin B and Pds1 triggers the separation of sister chromatids and the inactivation of Cdk1. By utilizing the same apparatus to degrade mitotic cyclins and anaphase inhibitors, eukaryotic cells ensure that preparations for chromosome rereplication cannot normally precede the separation of sister chromatids generated by a previous round of DNA replication. The recent discovery that destruction of I κ B and β -catenin are mediated by SCF suggests that this formula is not restricted to cell division but also has a key role in signal transduction. The persistence of APC/C in fully differentiated quiescent cells suggests that its powerful substrate recognition apparatus is not confined to proteins involved in cell cycle progression.

That proteolysis should have a key role in cell cycle regulation is in retrospect possibly not too surprising. What is still less clear is why so much of the proteolysis that orders the cell cycle should be mediated by large ubiquitin ligase complexes as opposed to more conventional proteases. A key advantage of these ligases is that they can, with great specificity, promote the complete annihilation of a protein without the need for cleavage sites within functional domains of that protein. This then may be one of the key advantages of separating the marking process from proteolysis itself. It is interesting in this regard that the proteolytic program that mediates apoptosis in contrast utilizes proteolytic cleavage. Ubiquitination requires ATP, which is in short supply in dying cells.

The cell cycle ends in an orgy of protein degradation that sets the scene for a new round of duplication. With the discovery of the apparatus responsible for this cleanup, we have embarked on a whole new area of cell biology.

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References

- Alexandru, G., W. Zachariae, A. Schleiffer, and K. Nasmyth. 1999. Sister chromatid separation and chromosome re-duplication are regulated by different mechanisms in response to spindle damage. *EMBO J.* **18**: 2707–2721.
- Amon, A., S. Irniger, and K. Nasmyth. 1994. Closing the cell cycle circle in yeast: G2 cyclin proteolysis initiated at mitosis persists until the activation of G1 cyclins in the next cycle. *Cell* **77**: 1037–1050.
- Aristarkhov, A., E. Eytan, A. Moghe, A. Admon, A. Herskho, and J.V. Ruderman. 1996. E2-C, a cyclin-selective ubiquitin carrier protein required for the destruction of mitotic cyclins. *Proc. Natl. Acad. Sci.* **93**: 4294–4299.
- Bai, C., P. Sen, K. Hofmann, L. Ma, M. Goebel, J.W. Harper, and S.J. Elledge. 1996. SKP1 connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box. *Cell* **86**: 263–274.
- Baumeister, W., J. Walz, F. Zuhl, and E. Seemuller. 1998. The proteasome: Paradigm of a self-compartmentalizing protease. *Cell* **92**: 367–380.
- Biggins, S., F.F. Severin, N. Bhalla, I. Sassoon, A.A. Hyman, and A.W. Murray. 1999. The conserved protein kinase Ipl1 regulates microtubule binding to kinetochores in budding yeast. *Genes & Dev.* **13**: 532–544.
- Borden, K.L. and P.S. Freemont. 1996. The RING finger domain: A recent example of a sequence-structure family. *Curr. Opin. Struct. Biol.* **6**: 395–401.
- Brandeis, M. and T. Hunt. 1996. The proteolysis of mitotic cyclins in mammalian cells persists from the end of mitosis until the onset of S phase. *EMBO J.* **15**: 5280–5289.
- Brown, K.D., R.M. Coulson, T.J. Yen, and D.W. Cleveland. 1994. Cyclin-like accumulation and loss of the putative kinetochore motor CENP-E results from coupling continuous synthesis with specific degradation at the end of mitosis. *J. Cell Biol.* **125**: 1303–1312.
- Charles, J.F., S.L. Jaspersen, R.L. Tinker-Kulberg, L. Hwang, A. Szidon, and D.O. Morgan. 1998. The Polo-related kinase Cdc5 activates and is destroyed by the mitotic cyclin destruction machinery in *S. cerevisiae*. *Curr. Biol.* **8**: 497–507.
- Chen, R.H., J.C. Waters, E.D. Salmon, and A.W. Murray. 1996. Association of spindle assembly checkpoint component XMad2 with unattached kinetochores. *Science* **274**: 242–246.
- Chen, R.H., A. Shevchenko, M. Mann, and A.W. Murray. 1998. Spindle checkpoint protein Xmad1 recruits Xmad2 to unattached kinetochores. *J. Cell Biol.* **143**: 283–295.
- Cheng, L., L. Hunke, and C.F.J. Hardy. 1998. Cell cycle regulation of the *Saccharomyces cerevisiae* polo-like kinase cdc5p. *Mol. J. Cell Biol.* **18**: 7360–7370.
- Cheng, L., T. Collyer, and C.F. Hardy. 1999. Cell cycle regulation of DNA replication initiator factor Dbf4p. *Mol. Cell Biol.* **19**: 4270–4278.
- Ciosk, R., W. Zachariae, C. Michaelis, A. Shevchenko, M. Mann, and K. Nasmyth. 1998. An ESP1/PDS1 complex regulates loss of sister chromatid cohesion at the metaphase to anaphase transition in yeast. *Cell* **93**: 1067–1076.
- Cohen-Fix, O. and D. Koshland. 1997. The anaphase inhibitor of *Saccharomyces cerevisiae* Pds1p is a target of the DNA damage checkpoint pathway. *Proc. Natl. Acad. Sci.* **94**: 14361–14366.
- . 1999. Pds1 of budding yeast has dual roles: Inhibition of anaphase and regulation of mitotic exit. *Genes & Dev.* **13**: (in press).
- Cohen-Fix, O., J.M. Peters, M.W. Kirschner, and D. Koshland. 1996. Anaphase initiation in *Saccharomyces cerevisiae* is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p. *Genes & Dev.* **10**: 3081–3093.
- Cohen-Fix, O. and D. Koshland. 1999. Pds1 of budding yeast has dual roles: Inhibition of anaphase and regulation of mitotic exit. *Genes Dev.* **13**: 1950–1959.
- Coux, O., K. Tanaka, and A.L. Goldberg. 1996. Structure and functions of the 20S and 26S proteasomes. *Annu. Rev. Biochem.* **65**: 801–847.
- Das, A.K., P.W. Cohen, and D. Barford. 1998. The structure of the tetratricopeptide repeats of protein phosphatase 5: Implications for TPR-mediated protein-protein interactions. *EMBO J.* **17**: 1192–1199.
- Dawson, I.A., S. Roth, and S. Artavanis-Tsakonas. 1995. The *Drosophila* cell cycle gene *fizzy* is required for normal degradation of cyclins A and B during mitosis and has homology to the CDC20 gene of *Saccharomyces cerevisiae*. *J. Cell Biol.*

- 129: 725–737.
- Descombes, P. and E.A. Nigg. 1998. The polo-like kinase Plx1 is required for M phase exit and destruction of mitotic regulators in *Xenopus* egg extracts. *EMBO J.* **17**: 1328–1335.
- Evans, T., E.T. Rosenthal, J. Youngblom, D. Distel, and T. Hunt. 1983. Cyclin: A protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. *Cell* **33**: 389–396.
- Fang, G., H. Yu, and M.W. Kirschner. 1998a. The checkpoint protein MAD2 and the mitotic regulator CDC20 form a ternary complex with the anaphase-promoting complex to control anaphase initiation. *Genes & Dev.* **12**: 1871–1883.
- . 1998b. Direct binding of CDC20 protein family members activates the anaphase-promoting complex in mitosis and G1. *Mol. Cell* **2**: 163–171.
- Feldman, R.M., C.C. Correll, K.B. Kaplan, and R.J. Deshaies. 1997. A complex of Cdc4p, Skp1p, and Cdc53p/cullin catalyzes ubiquitination of the phosphorylated CDK inhibitor Sic1p. *Cell* **91**: 221–230.
- Felix, M.A., J.C. Labbe, M. Doree, T. Hunt, and E. Karsenti. 1990. Triggering of cyclin degradation in interphase extracts of amphibian eggs by cdc2 kinase. *Nature* **346**: 379–382.
- Fesquet, D., P.J. Fitzpatrick, A.L. Johnson, K.M. Kramer, J.H. Toyn, and L.H. Johnston. 1999. A Bub2p-dependent spindle checkpoint pathway regulates the Dbf2p kinase in budding yeast. *EMBO J.* **18**: 2424–2434.
- Fraschini, R., E. Formenti, G. Lucchini, and S. Piatti. 1999. Budding yeast bub2 is localized at spindle pole bodies and activates the mitotic checkpoint via a different pathway from mad2. *J. Cell Biol.* **145**: 979–991.
- Funabiki, H., H. Yamano, K. Kumada, K. Nagao, T. Hunt, and M. Yanagida. 1996. Cut2 proteolysis required for sister-chromatid separation in fission yeast. *Nature* **381**: 438–441.
- Funabiki, H., H. Yamano, K. Nagao, H. Tanaka, H. Yasuda, T. Hunt, and M. Yanagida. 1997. Fission yeast Cut2 required for anaphase has two destruction boxes. *EMBO J.* **16**: 5977–5987.
- Furge, K.A., K. Wong, J. Armstrong, M. Balasubramanian, and C.F. Albright. 1998. Byr4 and Cdc16 form a two-component GTPase-activating protein for the Spg1 GTPase that controls septation in fission yeast. *Curr. Biol.* **8**: 947–954.
- Gallant, P. and E.A. Nigg. 1992. Cyclin B2 undergoes cell cycle-dependent nuclear translocation and, when expressed as a non-destructible mutant, causes mitotic arrest in HeLa cells. *J. Cell Biol.* **117**: 213–224.
- Glutzer, M., A.W. Murray, and M.W. Kirschner. 1991. Cyclin is degraded by the ubiquitin pathway. *Nature* **349**: 132–138.
- Gopalan, G., C.S. Chan, and P.J. Donovan. 1997. A novel mammalian, mitotic spindle-associated kinase is related to yeast and fly chromosome segregation regulators. *J. Cell Biol.* **138**: 643–656.
- Gorbsky, G.J., R.H. Chen, and A.W. Murray. 1998. Microinjection of antibody to Mad2 protein into mammalian cells in mitosis induces premature anaphase. *J. Cell Biol.* **141**: 1193–1205.
- Gottesfeld, J.M. and D.J. Forbes. 1997. Mitotic repression of the transcriptional machinery. *Trends Biochem. Sci.* **22**: 197–202.
- Grossberger, R., C. Gieffers, W. Zachariae, A.V. Podtelejnikov, A. Schleiffer, K. Nasmyth, M. Mann, and J.M. Peters. 1999. Characterization of the DOC1/APC10 subunit of the yeast and the human anaphase-promoting complex. *J. Biol. Chem.* **274**: 14500–14507.
- Haccard, O., B. Sarcevic, A. Lewellyn, R. Hartley, L. Roy, T. Izumi, E. Erikson, and J.L. Maller. 1993. Induction of metaphase arrest in cleaving *Xenopus* embryos by MAP kinase. *Science* **262**: 1262–1265.
- He, X., T.E. Patterson, and S. Sazer. 1997. The *Schizosaccharomyces pombe* spindle checkpoint protein mad2p blocks anaphase and genetically interacts with the anaphase-promoting complex. *Proc. Natl. Acad. Sci.* **94**: 7965–7970.
- Heichman, K.A. and J.M. Roberts. 1996. The yeast *CDC16* and *CDC27* genes restrict DNA replication to once per cell cycle. *Cell* **85**: 39–48.
- . 1998. CDC16 controls initiation at chromosome replication origins. *Mol. Cell* **1**: 457–463.
- Hershko, A., D. Ganoth, J. Pehrson, R.E. Palazzo, and L.H. Cohen. 1991. Methylated ubiquitin inhibits cyclin degradation in clam embryo extracts. *J. Biol. Chem.* **266**: 16376–16379.
- Hershko, A., D. Ganoth, V. Sudakin, A. Dahan, L.H. Cohen, F.C. Luca, J.V. Ruderman, and E. Eytan. 1994. Components of a system that ligates cyclin to ubiquitin and their regulation by the protein kinase cdc2. *J. Biol. Chem.* **269**: 4940–4946.
- Hicke, L. 1999. Gettin' down with ubiquitin: Turning off cell-surface receptors, transporters and channels. *Trends Cell Biol.* **9**: 107–112.
- Holloway, S.L., M. Glotzer, R.W. King, and A.W. Murray. 1993. Anaphase is initiated by proteolysis rather than by the inactivation of maturation-promoting factor. *Cell* **73**: 1393–1402.
- Hoyt, M.A. and J.R. Geiser. 1996. Genetic analysis of the mitotic spindle. *Annu. Rev. Genet.* **30**: 7–33.
- Hoyt, M.A., L. Totis, and B.T. Roberts. 1991. *S. cerevisiae* genes required for cell cycle arrest in response to loss of microtubule function. *Cell* **66**: 507–517.
- Hwang, L.H. and A.W. Murray. 1997. A novel yeast screen for mitotic arrest mutants identifies DOC1, a new gene involved in cyclin proteolysis. *Mol. Biol. Cell* **8**: 1877–1887.
- Hwang, L.H., L.F. Lau, D.L. Smith, C.A. Mistrot, K.G. Hardwick, E.S. Hwang, A. Amon, and A.W. Murray. 1998. Budding yeast Cdc20: A target of the spindle checkpoint. *Science* **279**: 1041–1044.
- Hyman, A.A. and P.K. Sorger. 1995. Structure and function of kinetochores in budding yeast. *Annu. Rev. Cell. Dev. Biol.* **11**: 471–495.
- Irner, S. and K. Nasmyth. 1997. The anaphase-promoting complex is required in G1 arrested yeast cells to inhibit B-type cyclin accumulation and to prevent uncontrolled entry into S-phase. *J. Cell Sci.* **110**: 1523–1531.
- Irner, S., S. Piatti, C. Michaelis, and K. Nasmyth. 1995. Genes involved in sister chromatid separation are needed for B-type cyclin proteolysis in budding yeast. *Cell* **81**: 269–278.
- Jaspersen, S.L., J.F. Charles, and D.O. Morgan. 1999. Inhibitory phosphorylation of the APC regulator Hct1 is controlled by the kinase Cdc28 and the phosphatase Cdc14. *Curr. Biol.* **9**: 227–236.
- Johnson, E.S. and G. Blobel. 1997. Ubc9p is the conjugating enzyme for the ubiquitin-like protein Smt3p. *J. Biol. Chem.* **272**: 26799–26802.
- Juang, Y.L., J. Huang, J.M. Peters, M.E. McLaughlin, C.Y. Tai, and D. Pellman. 1997. APC-mediated proteolysis of Ase1 and the morphogenesis of the mitotic spindle. *Science* **275**: 1311–1314.
- Kallio, M., J. Weinstein, J.R. Daum, D.J. Burke, and G.J. Gorbsky. 1998. Mammalian p55CDC mediates association of the spindle checkpoint protein Mad2 with the cyclosome/anaphase-promoting complex, and is involved in regulating anaphase onset and late mitotic events. *J. Cell Biol.* **141**: 1393–1406.
- Kamura, T., D.M. Koepp, M.N. Conrad, D. Skowyra, R.J. Moreland, O. Iliopoulos, W.S. Lane, W.G. Kaelin, Jr., S.J. Elledge,

- R.C. Conaway, J.W. Harper, and J.W. Conaway. 1999. Rbx1, a component of the VHL tumor suppressor complex and SCF ubiquitin ligase. *Science* **284**: 657–661.
- Kim, S.H., D.P. Lin, S. Matsumoto, A. Kitazono, and T. Matsumoto. 1998. Fission yeast Spl1: An effector of the Mad2-dependent spindle checkpoint. *Science* **279**: 1045–1047.
- Kimura, M., S. Kotani, T. Hattori, N. Sumi, T. Yoshioka, K. Todokoro, and Y. Okano. 1997. Cell cycle-dependent expression and spindle pole localization of a novel human protein kinase, Aik, related to Aurora of *Drosophila* and yeast Ipl1. *J. Biol. Chem.* **272**: 13766–13771.
- King, R.W., J.M. Peters, S. Tugendreich, M. Rolfe, P. Hieter, and M.W. Kirschner. 1995. A 20S complex containing CDC27 and CDC16 catalyzes the mitosis-specific conjugation of ubiquitin to cyclin B. *Cell* **81**: 279–288.
- Kitamura, K., H. Maekawa, and C. Shimoda. 1998. Fission yeast Ste9, a homolog of Hct1/Cdh1 and Fizzy-related, is a novel negative regulator of cell cycle progression during G1-phase. *Mol. Biol. Cell* **9**: 1065–1080.
- Knapp, D., L. Bhoite, D.J. Stillman, and K. Nasmyth. 1996. The transcription factor Swi5 regulates expression of the cyclin kinase inhibitor p40SIC1. *Mol. Cell Biol.* **16**: 5701–5707.
- Knoblich, J.A., K. Sauer, L. Jones, H. Richardson, R. Saint, and C.F. Lehner. 1994. Cyclin E controls S phase progression and its down-regulation during *Drosophila* embryogenesis is required for the arrest of cell proliferation. *Cell* **77**: 107–120.
- Koch, C. and K. Nasmyth. 1994. Cell cycle regulated transcription in yeast. *Curr. Opin. J. Cell Biol.* **6**: 451–459.
- Kominami, K., H. Seth-Smith, and T. Toda. 1998. Apc10 and Ste9/Srw1, two regulators of the APC-cyclosome, as well as the CDK inhibitor Rum1 are required for G1 cell-cycle arrest in fission yeast. *EMBO J.* **17**: 5388–5399.
- Kosako, H., Y. Gotoh, and E. Nishida. 1994. Mitogen-activated protein kinase kinase is required for the mos-induced metaphase arrest. *J. Biol. Chem.* **269**: 28354–28358.
- Kotani, S., S. Tugendreich, M. Fujii, P.M. Jorgensen, N. Watanabe, C. Hoog, P. Hieter, and K. Todokoro. 1998. PKA and MPF-activated polo-like kinase regulate anaphase-promoting complex activity and mitosis progression. *Mol. Cell* **1**: 371–380.
- Kramer, E.R., C. Gieffers, G. Holzl, M. Hengstschlager, and J.M. Peters. 1998a. Activation of the human anaphase-promoting complex by proteins of the CDC20/Fizzy family. *Curr. Biol.* **8**: 1207–1210.
- Kramer, K.M., D. Fesquet, A.L. Johnson, and L.H. Johnston. 1998b. Budding yeast RSI1/APC2, a novel gene necessary for initiation of anaphase, encodes an APC subunit. *EMBO J.* **17**: 498–506.
- Kumada, K., S. Su, M. Yanagida, and T. Toda. 1995. Fission yeast TPR-family protein nuc2 is required for G1-arrest upon nitrogen starvation and is an inhibitor of septum formation. *J. Cell. Sci.* **108**: 895–905.
- Kumada, K., T. Nakamura, K. Nagao, H. Funabiki, T. Nakagawa, and M. Yanagida. 1998. Cut1 is loaded onto the spindle by binding to Cut2 and promotes anaphase spindle movement upon Cut2 proteolysis. *Curr. Biol.* **8**: 633–641.
- Lamb, J.R., W.A. Michaud, R.S. Sikorski, and P.A. Hieter. 1994. Cdc16p, Cdc23p and Cdc27p form a complex essential for mitosis. *EMBO J.* **13**: 4321–4328.
- Lamb, J.R., S. Tugendreich, and P. Hieter. 1995. Tetratricopeptide repeat interactions: To TPR or not to TPR? *Trends Biochem. Sci.* **20**: 257–259.
- Lane, M.E., K. Sauer, K. Wallace, Y.N. Jan, C.F. Lehner, and H. Vaessin. 1996. Dacapo, a cyclin-dependent kinase inhibitor, stops cell proliferation during *Drosophila* development. *Cell* **87**: 1225–1235.
- Lee, G.W., F. Melchior, M.J. Matunis, R. Mahajan, Q. Tian, and P. Anderson. 1998. Modification of Ran GTPase-activating protein by the small ubiquitin-related modifier SUMO-1 requires Ubc9, an E2-type ubiquitin-conjugating enzyme homologue. *J. Biol. Chem.* **273**: 6503–6507.
- Li, L., B.R. Ernsting, M.J. Wishart, D.L. Lohse, and J.E. Dixon. 1997. A family of putative tumor suppressors is structurally and functionally conserved in humans and yeast. *J. Biol. Chem.* **272**: 29403–29406.
- Li, R. and A.W. Murray. 1991. Feedback control of mitosis in budding yeast. *Cell* **66**: 519–531.
- Li, Y. and R. Benezra. 1996. Identification of a human mitotic checkpoint gene: hSMAD2. *Science* **274**: 246–248.
- Lies, C.M., J. Cheng, S.W. James, N.R. Morris, M.J. O'Connell, and P.M. Mirabito. 1998. BIMAAPC3, a component of the *Aspergillus* anaphase promoting complex/cyclosome, is required for a G2 checkpoint blocking entry into mitosis in the absence of NIMA function. *J. Cell. Sci.* **111**: 1453–1465.
- Lim, H.H., P.Y. Goh, and U. Surana. 1998. Cdc20 is essential for the cyclosome-mediated proteolysis of both Pds1 and Clb2 during M phase in budding yeast. *Curr. Biol.* **8**: 231–234.
- Lorca, T., F.H. Cruzalegui, D. Fesquet, J.C. Cavadore, J. Mery, A. Means, and M. Doree. 1993. Calmodulin-dependent protein kinase II mediates inactivation of MPF and CSF upon fertilization of *Xenopus* eggs. *Nature* **366**: 270–273.
- Lorca, T., A. Castro, A.M. Martinez, S. Vigneron, N. Morin, S. Sigrist, C. Lehner, M. Doree, and J.C. Labbe. 1998. Fizzy is required for activation of the APC/cyclosome in *Xenopus* egg extracts. *EMBO J.* **17**: 3565–3575.
- Luca, F.C. and J.V. Ruderman. 1989. Control of programmed cyclin destruction in a cell-free system. *J. Cell Biol.* **109**: 1895–1909.
- Luca, F.C., E.K. Shibuya, C.E. Dohrmann, and J.V. Ruderman. 1991. Both cyclin A delta 60 and B delta 97 are stable and arrest cells in M-phase, but only cyclin B delta 97 turns on cyclin destruction. *EMBO J.* **10**: 4311–4320.
- Lupas, A., W. Baumeister, and K. Hofmann. 1997. A repetitive sequence in subunits of the 26S proteasome and 20S cyclosome (anaphase-promoting complex). *Trends Biochem. Sci.* **22**: 195–196.
- Mahaffey, D., Y. Yoo, and M. Rechsteiner. 1993. Ubiquitin metabolism in cycling *Xenopus* egg extracts. *J. Biol. Chem.* **268**: 21205–21211.
- Matsumoto, T. 1997. A fission yeast homolog of CDC20/p55CDC/Fizzy is required for recovery from DNA damage and genetically interacts with p34cdc2. *Mol. J. Cell Biol.* **17**: 742–750.
- Maxwell, P.H., M.S. Wiesener, G.W. Chang, S.C. Clifford, E.C. Vaux, M.E. Cockman, C.C. Wykoff, C.W. Pugh, E.R. Maher, and P.J. Ratcliffe. 1999. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* **399**: 271–275.
- McGarry, T.J. and M.W. Kirschner. 1998. Geminin, an inhibitor of DNA replication, is degraded during mitosis. *Cell* **93**: 1043–1053.
- Michaelis, C., R. Ciosk, and K. Nasmyth. 1997. Cohesins: Chromosomal proteins that prevent premature separation of sister chromatids. *Cell* **91**: 35–45.
- Moll, T., G. Tebb, U. Surana, H. Robitsch, and K. Nasmyth. 1991. The role of phosphorylation and the CDC28 protein kinase in cell cycle-regulated nuclear import of the *S. cerevisiae* transcription factor SWI5. *Cell* **66**: 743–758.
- Murray, A.W. and M.W. Kirschner. 1989. Cyclin synthesis drives the early embryonic cell cycle. *Nature* **339**: 275–280.
- Murray, A.W., M.J. Solomon, and M.W. Kirschner. 1989. The role of cyclin synthesis and degradation in the control of

- maturation promoting factor activity. *Nature* **339**: 280–286.
- Nakamura, N., M. Lowe, T.P. Levine, C. Rabouille, and G. Warren. 1997. The vesicle docking protein p115 binds GM130, a cis-Golgi matrix protein, in a mitotically regulated manner. *Cell* **89**: 445–455.
- Ohta, T., J.J. Michel, A.J. Schottelius, and Y. Xiong. 1999. ROC1, a homolog of APC11, represents a family of cullin partners with an associated ubiquitin ligase activity. *Mol. Cell* **3**: 535–541.
- Osaka, F., H. Seino, T. Seno, and F. Yamao. 1997. A ubiquitin-conjugating enzyme in fission yeast that is essential for the onset of anaphase in mitosis. *Mol. J. Cell Biol.* **17**: 3388–3397.
- Osmani, S.A. and X.S. Ye. 1996. Cell cycle regulation in *Aspergillus* by two protein kinases. *Biochem. J.* **317**: 633–641.
- Osmani, S.A., D.B. Engle, J.H. Doonan, and N.R. Morris. 1988. Spindle formation and chromatin condensation in cells blocked at interphase by mutation of a negative cell cycle control gene. *Cell* **52**: 241–251.
- Osmani, A.H., S.L. McGuire, and S.A. Osmani. 1991. Parallel activation of the NIMA and p34cdc2 cell cycle-regulated protein kinases is required to initiate mitosis in *A. nidulans*. *Cell* **67**: 283–291.
- Patra, D. and W.G. Dunphy. 1996. Xe-p9, a *Xenopus* Suc1/Cks homolog, has multiple essential roles in cell cycle control. *Genes & Dev.* **10**: 1503–1515.
- . 1998. Xe-p9, a *Xenopus* Suc1/Cks protein, is essential for the Cdc2-dependent phosphorylation of the anaphase-promoting complex at mitosis. *Genes & Dev.* **12**: 2549–2559.
- Patton, E.E., A.R. Willems, D. Sa, L. Kuras, D. Thomas, K.L. Craig, and M. Tyers. 1998. Cdc53 is a scaffold protein for multiple Cdc34/Skp1/F-box protein complexes that regulate cell division and methionine biosynthesis in yeast. *Genes & Dev.* **12**: 692–705.
- Pause, A., S. Lee, R.A. Worrell, D.Y. Chen, W.H. Burgess, W.M. Linehan, and R.D. Klausner. 1997. The von Hippel-Lindau tumor-suppressor gene product forms a stable complex with human CUL-2, a member of the Cdc53 family of proteins. *Proc. Natl. Acad. Sci.* **94**: 2156–2161.
- Pellman, D., M. Bagget, Y.H. Tu, G.R. Fink, and H. Tu. 1995. Two microtubule-associated proteins required for anaphase spindle movement in *Saccharomyces cerevisiae*. *J. Cell Biol.* **130**: 1373–1385.
- Peters, J.M., R.W. King, C. Hoog, and M.W. Kirschner. 1996. Identification of BIME as a subunit of the anaphase-promoting complex. *Science* **274**: 1199–1201.
- Pichler, S., S. Piatti, and K. Nasmyth. 1997. Is the yeast anaphase promoting complex needed to prevent re-replication during G2 and M phases? *EMBO J.* **16**: 5988–5997.
- Posada, J., N. Yew, N.G. Ahn, G.F. Vande Woude, and J.A. Cooper. 1993. Mos stimulates MAP kinase in *Xenopus* oocytes and activates a MAP kinase kinase in vitro. *Mol. J. Cell Biol.* **13**: 2546–2553.
- Prinz, S., E.S. Hwang, R. Visintin, and A. Amon. 1998. The regulation of Cdc20 proteolysis reveals a role for APC components Cdc23 and Cdc27 during S phase and early mitosis. *Curr. Biol.* **8**: 750–760.
- Pu, R.T. and S.A. Osmani. 1995. Mitotic destruction of the cell cycle regulated NIMA protein kinase of *Aspergillus nidulans* is required for mitotic exit. *EMBO J.* **14**: 995–1003.
- Qian, Y.W., E. Erikson, and J.L. Maller. 1998. Purification and cloning of a protein kinase that phosphorylates and activates the polo-like kinase Plx1. *Science* **282**: 1701–1704.
- Roghi, C., R. Giet, R. Uzbekov, N. Morin, I. Chartrain, R. Le Guellec, A. Couturier, M. Doree, M. Philippe, and C. Pri-
gent. 1998. The *Xenopus* protein kinase pEg2 associates with the centrosome in a cell cycle-dependent manner, binds to the spindle microtubules and is involved in bipolar mitotic spindle assembly. *J. Cell Sci.* **111**: 557–572.
- Sagata, N., N. Watanabe, G.F. Vande Woude, and Y. Ikawa. 1989. The c-mos proto-oncogene product is a cytostatic factor responsible for meiotic arrest in vertebrate eggs. *Nature* **342**: 512–518.
- Sassoon, I., F.F. Severin, P.D. Andrews, M.R. Taba, K.B. Kaplan, A.J. Ashford, M.J. Stark, P.K. Sorger, and A.A. Hyman. 1999. Regulation of *Saccharomyces cerevisiae* kinetochores by the type 1 phosphatase Glc7p. *Genes & Dev.* **13**: 545–555.
- Scheffner, M., U. Nuber, and J.M. Huibregtse. 1995. Protein ubiquitination involving an E1-E2-E3 enzyme ubiquitin thioester cascade. *Nature* **373**: 81–83.
- Schwab, M., A.S. Lutum, and W. Seufert. 1997. Yeast Hct1 is a regulator of Clb2 cyclin proteolysis. *Cell* **90**: 683–693.
- Schwarz, S.E., K. Matuschewski, D. Liakopoulos, M. Scheffner, and S. Jentsch. 1998. The ubiquitin-like proteins SMT3 and SUMO-1 are conjugated by the UBC9 E2 enzyme. *Proc. Natl. Acad. Sci.* **95**: 560–564.
- Seol, J.H., R. Feldmann, W. Zachariae, A. Shevchenko, C.C. Correl, S. Lyapina, Y. Chi, M. Galova, J. Claypool, S. Sandmeyer, A. Shevchenko, K. Nasmyth, and R.J. Deshaies. 1999. Cdc53/cullin and the essential Hrt1 RING-H2 subunit of SCF define a ubiquitin ligase module that activates the E2 enzyme Cdc34. *Genes & Dev.* **13**: 1614–1626.
- Seufert, W., B. Futcher, and S. Jentsch. 1995. Role of a ubiquitin-conjugating enzyme in degradation of S- and M-phase cyclins. *Nature* **373**: 78–81.
- Shirayama, M., W. Zachariae, R. Ciosk, and K. Nasmyth. 1998. The Polo-like kinase Cdc5p and the WD-repeat protein Cdc20p/fizzy are regulators and substrates of the anaphase promoting complex in *Saccharomyces cerevisiae*. *EMBO J.* **17**: 1336–1349.
- Shou, W., J.H. Seol, A. Shevchenko, C. Baskerville, D. Moazed, Z.W. Chen, J. Jang, H. Charbonneau, and R.J. Deshaies. 1999. Exit from mitosis is triggered by Tem1-dependent release of the protein phosphatase Cdc14 from nucleolar RENT complex. *Cell* **97**: 233–244.
- Shteinberg, M., Y. Protopopov, T. Listovsky, M. Brandeis, and A. Hershko. 1999. Phosphorylation of the cyclosome is required for its stimulation by Fizzy/cdc20. *Biochem. Biophys. Res. Commun.* **260**: 193–198.
- Sigrist, S.J. and C.F. Lehner. 1997. *Drosophila* fizzy-related down-regulates mitotic cyclins and is required for cell proliferation arrest and entry into endocycles. *Cell* **90**: 671–681.
- Sigrist, S., H. Jacobs, R. Stratmann, and C.F. Lehner. 1995. Exit from mitosis is regulated by *Drosophila* fizzy and the sequential destruction of cyclins A, B and B3. *EMBO J.* **14**: 4827–4838.
- Skowyra, D., K.L. Craig, M. Tyers, S.J. Elledge, and J.W. Harper. 1997. F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex. *Cell* **91**: 209–219.
- Skowyra, D., D.M. Koepp, T. Kamura, M.N. Conrad, R.C. Conaway, J.W. Conaway, S.J. Elledge, and J.W. Harper. 1999. Reconstitution of G1 cyclin ubiquitination with complexes containing SCF-Grr1 and Rbx1. *Science* **284**: 662–665.
- Stebbins, C.E., W.G. Kaelin, Jr., and N.P. Pavletich. 1999. Structure of the VHL-ElonginC-elonginB complex: Implications for VHL tumor suppressor function. *Science* **284**: 455–461.
- Stratmann, R. and C.F. Lehner. 1996. Separation of sister chromatids in mitosis requires the *Drosophila* pimpls product, a protein degraded after the metaphase/anaphase transition. *Cell* **84**: 25–35.

- Sudakin, V., D. Ganioth, A. Dahan, H. Heller, J. Hershko, F.C. Luca, J.V. Ruderman, and A. Hershko. 1995. The cyclosome, a large complex containing cyclin-selective ubiquitin ligase activity, targets cyclins for destruction at the end of mitosis. *Mol. Biol. Cell* **6**: 185–197.
- Surana, U., A. Amon, C. Dowzer, J. McGrew, B. Byers, and K. Nasmyth. 1993. Destruction of the CDC28/CLB mitotic kinase is not required for the metaphase to anaphase transition in budding yeast. *EMBO J.* **12**: 1969–1978.
- Tan, P., S.Y. Fuchs, A. Chen, K. Wu, C. Gomez, Z. Ronai, and Z.Q. Pan. 1999. Recruitment of a ROC1-CUL1 ubiquitin ligase by Skp1 and HOS to catalyze the ubiquitination of I kappa B alpha. *Mol. Cell* **3**: 527–533.
- Taylor, S.S. and F. McKeon. 1997. Kinetochore localization of murine Bub1 is required for normal mitotic timing and checkpoint response to spindle damage. *Cell* **89**: 727–735.
- . 1998. The human homologue of Bub3 is required for kinetochore localization of Bub1 and a Mad3/Bub1-related protein kinase. *J. Cell Biol.* **142**: 1–11.
- Tinker-Kulberg, R.L. and D.O. Morgan. 1999. Pds1 and Esp1 control both anaphase and mitotic exit in normal cells and following DNA damage. *Genes & Dev.* **13**: (in press).
- Townsley, F.M., A. Aristarkhov, S. Beck, A. Hershko, and J.V. Ruderman. 1997. Dominant-negative cyclin-selective ubiquitin carrier protein E2- C/UbcH10 blocks cells in metaphase. *Proc. Natl. Acad. Sci.* **94**: 2362–2367.
- Townsley, F.M. and J.V. Ruderman. 1998. Functional analysis of the *Saccharomyces cerevisiae* UBC11 gene. *Yeast* **14**: 747–757.
- Tugendreich, S., J. Tomkiel, W. Earnshaw, and P. Hieter. 1995. CDC27Hs colocalizes with CDC16Hs to the centrosome and mitotic spindle and is essential for the metaphase to anaphase transition. *Cell* **81**: 261–268.
- Uhlmann, F., F. Lottspeich, and K. Nasmyth. 1999. Sister chromatid separation at anaphase onset triggered by cleavage of the cohesin subunit Scc1p. *Nature* **400**: 37–42.
- Visintin, R., S. Prinz, and A. Amon. 1997. CDC20 and CDH1: A family of substrate-specific activators of APC- dependent proteolysis. *Science* **278**: 460–463.
- Visintin, R., K. Craig, E.S. Hwang, S. Prinz, M. Tyers, and A. Amon. 1998. The phosphatase Cdc14 triggers mitotic exit by reversal of Cdk- dependent phosphorylation. *Mol. Cell* **2**: 709–718.
- Visintin, R., E.S. Hwang, and A. Amon. 1999. Cfl1 prevents premature exit from mitosis by anchoring Cdc14 phosphatase in the nucleolus. *Nature* **398**: 818–823.
- Vorlauffer, E. and J.M. Peters. 1998. Regulation of the cyclin B degradation system by an inhibitor of mitotic proteolysis. *Mol. Biol. Cell* **9**: 1817–1831.
- Wang, Y. and D.J. Burke. 1995. Checkpoint genes required to delay cell division in response to nocodazole respond to impaired kinetochore function in the yeast *Saccharomyces cerevisiae*. *Mol. J. Cell Biol.* **15**: 6838–6844.
- Wassmann, K. and R. Benezra. 1998. Mad2 transiently associates with an APC/p55Cdc complex during mitosis. *Proc. Natl. Acad. Sci.* **95**: 11193–11198.
- Watanabe, N., T. Hunt, Y. Ikawa, and N. Sagata. 1991. Independent inactivation of MPF and cytoskeletal factor (Mos) upon fertilization of *Xenopus* eggs. *Nature* **352**: 247–248.
- Waters, J.C., R.H. Chen, A.W. Murray, and E.D. Salmon. 1998. Localization of Mad2 to kinetochores depends on microtubule attachment, not tension. *J. Cell Biol.* **141**: 1181–1191.
- Weinstein, J. 1997. Cell cycle-regulated expression, phosphorylation, and degradation of p55Cdc. A mammalian homolog of CDC20/Fizzy/slp1. *J. Biol. Chem.* **272**: 28501–28511.
- Weiss, E. and M. Winey. 1996. The *Saccharomyces cerevisiae* spindle pole body duplication gene MPS1 is part of a mitotic checkpoint. *J. Cell Biol.* **132**: 111–123.
- Yamada, H., K. Kumada, and M. Yanagida. 1997. Distinct subunit functions and cell cycle regulated phosphorylation of 20S APC/cyclosome required for anaphase in fission yeast. *J. Cell Sci.* **110**: 1793–1804.
- Yamaguchi, S., H. Murakami, and H. Okayama. 1997. A WD-repeat protein controls the cell cycle and differentiation by negatively regulating Cdc2/B-type cyclin complexes. *Mol. Biol. Cell* **8**: 2475–2486.
- Yamamoto, A., V. Guacci, and D. Koshland. 1996a. Pds1p is required for faithful execution of anaphase in the yeast, *Saccharomyces cerevisiae*. *J. Cell Biol.* **133**: 85–97.
- Yamamoto, A., V. Guacci, and D. Koshland. 1996b. Pds1p, an inhibitor of anaphase in budding yeast, plays a critical role in the APC and checkpoint pathway(s). *J. Cell Biol.* **133**: 99–110.
- Yamano, H., J. Gannon, and T. Hunt. 1996. The role of proteolysis in cell cycle progression in *Schizosaccharomyces pombe*. *EMBO J.* **15**: 5268–5279.
- Yamano, H., C. Tsurumi, J. Gannon, and T. Hunt. 1998. The role of the destruction box and its neighbouring lysine residues in cyclin B for anaphase ubiquitin-dependent proteolysis in fission yeast: Defining the D-box receptor. *EMBO J.* **17**: 5670–5678.
- Yamashita, Y.M., Y. Nakaseko, I. Samejima, K. Kumada, H. Yamada, D. Michaelson, and M. Yanagida. 1996. 20S cyclosome complex formation and proteolytic activity inhibited by the cAMP/PKA pathway. *Nature* **384**: 276–279.
- Yanagida, M. 1998. Fission yeast cut mutations revisited: control of anaphase. *Trends Cell Biol.* **8**: 144–149.
- Ye, X.S., R.R. Fincher, A. Tang, A.H. Osmani, and S.A. Osmani. 1998. Regulation of the anaphase-promoting complex/cyclosome by bimAAPC3 and proteolysis of NIMA. *Mol. Biol. Cell* **9**: 3019–3030.
- Yu, H., R.W. King, J.M. Peters, and M.W. Kirschner. 1996. Identification of a novel ubiquitin-conjugating enzyme involved in mitotic cyclin degradation. *Curr. Biol.* **6**: 455–466.
- Yu, H., J.M. Peters, R.W. King, A.M. Page, P. Hieter, and M.W. Kirschner. 1998. Identification of a cullin homology region in a subunit of the anaphase- promoting complex. *Science* **279**: 1219–1222.
- Zachariae, W. and K. Nasmyth. 1996. TPR proteins required for anaphase progression mediate ubiquitination of mitotic B-type cyclins in yeast. *Mol. Biol. Cell* **7**: 791–801.
- Zachariae, W., T.H. Shin, M. Galova, B. Obermaier, and K. Nasmyth. 1996. Identification of subunits of the anaphase-promoting complex of *Saccharomyces cerevisiae*. *Science* **274**: 1201–1204.
- Zachariae, W., M. Schwab, K. Nasmyth, and W. Seufert. 1998a. Control of cyclin ubiquitination by CDK-regulated binding of Hct1 to the anaphase promoting complex. *Science* **282**: 1721–1724.
- Zachariae, W., A. Shevchenko, P.D. Andrews, R. Ciosk, M. Galova, M.J. Stark, M. Mann, and K. Nasmyth. 1998b. Mass spectrometric analysis of the anaphase-promoting complex from yeast: Identification of a subunit related to cullins. *Science* **279**: 1216–1219.
- Zou, H., T.J. McGarry, T. Bernal, and M.W. Kirschner. 1999. Identification of a vertebrate sister-chromatid separation inhibitor encoded by a tumor transforming gene. *Science* **285**: 418–422.

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References

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