Emerging regulatory mechanisms in ubiquitin-dependent cell cycle control

Annamaria Mocciaro and Michael Rape*

Department of Molecular Cell Biology, University of California at Berkeley, Berkeley, CA 94720-3202, USA

*Author for correspondence (mrape@berkeley.edu)

Journal of Cell Science 125, 255–263

doi:10.1242/jcs.091199

Summary

The covalent modification of proteins with ubiquitin is required for accurate cell division in all eukaryotes. Ubiquitylation depends on an enzymatic cascade, in which E3 enzymes recruit specific substrates for modification. Among ~600 human E3s, the SCF (Skp1–cullin1–F-box) and the APC/C (anaphase-promoting complex/cyclosome) are known for driving the degradation of cell cycle regulators to accomplish irreversible cell cycle transitions. The cell cycle machinery reciprocally regulates the SCF and APC/C through various mechanisms, including the modification of these E3s or the binding of specific inhibitors. Recent studies have provided new insight into the intricate relationship between ubiquitylation and the cell division apparatus as they revealed roles for atypical ubiquitin chains, new mechanisms of substrate and E3 regulation, as well as extensive crosstalk between ubiquitylation enzymes. Here, we review these emerging regulatory mechanisms of ubiquitin-dependent cell cycle control and discuss how their manipulation might provide therapeutic benefits in the future.

This article is part of a Minifocus on Ubiquitin. For further reading, please see related articles: 'Ubiquitin and SUMO in DNA repair at a glance' by Helle D. Ulrich (*J. Cell Sci.* **125**, 249-254). 'The role of ubiquitylation in receptor endocytosis and endosomal sorting' by Kaisa Haglund and Ivan Dikic (*J. Cell Sci.* **125**, 265-275). 'Cellular functions of the DUBs' by Michael J. Clague et al. (*J. Cell Sci.* **125**, 277-286). 'HECT and RING finger families of E3 ubiquitin ligases at a glance' by Meredith B. Metzger et al. (*J. Cell Sci.* **125**, 531-537). 'Non-canonical ubiquitin-based signals for proteasomal degradation' by Yelena Kravtsova-Ivantsiv and Aaron Ciechanover (*J. Cell Sci.* **125**, 539-548). 'No one can whistle a symphony alone – how different ubiquitin linkages cooperate to orchestrate NF-κB activity' by Anna C. Schmukle and Henning Walczak (*J. Cell Sci.* **125**, 549-559).

Key words: SCF, Anaphase-promoting complex, Lysine 11, Ubiquitin

Introduction

Accurate cell division is no trivial task: cells need to duplicate their genomic material, correct mistakes made by sloppy DNA polymerases, repair damage caused by harsh environments and yet still distribute their chromosomes into identical daughter cells. Errors in this program can be deadly for the cell, or, if they result in transformation, have detrimental effects on the organism. To prevent this from happening, the cell division machinery is subject to multiple layers of regulation, with ubiquitylation being of central importance.

The post-translational modification with ubiquitin controls the stability, activity or localization of numerous proteins, including multiple cell cycle regulators. It is catalyzed by an enzymatic cascade composed of E1 ubiquitin-activating enzymes, E2 ubiquitin-conjugating enzymes and E3 ubiquitin ligases (Deshaies and Joazeiro, 2009; Rotin and Kumar, 2009; Schulman and Harper, 2009; Ye and Rape, 2009). Together, these enzymes promote the formation of an isopeptide bond between a lysine residue within the substrate and the C-terminus of ubiquitin. The covalent addition of a single ubiquitin, referred to as monoubiquitylation, can alter protein localization or its interactions (Mukhopadhyay and Riezman, 2007). Attachment of further ubiquitin molecules to one of the seven lysine residues or the N-terminus of a substrate-linked ubiquitin results in formation of polymeric chains (Fig. 1) (Ye and Rape, 2009). When connected through lysine 48 (K48) of ubiquitin, these chains trigger degradation of the substrate by the proteasome (Chau et al., 1989), but when linked through K63, they act as a molecular scaffold that orchestrates kinase activation or DNA repair (Mukhopadhyay and Riezman, 2007). K48- and K63-linked ubiquitin chains have long been recognized as essential regulators of cell division, as they provide a signal for the degradation of inhibitors of cell cycle progression or the activation of cell cycle checkpoints, respectively (Fig. 1).

Among the ~600 human E3s, two enzymes – the SCF (Skp1–cullin1–F-box) and APC/C (anaphase-promoting complex/cyclosome) – are well known for their roles in cell cycle control. These E3s share similar domain architectures, as they are composed of a cullin (in the case of SCF) or cullin-related (in the case of the APC/C) scaffold, a RING domain for binding the ubiquitin-charged E2 and a module for substrate recruitment (Box 1) (Petroski and Deshaies, 2005a; Schreiber et al., 2011). The SCF and APC/C regulate cell division by triggering the degradation of cyclins, Aurora or Polo-like kinases, Cdc25 phosphatases and cyclin-dependent kinase (CDK) inhibitors (Petroski and Deshaies, 2005a; Sullivan and Morgan, 2007). Despite similarities in structure and function, the regulatory mechanisms that ensure proper activation of the SCF and the APC/C are strikingly different: in the case of SCF, the substrate usually needs to be phosphorylated to be recognized by the E3, and mutations in the phosphorylation sites of SCF substrates can result in unrestricted cell division (Petroski and Deshaies, 2005a). By contrast, most APC/C substrates do not require post-translational modifications for E3 binding (Sullivan and Morgan, 2007). Instead, it is the APC/C itself whose activity is controlled at distinct stages of the cell cycle program through APC/C phosphorylation or the binding of inhibitors to the enzyme.

Analyses of the SCF and the APC/C have further shown a reciprocal relationship between ubiquitylation enzymes and the cell cycle machinery: E3s regulate cell division but, at the same time, the cell cycle controls ubiquitylation. Recent studies have

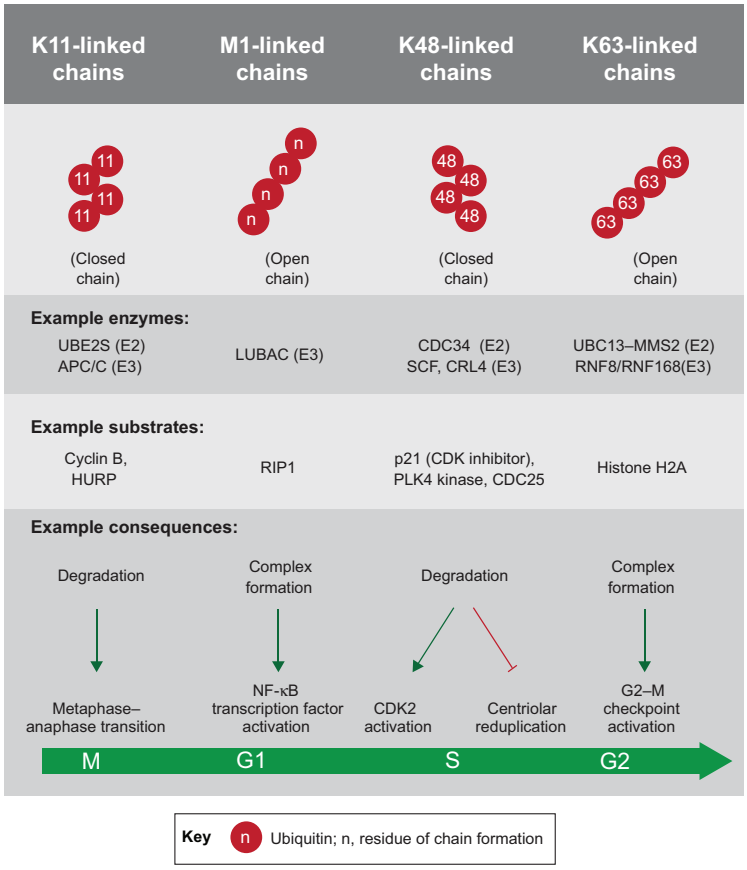


Fig. 1. Diversity of ubiquitin chain topologies with roles in cell cycle control. Ubiquitin chains of different topologies have distinct functional consequences. When linked through K48 of ubiquitin, ubiquitin chains trigger proteasomal degradation. K48-linked chains are important, for example, at the G1–S transition, when they trigger the degradation of CDK2 inhibitors to promote S-phase entry. K11-linked ubiquitin chains are assembled by the APC/C and also trigger degradation by the 26S proteasome. The K11-linked chains are important during mitosis, when they promote the metaphase-anaphase transition. By contrast, both K63- and M1-linked ubiquitin chains act in a non-proteolytic manner and regulate complex formation or kinase activation. In this way, M1-linked ubiquitin chains drive activation of the NF-κB transcription factor, which leads to the synthesis of important cell cycle regulators. K63-linked chains are assembled, for example, at sites of DNA damage, and they are required for establishing a G2–M checkpoint that inhibits cell division in the face of DNA damage.

provided further insight into this intricate connection by revealing the importance of atypical ubiquitin chains, new means of substrate and E3 regulation, and crosstalk between multiple cell-cycle-dependent ubiquitylation enzymes. In this Commentary, we will review these emerging regulatory mechanisms of ubiquitin-dependent cell cycle control. In addition, we will discuss how cells utilize these processes to accomplish their most important task – the accurate distribution of genomic material to their progeny.

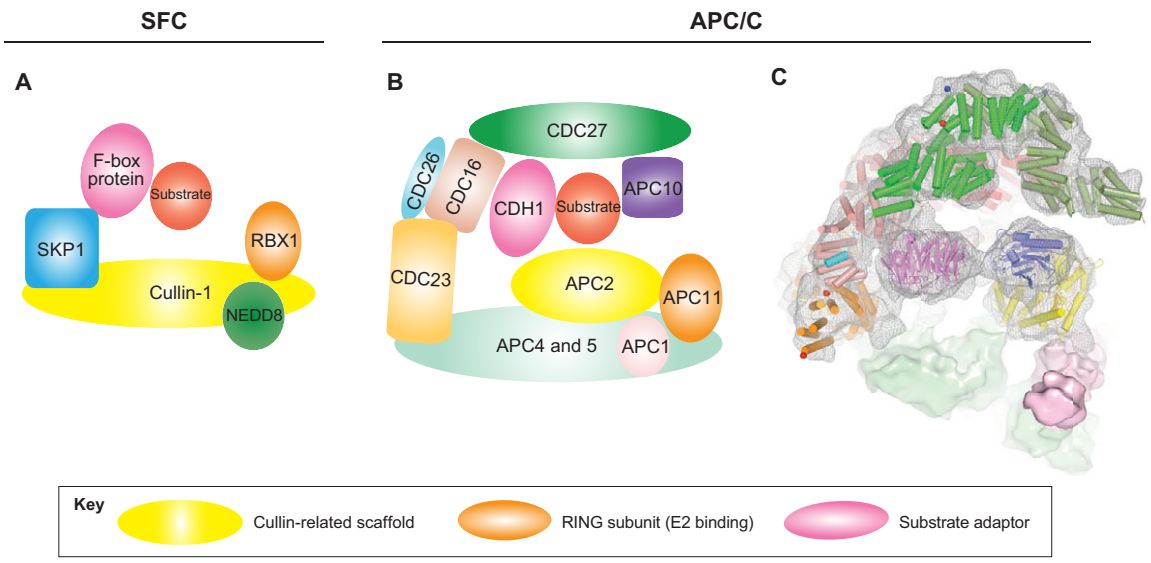
Diversity: new signals in cell cycle control

Ubiquitin-dependent proteolysis of cell cycle regulators helps to establish irreversible cell cycle transitions and is a prerequisite for the unidirectional progression through the cell cycle program. Classic experiments showed that proteasomal degradation of yeast cell cycle regulators depends on K48-linked ubiquitin chains (Chau et al., 1989; Glotzer et al., 1991; Thrower et al., 2000), and K48 is the only lysine residue of ubiquitin that is required for cell division in yeast (Sloper-Mould et al., 2001). In agreement with these observations, the yeast SCF and APC/C trigger degradation of their substrates through the formation of K48-linked ubiquitin chains (Petroski and Deshaies, 2005b; Rodrigo-Brenni and Morgan, 2007). It thus came as a surprise that, in higher eukaryotes, the APC/C was found to assemble ubiquitin chains of a different topology, namely those linked through K11, instead of K48-linked chains (Jin et al., 2008).

K11 linkages had been previously detected in biochemical experiments and cells, yet their functions remained poorly understood (Baboshina and Haas, 1996; Xu et al., 2009). Because levels of K11 linkages increase in response to proteasome inhibition, it has been suggested that they are involved in mediating proteolysis (Bennett et al., 2007; Kaiser et al., 2011; Xu et al., 2009; Ziv et al., 2011). Indeed, when homogeneous K11-linked ubiquitin chains were discovered as the product of the human APC/C, it was found that they target mitotic regulators for proteasomal degradation (Jin et al., 2008). Using linkage-specific antibodies, it was observed that the abundance of K11-linked chains increases dramatically when cells activate the APC/C during mitosis (Matsumoto et al., 2010). Conversely, a blockade in K11-linked chain formation stabilizes substrates of the APC/C and causes a cell cycle arrest that is reminiscent of APC/C inhibition (Jin et al., 2008; Williamson et al., 2009). Thus, in higher eukaryotes, K11-linked ubiquitin chains constitute a proteolytic signal that is required for cell division.

The APC/C catalyzes the formation of K11-linked chains by employing a pair of dedicated E2 enzymes, the chain-initiating UBE2C (also known as UBCH10) and the chain-elongating UBE2S (Ye and Rape, 2009). To rapidly catalyze chain initiation (i.e. the modification of lysine residues on the substrate with ubiquitin) UBE2C depends on stretches of conserved and positively charged substrate residues that are referred to as initiation motifs (Jin et al., 2008; Williamson et al., 2011). Initiation motifs are distinct from the D-boxes that mediate the binding of a substrate to the APC/C (Glotzer et al., 1991), and blocking initiation motifs can inhibit degradation without affecting the affinity of a particular substrate for the APC/C (Williamson, 2011). Following chain initiation, UBE2S rapidly extends K11-linked ubiquitin chains (Garnett et al., 2009; Williamson et al., 2009; Wu, T. et al., 2010). UBE2S does not recognize the substrate directly, but instead depends on the recognition of a specific surface on ubiquitin, the TEK-box, for

Box 1. Structural highlights on the APC/C



Although the SCF and APC/C are structurally similar, they differ in complexity. The SCF has only three core subunits (cullin 1, RBX1 and SKP1) but cooperates with multiple different substrate adaptors, the F-box proteins (see figure panel A). The human genome encodes 68 different F-box proteins. By contrast, the human APC/C comprises 15 core subunits and only two substrate adaptors. Its core subunits can be grouped into smaller subcomplexes that are charged with substrate binding or ubiquitin transfer (see figure panel B).

Owing to its complexity, the APC/C has long eluded structural analyses in reconstituted systems, which has left the field with diverse cryo-electron microscopy structures of APC/C complexes isolated from many different sources. In a breakthrough study, the yeast APC/C was recently reconstituted from recombinant subcomplexes and its structure was resolved by a combination of electron microscopy and X-ray crystallography (see figure panel C) (da Fonseca et al., 2011; Schreiber et al., 2011). The catalytic core of the APC/C, its cullin-related subunit APC2 and its RING protein APC11, localize to a platform domain, whereas the tetratricopeptide repeat (TPR) subunits involved in substrate recruitment [APC6 (CDC16), APC8 (CDC23) and APC3 (CDC27)] are sequestered in the 'arc lamp' domain. The degrons of APC/C substrates, referred to as D-boxes, are recognized by two APC/C components, the core subunit APC10 and the adaptor subunit CDC20 or CDH1 (FZR1), which supports previous data that linked APC10 to substrate recognition (Carroll and Morgan, 2002; Passmore et al., 2003). As the D-box coreceptor and the RING domain are in proximity to each other, the structural analysis of APC/C strengthened the notion that initiation motifs (i.e. those recognized by UBE2C, which binds to the RING domain) are typically close to D-boxes [i.e. the regions that are recognized by the substrate coreceptor (Williamson et al., 2011)]. Yeast and human APC/C differ in many aspects, with many subunits, substrates and regulatory mechanisms that are unique to human APC/C. Thus, a structural elucidation of reconstituted human APC/C will be a crucial step in elucidating the inner workings of this essential cell cycle regulator.

chain elongation through a mechanism of substrate-assisted catalysis (Wickliffe et al., 2011). Together, UBE2C and UBE2S assemble the majority of K11-linked chains during mitosis (Matsumoto et al., 2010), thereby driving the degradation of most APC/C substrates and steering progression of cells through division (Garnett et al., 2009; Matsumoto et al., 2010; Williamson et al., 2009).

Underscoring the importance of K11-linked chains for cell division, several studies have shown that the expression and stability of UBE2C and UBE2S are tightly regulated (Mathe et al., 2004; Rape and Kirschner, 2004; Whitfield et al., 2002; Williamson et al., 2009). The aberrant accumulation of UBE2C leads to premature APC/C activation in mitosis and inaccurate sister chromatid separation (Miniowitz-Shemtov et al., 2010; Reddy et al., 2007; Summers et al., 2008; van Ree et al., 2010). Accordingly, increased levels of UBE2C and UBE2S are frequently observed in cancer, and overexpression of either of these E2s has been found to promote tumorigenesis in mice (Jung et al., 2006; Tedesco et al., 2007; van Ree et al., 2010; Wagner et al., 2004).

At the same time as studies identified K11-linked chains as regulators of cell division, another type of ubiquitin linkage was found to control proliferation indirectly: ubiquitin chains that are connected through isopeptide bonds between the N-terminus of one ubiquitin and the C-terminus of another result in activation of NF-κB, a transcription factor that has roles in promoting proliferation and survival (Tokunaga et al., 2009). These linear (or M1-linked) ubiquitin chains are assembled on NF-κB essential modulator (NEMO), a subunit of the IκBα-kinase (IKK), by the oligomeric E3 LUBAC (linear ubiquitin chain assembly complex) (Gerlach et al., 2011; Ikeda et al., 2011; Tokunaga et al., 2011; Tokunaga et al., 2009). Interestingly, the M1-linked ubiquitin chains are recognized by specific domains, such as the ubiquitin-binding in ABIN and NEMO (UBAN) domain of NEMO itself (Rahighi et al., 2009). The interaction of M1-linked chains with NEMO results in the activation of IKK, which ultimately triggers the phosphorylation-dependent degradation of an NF-κB inhibitor, activation of NF-κB-dependent transcription and cell division. Thus, NEMO is both a substrate and effector of M1-linked chain formation, placing it at a central node for signal transduction through this atypical ubiquitin chain type.

Together, the discoveries of M1- and K11-linked chains expanded the repertoire of post-translational modifications that have important roles in cell division control. These chain types act in parallel to canonical K48-linked chains, which drive degradation of SCF substrates, or K63-linked chains, which regulate the G2–M cell cycle checkpoint (Fig. 1). Cell division is, therefore, controlled by a diverse set of ubiquitin chains with distinct topologies.

Focus: specific targeting of active cell cycle regulators

To ensure accurate division, the formation of the various ubiquitin chain types needs to be tightly controlled. For example, the APC/C, which is the E3 responsible for K11-linked chain formation, is only activated by the cyclin-B1–CDK1 complex after cells have entered mitosis. The formation of K11-linked ubiquitin chains then triggers degradation of cyclin B1 and subsequent inhibition of CDK1, an event that is essential for exit from mitosis (Glotzer et al., 1991; Murray et al., 1989). This regulatory circuit ensures that the APC/C only ubiquitylates active cyclin B1 and prevents unscheduled APC/C activation from interfering with cyclin B1 accumulation and cell cycle progression. In variations on this theme, ubiquitylation often achieves temporal and spatial regulation of cell division by selectively targeting active cell cycle regulators, as discussed below and in Fig. 2.

The cullin-RING E3 ubiquitin ligase 4 (CRL4) together with its substrate recognition factor CDT2 ($CRL4^{CDT2}$) controls the activity of proteins that regulate DNA replication and repair (Abbas and Dutta, 2011; Havens and Walter, 2011). Reminiscent of the APC/C and the SCF, $CRL4^{CDT2}$ contains a cullin scaffold, a RING subunit, an adaptor and the substrate receptor CDT2. $CRL4^{CDT2}$ ubiquitylates and marks for degradation the CDK inhibitor p21 (also known as CDKN1A or WAF) (Abbas et al., 2008), the transcription factor E2F1 (Shibutani et al., 2008), DNA polymerase η (Kim and Michael, 2008), chromatin licensing and DNA replication factor 1 (CDT1) (Zhong et al., 2003), and the SET-domain-containing methyltransferase 8 (SETD8) (Abbas et al., 2010; Arias and Walter, 2006; Centore et al., 2010; Jorgensen et al., 2011; Oda et al., 2010; Senga et al., 2006; Tardat et al., 2010). By targeting these proteins, $CRL4^{CDT2}$ contributes to limiting DNA replication to once per cell cycle (Abbas and Dutta, 2011; Havens and Walter, 2011).

Most of the $CRL4^{CDT2}$ substrates are targeted to their location on chromatin, and hence are activated, by the DNA polymerase processivity factor proliferating cell nuclear antigen (PCNA). Importantly, this high-affinity interaction with PCNA is now known to be a prerequisite for $CRL4^{CDT2}$-driven ubiquitylation and proteolysis (Fig. 2) (Arias and Walter, 2006; Senga et al., 2006). All $CRL4^{CDT2}$ substrates mentioned above contain a conserved sequence, the PIP box degron, which mediates binding of the substrates to PCNA and at the same time contributes to their recognition by CDT2 (Havens and Walter, 2009). By coupling ubiquitylation to PCNA binding, $CRL4^{CDT2}$ ensures that only the chromatin-bound and active substrate pool is targeted for degradation (Arias and Walter, 2006; Havens and Walter, 2009). Employing a localized co-factor for ubiquitylation, therefore, provides a mechanism by which cells achieve selective degradation of active cell cycle regulators.

An alternative mechanism by which ubiquitylation occurs on active cell cycle regulators is illustrated by a proteolytic circuit that controls centrosome duplication. During mitosis, cells contain two

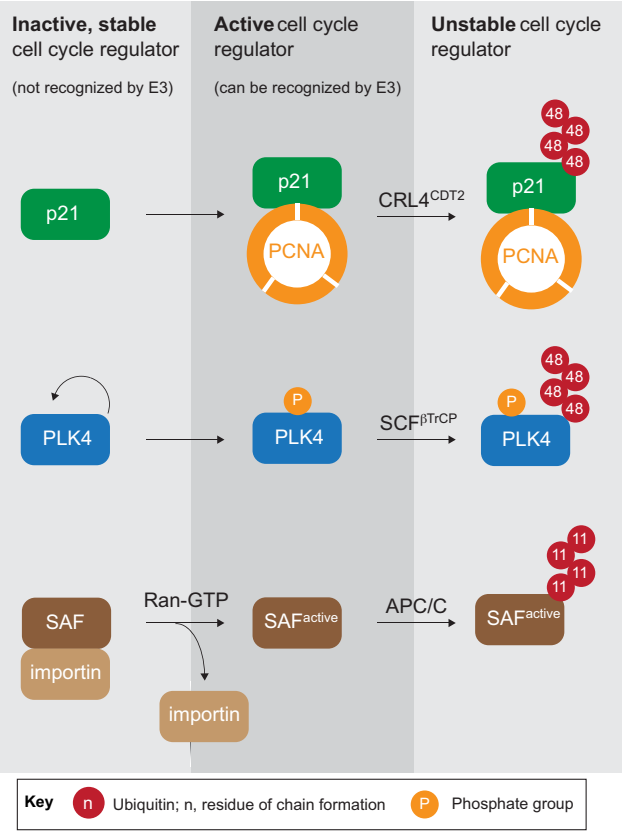


Fig. 2. Mechanisms of ubiquitin-dependent feedback regulation. Different mechanisms allow E3 enzymes to destabilize active cell cycle regulators. These include the involvement of essential cofactors, such as PCNA, autophosphorylation, as observed in the case of PLK4, and coupled activation and degradation, as observed for spindle assembly factors (SAFs). In all cases, the inactive cell cycle regulator is not recognized by the E3, and hence, it is spared from ubiquitylation and subsequent degradation.

centrosomes that act as microtubule-nucleating centers at spindle poles. Following cell division, the single centrosome in each daughter needs to be duplicated once, but only once, in a process that is initiated by the Polo-like kinase 4 (PLK4) (Kleylein-Sohn et al., 2007). Cancer cells often have compromised mechanisms that restrict centrosome duplication, which leads to multipolar spindles and chromosome missegregation (Ganem et al., 2009). To prevent centrosome overduplication, the activities of PLK4 (Cunha-Ferreira et al., 2009; Guderian et al., 2010; Holland et al., 2010; Rogers et al., 2009), its effector spindle assembly 6 homolog (SAS6) (Puklowski et al., 2011; Strnad et al., 2007) or the centriolar coiled coil protein of 110kDa (CP110) (D'Angiolella et al., 2010) are controlled by ubiquitin-dependent degradation.

Among the mechanisms protecting cells against supernumerous centrosomes, regulation of PLK4 is particularly important, as high levels of this kinase are sufficient to drive centrosome overduplication (Kleylein-Sohn et al., 2007). However, the activation of PLK4 not only induces the phosphorylation of centrosomal proteins, but it also leads to robust autophosphorylation (Guderian et al., 2010; Holland et al., 2010). The autophosphorylation of PLK4 then generates a degron that can be recognized by the E3 SCF in conjunction with its recognition factor β-transducin-repeat-containing ($SCF^{βTrCP}$) (βTrCP is also known as FBW1A), which subsequently targets PLK4 for

degradation (Fig. 2) (Cunha-Ferreira et al., 2009; Rogers et al., 2009). Degradation coupled to autophosphorylation thus ensures that only the active kinase is subject to ubiquitin-dependent degradation.

During mitosis, microtubules not only originate from centrosomes but also from chromosomes in a process that depends on the GTPase Ran (Kalab and Heald, 2008). When charged with GTP, Ran activates a set of spindle assembly factors (SAFs), allowing them to nucleate and stabilize microtubules. Several of these SAFs are later targeted for degradation through APC/C-dependent ubiquitylation (Fig. 2) (Song and Rape, 2010; Stewart and Fang, 2005a). Both the activity and stability of SAFs are controlled by inhibitors, the importins, which bind to sequence motifs required for microtubule binding and APC/C recognition. By dissociating importins, Ran activates these SAFs, yet at the same time, it enables their recognition by the APC/C (Song and Rape, 2010). Coupling activation and degradation, therefore, provides another mechanism to remove active cell cycle regulators. Together, these mechanisms underscore the idea that regulated ubiquitylation is an effective mechanism for implementing precise temporal and spatial control in cell division.

Independence: autocatalytic mechanisms of cell cycle control

In the above sections, we focused on the regulation that the ubiquitylation machinery can impose on substrates. However, as E3s drive crucial processes such as CDK inactivation or spindle formation, the activities of the enzymes themselves also have to be tightly regulated. Although this can be achieved by several mechanisms, E3s with roles in cell cycle control often employ autocatalytic means of activation and inhibition, and the APC/C provides a prime example of this.

During the early stages of mitosis, the activity of the APC/C is kept in check by an inhibitory network referred to as the spindle assembly checkpoint (SAC). Components of the SAC interfere with substrate recognition by the APC/C and thereby delay sister chromatid separation until all chromosomes have achieved bipolar attachment to the spindle (Musacchio and Salmon, 2007). Once chromosome attachment has been completed, the APC/C is rapidly activated, which requires the dissociation of inhibitory complexes between CDC20, the substrate-targeting subunit of the APC/C, and the SAC components MAD2 (also known as MAD2L1) and BubR1 (also known as BUB1B). Intriguingly, the dissociation of checkpoint complexes is brought about by the APC/C itself, which together with $p31^{comet}$ (also known as MAD2L1 and MAD2L1BP), UBE2C and UBE2S, catalyzes the ubiquitylation and degradation of CDC20 and potentially other proteins (Miniowitz-Shemtov et al., 2010; Reddy et al., 2007; Summers et al., 2008; Teichner et al., 2011; Williamson et al., 2009; Xia et al., 2004; Zeng et al., 2010). This APC/C-dependent ubiquitylation is likely to promote the exchange of checkpoint-inhibited CDC20 with newly synthesized, uninhibited CDC20, thereby leading to further APC/C activation. Shortly thereafter, the APC/C ensures the permanent nature of checkpoint-inactivation by initiating the degradation of the checkpoint components cyclin B1, monopolar spindle 1 (MPS1, also known as TKK), BUB1 and Aurora B (Palframan et al., 2006; Qi and Yu, 2007; Stewart and Fang, 2005b). Thus, the APC/C itself initiates events that lead to spindle checkpoint inactivation and full APC/C activation, a layout that has the potential for positive-feedback regulation.

Once the APC/C has been turned on, the sequential degradation of its many substrates ensures the ordered progression of cells through mitosis. After the APC/C has completed this task, its attention is focused on itself and it initiates its own inactivation. The APC/C accomplishes this feat by catalyzing the ubiquitylation of its substrate adaptors, CDC20 and CDH1 (Cdc20 homolog 1, also known as FZR1), as well as that of its E2s, UBE2C and UBE2S (Listovsky et al., 2004; Mathe et al., 2004; Nilsson et al., 2008; Pfleger and Kirschner, 2000; Rape and Kirschner, 2004; Williamson et al., 2009). Substrates can competitively inhibit these degradation reactions, thereby ensuring that the APC/C is not prematurely inactivated (Rape and Kirschner, 2004; Williamson et al., 2011). The decrease in APC/C activity also stabilizes the deubiquitylating enzyme USP37 (for ubiquitin specific peptidase 37) (Huang et al., 2011). USP37 binds to the APC/C, protecting its substrate cyclin A from ubiquitylation, and thereby allows cyclin A to team up with the CDK2 kinase to completely shut off APC/C activity. These intrinsic mechanisms of APC/C inactivation are overlaid by control reactions that are coupled to the environment of the cell, such as the growth-factor-dependent synthesis of the APC/C inhibitor EMI1 (also known as FBXO5 and FBX5) (Hsu et al., 2002). Thus, inactivation of the APC/C is brought about by a complex series of events that, to a large extent, depends on autocatalytic mechanisms.

Similar to the APC/C, the SCF is subject to autocatalytic regulation. The SCF is activated by conjugation of the ubiquitin-like protein NEDD8 to a conserved lysine residue in cullin 1 (CUL1) (see figure in Box 1). The neddylation of CUL1 increases the flexibility of the RING-subunit ring-box 1 (RBX1) and prevents binding of the SCF inhibitor CAND1 (for cullin-associated and neddylation-dissociated 1) (Duda et al., 2008; Liakopoulos et al., 1998; Liu et al., 2002; Saha and Deshaies, 2008). Together with a cofactor, RBX1 itself catalyzes the transfer of NEDD8 to CUL1, thereby turning on its own SCF (Scott et al., 2010). Extending the similarities to the APC/C, the SCF also promotes the turnover of its own substrate-targeting factors, the F-box proteins (Bennett et al., 2010; Lee et al., 2011). Thus, through various mechanisms, the APC/C and SCF control their own activity. By providing feedback control, such autocatalytic regulatory circuits are likely to be of central importance for accurate cell cycle progression.

Team work: coordinated action of ubiquitylation enzymes

With the advent of global analysis tools, crosstalk between ubiquitylation enzymes has been recognized to provide another layer of ubiquitin-dependent cell cycle control. For the SCF and the APC/C, different mechanisms of such coordination have been reported: the two E3s can regulate each other's activity, as demonstrated by the degradation of F-box proteins by the APC/C (Bashir et al., 2004; Puklowski et al., 2011; Wei et al., 2004), they can target the same substrates (Young and Pagano, 2010), and their activities in the cell can be coordinated to regulate the same process, as shown for the response of cells to the anti-tubulin chemotherapeutic taxol (Inuzuka et al., 2011; Wertz et al., 2011).

Crosstalk between cell-cycle-dependent ubiquitylation enzymes is facilitated by their sequestration in overlapping protein complexes, a feature that is prominently displayed by E3s and opposing deubiquitylating enzymes (DUBs) (Sowa et al., 2009). Such E3–DUB pairs can achieve transient substrate modification, as observed, for example, for the reversible ubiquitylation of spliceosomal proteins through the E3 PRP19 [officially known as

pre-mRNA processing factor 19 homolog (PRPF19)] and the DUB USP4 (Song et al., 2010). Alternatively, DUBs and E3s can regulate each other: the SCF-associated DUB USP15, for example, controls the stability of RBX1, the RING subunit of the SCF (Hetfeld et al., 2005), and USP44 protects the APC/C activator CDC20 from ubiquitylation (Stegmeier et al., 2007; Zhang, 2011). This interplay between DUBs and E3s works in both ways: the APC/C targets the DUB USP37 for degradation during mitosis, yet in late G1 phase, a stabilized USP37 helps to shut off the APC/C (Huang et al., 2011). Thus, by reverting ubiquitylation, DUBs can terminate ubiquitin-dependent signaling, set a threshold for the E3-activity required for measurable substrate modification or protect the E3 from degradation.

As mentioned above, the APC/C and SCF can target the same proteins, but do so mostly at different cell cycle stages. However, during mitosis, the APC/C cooperates with other E3s, such as the essential cullin RING E3 ligase CRL3. Together with specific substrate adaptors, CRL3 ubiquitylates the kinase Aurora B, which is an essential regulator of kinetochore function, spindle checkpoint activity and cytokinesis (Maerki et al., 2009; Sumara et al., 2007). The CRL3-dependent ubiquitylation of Aurora B removes the kinase from prometaphase chromosomes and helps to target it to the spindle midzone in anaphase, a re-localization characteristic for so-called passenger proteins. Ubiquitylated Aurora B was proposed to interact with the ubiquitin-dependent segregase p97 (also known as VCP) (Ramadan et al., 2007), which might provide energy in form of ATP hydrolysis to separate Aurora B from its binding partners at chromosomes. At later stages in mitosis, Aurora B is targeted for degradation by the APC/C (Stewart and Fang, 2005b). Although it remains to be tested, it is an interesting hypothesis that the same Aurora B molecule is dissociated from chromatin by CRL3 and then sent for degradation by the APC/C.

Taxol is a chemotherapeutic agent that interferes with cancer cell proliferation by activating the spindle checkpoint, which in turn inhibits the APC/C and leads to mitotic arrest. Upon prolonged exposure to taxol, cells commit to apoptosis and die or, in a scenario that is less advantageous for the organism, they slowly reduce CDK1 activity and 'slip' out of mitosis (Fig. 3). The decision between death and slippage is determined by the stability of the caspase inhibitor myeloid cell leukemia sequence 1 (MCL1). In cells that sustain taxol-induced APC/C inhibition, MCL1 is eventually phosphorylated by several kinases and targeted for ubiquitylation by the SCF^{FBW7} complex (Inuzuka et al., 2011; Wertz et al., 2011). The ensuing degradation of MCL1 triggers apoptosis. Cancer cells can subvert this mechanism and obtain resistance to taxol by stabilizing MCL1 through mutations in FBW7, overexpression of the deubiquitylating enzyme USP9X, or premature APC/C activation as the result of a defective spindle checkpoint (Petroski and Deshaies, 2005a; Schwickart et al., 2010; van Ree et al., 2010; Wagner et al., 2004). Thus, APC/C inhibition and SCF activation have to act in concert to guarantee the appropriate response to permanent spindle damage, as caused by anti-tubulin chemotherapeutics. Thus, not only collaboration, but also coordination between multiple ubiquitylation enzymes is important for proper cell division control.

Perspectives

Among the many recent findings that have improved the understanding of ubiquitin-dependent cell cycle control, we have focused our discussion on new regulatory motifs that we believe will have general importance for accurate cell division. The discoveries described here raise many new issues that will need to be addressed in the future.

Despite being at the center of intense study for several years, aspects of APC/C and SCF regulation still remain relatively poorly understood. Why, for example, does the APC/C of higher eukaryotes synthesize K11-linked ubiquitin chains instead of the more canonical K48-linked chains? Is there a specific receptor that recognizes K11-linked, but not K48-linked, ubiquitin chains? What allows the APC/C to turn against its own inhibitor and catalyze spindle checkpoint inactivation, and could this mechanism provide the basis for positive feedback and switch-like APC/C-activation? So far, the APC/C is the only known E3 that assembles homogeneous K11-linked chains. As the APC/C and K11-linked chains are essential for cell division, a better understanding of the function and regulation of these atypical chains might provide specific approaches to interfering with the proliferation of rapidly dividing cells, such as those observed in tumors.

As demonstrated by the many examples of crosstalk between E3s and DUBs, future studies of ubiquitin-dependent cell cycle control should also go beyond a single enzyme and include a system-wide analysis of the ubiquitylation networks that are linked to cell cycle control. This will require a combination of modern proteomic approaches (Bennett et al., 2010; Kaiser et al., 2011), genetic analyses through high-throughput small interfering RNA screens (Neumann et al., 2010; Song et al., 2010) and biochemical dissection of enzyme activities (Wickliffe et al., 2011). We expect such studies to provide mechanistic insight into the complexities and redundancies of cell cycle control, but also to reveal how these mechanisms could be exploited to target aberrant cell division in disease.

Indeed, an emphasis should be placed on the development of small molecules that interfere with the activity of ubiquitylation enzymes that are essential for cell division or are misregulated in

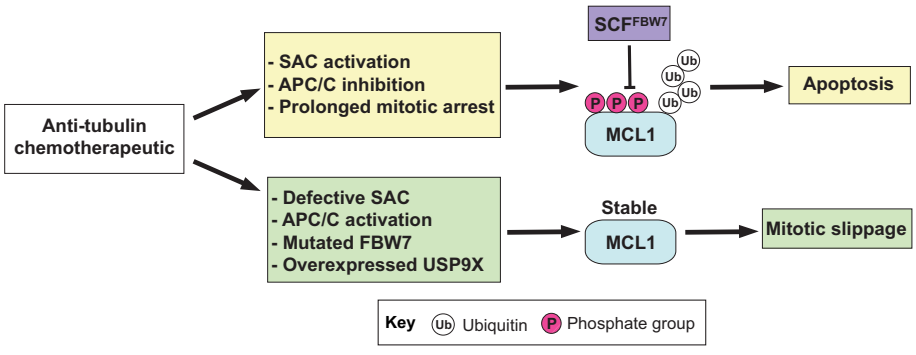


Fig. 3. Coordination of APC/C and SCF activities in cells experiencing spindle damage. Spindle damage, as induced by anti-tubulin chemotherapeutics such as taxol, activates the spindle assembly checkpoint (SAC), which in turn inhibits the APC/C. If cells can sustain APC/C inhibition, the caspase inhibitor MCL1 is phosphorylated and targeted for ubiquitylation by SCF^{FBW7}. Degradation of MCL1 leads to cell death. Cancer cells can bypass this protective mechanism by impeding SAC function, mutating FBW7, or overexpressing the DUB USP9X. These cells can eventually downregulate CDK1 activity and slip out of mitosis without undergoing apoptosis.

disease. Recent studies have identified molecules that target enzymes of all stages of the ubiquitylation cascade, including the E1 involved in the conjugation of NEDD8, the E2 UBE2R1, the E3s SCF^{CDC4} and APC/C, the DUB USP7, and the proteasome (Ceccarelli et al., 2011; Orlicky et al., 2010; Soucy et al., 2009; Wu, W. K. et al., 2010; Zeng et al., 2010). These molecules have provided insight into ubiquitin-dependent cell cycle control: for example, MLN4942, an inhibitor of the NEDD8 E1, or the UBE2R1 inhibitor CC0651 have underscored the importance of SCF for cell division and survival (Ceccarelli et al., 2011; Soucy et al., 2009). In addition, use of the the APC/C inhibitor TAME has confirmed that the APC/C triggers spindle checkpoint inactivation (Zeng et al., 2010), a concept that had previously been contested (Nilsson et al., 2008). Importantly, the fact that the proteasome inhibitor Velcade has been successful in treating multiple myeloma predicts that compounds against essential ubiquitylation enzymes will be useful tools in our armory against hyperproliferative diseases. We are optimistic that our increasing knowledge about ubiquitin-dependent mechanisms of cell cycle control will be translated into effective therapeutic strategies against cancer cells – namely cells that do not live up to the task of achieving accurate cell division – in the future.

Acknowledgements

We are grateful to Julia Schaletzky for many discussions and for comments on this manuscript. We thank all members of the Rape lab for their discussions and ideas.

Funding

Work in our laboratory is funded by grants from the National Institutes of Health (NIH Director's New Innovator Award) [grant number NIGMS RO1] and the March of Dimes Foundation. A.M. is a Siebel Fellow. Deposited in PMC for release after 12 months.

References

Abbas, T. and Dutta, A. (2011). CRL4Cdt2: master coordinator of cell cycle progression and genome stability. *Cell Cycle* **10**, 241-249.

Abbas, T., Sivaprasad, U., Terai, K., Amador, V., Pagano, M. and Dutta, A. (2008). PCNA-dependent regulation of p21 ubiquitylation and degradation via the CRL4Cdt2 ubiquitin ligase complex. *Genes Dev*. **22**, 2496-2506.

Abbas, T., Shibata, E., Park, J., Jha, S., Karnani, N. and Dutta, A. (2010). CRL4(Cdt2) regulates cell proliferation and histone gene expression by targeting PR-Set7/Set8 for degradation. *Mol. Cell* **40**, 9-21.

Arias, E. E. and Walter, J. C. (2006). PCNA functions as a molecular platform to trigger Cdt1 destruction and prevent re-replication. *Nat. Cell Biol*. **8**, 84-90.

Baboshina, O. V. and Haas, A. L. (1996). Novel multiubiquitin chain linkages catalyzed by the conjugating enzymes E2EPF and RAD6 are recognized by 26 S proteasome subunit 5. *J. Biol. Chem*. **271**, 2823-2831.

Bashir, T., Dorrello, N. V., Amador, V., Guardavaccaro, D. and Pagano, M. (2004). Control of the SCF(Skp2-Cks1) ubiquitin ligase by the APC/C(Cdh1) ubiquitin ligase. *Nature* **428**, 190-193.

Bennett, E. J., Shaler, T. A., Woodman, B., Ryu, K. Y., Zaitseva, T. S., Becker, C. H., Bates, G. P., Schulman, H. and Kopito, R. R. (2007). Global changes to the ubiquitin system in Huntington's disease. *Nature* **448**, 704-708.

Bennett, E. J., Rush, J., Gygi, S. P. and Harper, J. W. (2010). Dynamics of cullin-RING ubiquitin ligase network revealed by systematic quantitative proteomics. *Cell* **143**, 951-965.

Carroll, C. W. and Morgan, D. O. (2002). The Doc1 subunit is a processivity factor for the anaphase-promoting complex. *Nat. Cell Biol*. **4**, 880-887.

Ceccarelli, D. F., Tang, X., Pelletier, B., Orlicky, S., Xie, W., Plantevin, V., Neculai, D., Chou, Y. C., Ogunjimi, A., Al-Hakim, A. et al. (2011). An allosteric inhibitor of the human cdc34 ubiquitin-conjugating enzyme. *Cell* **145**, 1075-1087.

Centore, R. C., Havens, C. G., Manning, A. L., Li, J. M., Flynn, R. L., Tse, A., Jin, J., Dyson, N. J., Walter, J. C. and Zou, L. (2010). CRL4(Cdt2)-mediated destruction of the histone methyltransferase Set8 prevents premature chromatin compaction in S phase. *Mol. Cell* **40**, 22-33.

Chau, V., Tobias, J. W., Bachmair, A., Marriott, D., Ecker, D. J., Gonda, D. K. and Varshavsky, A. (1989). A multiubiquitin chain is confined to specific lysine in a targeted short-lived protein. *Science* **243**, 1576-1583.

Cunha-Ferreira, I., Rodrigues-Martins, A., Bento, I., Riparbelli, M., Zhang, W., Laue, E., Callaini, G., Glover, D. M. and Bettencourt-Dias, M. (2009). The SCF/Slimb ubiquitin ligase limits centrosome amplification through degradation of SAK/PLK4. *Curr. Biol*. **19**, 43-49.

D'Angiolella, V., Donato, V., Vijayakumar, S., Saraf, A., Florens, L., Washburn, M. P., Dynlacht, B. and Pagano, M. (2010). SCF(Cyclin F) controls centrosome homeostasis and mitotic fidelity through CP110 degradation. *Nature* **466**, 138-142.

da Fonseca, P. C., Kong, E. H., Zhang, Z., Schreiber, A., Williams, M. A., Morris, E. P. and Barford, D. (2011). Structures of APC/C(Cdh1) with substrates identify Cdh1 and Apc10 as the D-box co-receptor. *Nature* **470**, 274-278.

Deshaies, R. J. and Joazeiro, C. A. (2009). RING domain E3 ubiquitin ligases. *Annu. Rev. Biochem*. **78**, 399-434.

Duda, D. M., Borg, L. A., Scott, D. C., Hunt, H. W., Hammel, M. and Schulman, B. A. (2008). Structural insights into NEDD8 activation of cullin-RING ligases: conformational control of conjugation. *Cell* **134**, 995-1006.

Ganem, N. J., Godinho, S. A. and Pellman, D. (2009). A mechanism linking extra centrosomes to chromosomal instability. *Nature* **460**, 278-282.

Garnett, M. J., Mansfeld, J., Godwin, C., Matsusaka, T., Wu, J., Russell, P., Pines, J. and Venkitaraman. A. R. (2009). UBE2S elongates ubiquitin chains on APC/C substrates to promote mitotic exit. *Nat. Cell Biol*. **11**, 1363-1369.

Gerlach, B., Cordier, S. M., Schmukle, A. C., Emmerich, C. H., Rieser, E., Haas, T. L., Webb, A. I., Rickard, J. A., Anderton, H., Wong, W. W. et al. (2011). Linear ubiquitination prevents inflammation and regulates immune signalling. *Nature* **471**, 591-596.

Glotzer, M., Murray, A. W. and Kirschner, M. W. (1991). Cyclin is degraded by the ubiquitin pathway. *Nature* **349**, 132-138.

Guderian, G., Westendorf, J., Uldschmid, A. and Nigg, E. A. (2010). Plk4 trans-autophosphorylation regulates centriole number by controlling betaTrCP-mediated degradation. *J. Cell Sci*. **123**, 2163-2169.

Havens, C. G. and Walter, J. C. (2009). Docking of a specialized PIP Box onto chromatin-bound PCNA creates a degron for the ubiquitin ligase CRL4Cdt2. *Mol. Cell* **35**, 93-104.

Havens, C. G. and Walter, J. C. (2011). Mechanism of CRL4Cdt2, a PCNA-dependent E3 ubiquitin ligase. *Genes Dev*. **25**, 1568-1582.

Hetfeld, B. K., Helfrich, A., Kapelari, B., Scheel, H., Hofmann, K., Guterman, A., Glickman, M., Schade, R., Kloetzel, P. M. and Dubiel, W. (2005). The zinc finger of the CSN-associated deubiquitinating enzyme USP15 is essential to rescue the E3 ligase Rbx1. *Curr. Biol*. **15**, 1217-1221.

Holland, A. J., Lan, W., Niessen, S., Hoover, H. and Cleveland, D. W. (2010). Polo-like kinase 4 kinase activity limits centrosome overduplication by autoregulating its own stability. *J. Cell Biol*. **188**, 191-198.

Hsu, J. Y., Reimann, J. D., Sorensen, C. S., Lukas, J. and Jackson, P. K. (2002). E2F-dependent accumulation of hEmi1 regulates S phase entry by inhibiting APC(Cdh1). *Nat. Cell Biol*. **4**, 358-366.

Huang, X., Summers, M. K., Pham, V., Lill, J. R., Liu, J., Lee, G., Kirkpatrick, D. S., Jackson, P. K., Fang, G. and Dixit, V. M. (2011). Deubiquitinase USP37 is activated by CDK2 to antagonize APC(CDH1) and promote S phase entry. *Mol. Cell* **42**, 511-523.

Ikeda, F., Deribe, Y. L., Skanland, S. S., Stieglitz, B., Grabbe, C., Franz-Wachtel, M., van Wijk, S. J., Goswami, P., Nagy, V., Terzic, J. et al. (2011). SHARPIN forms a linear ubiquitin ligase complex regulating NF-kappaB activity and apoptosis. *Nature* **471**, 637-641.

Inuzuka, H., Shaik, S., Onoyama, I., Gao, D., Tseng, A., Maser, R. S., Zhai, B., Wan, L., Gutierrez, A., Lau, A. W. et al. (2011). SCF(FBW7) regulates cellular apoptosis by targeting MCL1 for ubiquitylation and destruction. *Nature* **471**, 104-109.

Jin, L., Williamson, A., Banerjee, S., Philipp, I. and Rape, M. (2008). Mechanism of ubiquitin-chain formation by the human anaphase-promoting complex. *Cell* **133**, 653-665.

Jorgensen, S., Eskildsen, M., Fugger, K., Hansen, L., Larsen, M. S., Kousholt, A. N., Syljuasen, R. G., Trelle, M. B., Jensen, O. N., Helin, K. et al. (2011). SET8 is degraded via PCNA-coupled CRL4(CDT2) ubiquitylation in S phase and after UV irradiation. *J. Cell Biol*. **192**, 43-54.

Jung, C. R., Hwang, K. S., Yoo, J., Cho, W. K., Kim, J. M., Kim, W. H. and Im, D. S. (2006). E2-EPF UCP targets pVHL for degradation and associates with tumor growth and metastasis. *Nat. Med*. **12**, 809-816.

Kaiser, S. E., Riley, B. E., Shaler, T. A., Trevino, R. S., Becker, C. H., Schulman, H. and Kopito, R. R. (2011). Protein standard absolute quantification (PSAQ) method for the measurement of cellular ubiquitin pools. *Nat. Methods* **8**, 691-696.

Kalab, P. and Heald, R. (2008). The RanGTP gradient-a GPS for the mitotic spindle. *J. Cell Sci*. **121**, 1577-1586.

Kim, S. H. and Michael, W. M. (2008). Regulated proteolysis of DNA polymerase eta during the DNA-damage response in C. elegans. *Mol. Cell* **32**, 757-766.

Kleylein-Sohn, J., Westendorf, J., Le Clech, M., Habedanck, R., Stierhof, Y. D. and Nigg, E. A. (2007). Plk4-induced centriole biogenesis in human cells. *Dev. Cell* **13**, 190-202.

Lee, J. E., Sweredoski, M. J., Graham, R. L., Kolawa, N. J., Smith, G. T., Hess, S. and Deshaies, R. J. (2011). The steady-state repertoire of human SCF ubiquitin ligase complexes does not require ongoing Nedd8 conjugation. *Mol. Cell. Proteomics* **10**, M110 006460.

Liakopoulos, D., Doenges, G., Matuschewski, K. and Jentsch, S. (1998). A novel protein modification pathway related to the ubiquitin system. *EMBO J*. **17**, 2208-2214.

Listovsky, T., Oren, Y. S., Yudkovsky, Y., Mahbubani, H. M., Weiss, A. M., Lebendiker, M. and Brandeis, M. (2004). Mammalian Cdh1/Fzr mediates its own degradation. *EMBO J*. **23**, 1619-1626.

Liu, J., Furukawa, M., Matsumoto, T. and Xiong, Y. (2002). NEDD8 modification of CUL1 dissociates p120(CAND1), an inhibitor of CUL1-SKP1 binding and SCF ligases. *Mol. Cell* **10**, 1511-1518.

Maerki, S., Olma, M. H., Staubli, T., Steigemann, P., Gerlich, D. W., Quadroni, M., Sumara, I. and Peter, M. (2009). The Cul3-KLHL21 E3 ubiquitin ligase targets aurora

B to midzone microtubules in anaphase and is required for cytokinesis. *J. Cell Biol*. **187**, 791-800.

Mathe, E., Kraft, C., Giet, R., Deak, P., Peters, J. M. and Glover, D. M. (2004). The E2-C vihar is required for the correct spatiotemporal proteolysis of cyclin B and itself undergoes cyclical degradation. *Curr. Biol*. **14**, 1723-1733.

Matsumoto, M. L., Wickliffe, K. E., Dong, K. C., Yu, C., Bosanac, I., Bustos, D., Phu, L., Kirkpatrick, D. S., Hymowitz, S. G., Rape, M. et al. (2010). K11-linked polyubiquitination in cell cycle control revealed by a K11 linkage-specific antibody. *Mol. Cell* **39**, 477-484.

Miniowitz-Shemtov, S., Teichner, A., Sitry-Shevah, D. and Hershko, A. (2010). ATP is required for the release of the anaphase-promoting complex/cyclosome from inhibition by the mitotic checkpoint. *Proc. Natl. Acad. Sci. USA* **107**, 5351-5356.

Mukhopadhyay, D. and Riezman, H. (2007). Proteasome-independent functions of ubiquitin in endocytosis and signaling. *Science* **315**, 201-205.

Murray, A. W., Solomon, M. J. and Kirschner, M. W. (1989). The role of cyclin synthesis and degradation in the control of maturation promoting factor activity. *Nature* **339**, 280-286.

Musacchio, A. and Salmon, E. D. (2007). The spindle-assembly checkpoint in space and time. *Nat. Rev. Mol. Cell Biol*. **8**, 379-393.

Neumann, B., Walter, T., Heriche, J. K., Bulkescher, J., Erfle, H., Conrad, C., Rogers, P., Poser, I., Held, M., Liebel, U. et al. (2010). Phenotypic profiling of the human genome by time-lapse microscopy reveals cell division genes. *Nature* **464**, 721-727.

Nilsson, J., Yekezare, M., Minshull, J. and Pines, J. (2008). The APC/C maintains the spindle assembly checkpoint by targeting Cdc20 for destruction. *Nat. Cell Biol*. **10**, 1411-1420.

Oda, H., Hubner, M. R., Beck, D. B., Vermeulen, M., Hurwitz, J., Spector, D. L. and Reinberg, D. (2010). Regulation of the histone H4 monomethylase PR-Set7 by CRL4(Cdt2)-mediated PCNA-dependent degradation during DNA damage. *Mol. Cell* **40**, 364-376.

Orlicky, S., Tang, X., Neduva, V., Elowe, N., Brown, E. D., Sicheri, F. and Tyers, M. (2010). An allosteric inhibitor of substrate recognition by the SCF(Cdc4) ubiquitin ligase. *Nat. Biotechnol*. **28**, 733-737.

Palframan, W. J., Meehl, J. B., Jaspersen, S. L., Winey, M. and Murray, A. W. (2006). Anaphase inactivation of the spindle checkpoint. *Science* **313**, 680-684.

Passmore, L. A., McCormack, E. A., Au, S. W., Paul, A., Willison, K. R., Harper, J. W. and Barford, D. (2003). Doc1 mediates the activity of the anaphase-promoting complex by contributing to substrate recognition. *EMBO J*. **22**, 786-796.

Petroski, M. D. and Deshaies, R. J. (2005a). Function and regulation of cullin-RING ubiquitin ligases. *Nat. Rev. Mol. Cell Biol*. **6**, 9-20.

Petroski, M. D. and Deshaies, R. J. (2005b). Mechanism of lysine 48-linked ubiquitin-chain synthesis by the cullin-RING ubiquitin-ligase complex SCF-Cdc34. *Cell* **123**, 1107-1120.

Pfleger, C. M. and Kirschner, M. W. (2000). The KEN box: an APC recognition signal distinct from the D box targeted by Cdh1. *Genes Dev*. **14**, 655-665.

Puklowski, A., Homsi, Y., Keller, D., May, M., Chauhan, S., Kossatz, U., Grunwald, V., Kubicka, S., Pich, A., Manns, M. P. et al. (2011). The SCF-FBXW5 E3-ubiquitin ligase is regulated by PLK4 and targets HsSAS-6 to control centrosome duplication. *Nat. Cell Biol*. **13**, 1004-1009.

Qi, W. and Yu, H. (2007). KEN-box-dependent degradation of the Bub1 spindle checkpoint kinase by the anaphase-promoting complex/cyclosome. *J. Biol. Chem*. **282**, 3672-3679.

Rahighi, S., Ikeda, F., Kawasaki, M., Akutsu, M., Suzuki, N., Kato, R., Kensche, T., Uejima, T., Bloor, S., Komander, D. et al. (2009). Specific recognition of linear ubiquitin chains by NEMO is important for NF-kappaB activation. *Cell* **136**, 1098-1109.

Ramadan, K., Bruderer, R., Spiga, F. M., Popp, O., Baur, T., Gotta, M. and Meyer, H. H. (2007). Cdc48/p97 promotes reformation of the nucleus by extracting the kinase Aurora B from chromatin. *Nature* **450**, 1258-1262.

Rape, M. and Kirschner, M. W. (2004). Autonomous regulation of the anaphase-promoting complex couples mitosis to S-phase entry. *Nature* **432**, 588-595.

Reddy, S. K., Rape, M., Margansky, W. A. and Kirschner, M. W. (2007). Ubiquitination by the anaphase-promoting complex drives spindle checkpoint inactivation. *Nature* **446**, 921-925.

Rodrigo-Brenni, M. C. and Morgan, D. O. (2007). Sequential E2s drive polyubiquitin chain assembly on APC targets. *Cell* **130**, 127-139.

Rogers, G. C., Rusan, N. M., Roberts, D. M., Peifer, M. and Rogers, S. L. (2009). The SCF Slimb ubiquitin ligase regulates Plk4/Sak levels to block centriole reduplication. *J. Cell Biol*. **184**, 225-239.

Rotin, D. and Kumar, S. (2009). Physiological functions of the HECT family of ubiquitin ligases. *Nat. Rev. Mol. Cell Biol*. **10**, 398-409.

Saha, A. and Deshaies, R. J. (2008). Multimodal activation of the ubiquitin ligase SCF by Nedd8 conjugation. *Mol. Cell* **32**, 21-31.

Schreiber, A., Stengel, F., Zhang, Z., Enchev, R. I., Kong, E. H., Morris, E. P., Robinson, C. V., da Fonseca, P. C. and Barford, D. (2011). Structural basis for the subunit assembly of the anaphase-promoting complex. *Nature* **470**, 227-232.

Schulman, B. A. and Harper, J. W. (2009). Ubiquitin-like protein activation by E1 enzymes: the apex for downstream signalling pathways. *Nat. Rev. Mol. Cell Biol*. **10**, 319-331.

Schwickart, M., Huang, X., Lill, J. R., Liu, J., Ferrando, R., French, D. M., Maecker, H., O'Rourke, K., Bazan, F., Eastham-Anderson, J. et al. (2010). Deubiquitinase USP9X stabilizes MCL1 and promotes tumour cell survival. *Nature* **463**, 103-107.

Scott, D. C., Monda, J. K., Grace, C. R., Duda, D. M., Kriwacki, R. W., Kurz, T. and Schulman, B. A. (2010). A dual E3 mechanism for Rub1 ligation to Cdc53. *Mol. Cell* **39**, 784-796.

Senga, T., Sivaprasad, U., Zhu, W., Park, J. H., Arias, E. E., Walter, J. C. and Dutta, A. (2006). PCNA is a cofactor for Cdt1 degradation by CUL4/DDB1-mediated N-terminal ubiquitination. *J. Biol. Chem*. **281**, 6246-6252.

Shibutani, S. T., de la Cruz, A. F. A., Tran, V., Turbyfill, W. J., 3rd, Reis, T., Edgar, B. A. and Duronio, R. J. (2008). Intrinsic negative cell cycle regulation provided by PIP box- and Cul4Cdt2-mediated destruction of E2f1 during S phase. *Dev. Cell* **15**, 890-900.

Sloper-Mould, K. E., Jemc, J. C., Pickart, C. M. and Hicke, L. (2001). Distinct functional surface regions on ubiquitin. *J. Biol. Chem*. **276**, 30483-30489.

Song, E. J., Werner, S, L., Neubauer, J., Stegmeier, F., Aspden, J., Rio, D., Harper, J, W., Elledge, S. J., Kirschner, M. W. and Rape, M. (2010). The Prp19 complex and the Usp4Sart3 deubiquitinating enzyme control reversible ubiquitination at the spliceosome. *Genes Dev*. **24**, 1434-1447.

Song, L. and Rape, M. (2010). Regulated degradation of spindle assembly factors by the anaphase-promoting complex. *Mol. Cell* **38**, 369-382.

Soucy, T. A., Smith, P. G., Milhollen, M. A., Berger, A. J., Gavin, J. M., Adhikari, S., Brownell, J. E., Burke, K. E., Cardin, D. P., Critchley, S. et al. (2009). An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. *Nature* **458**, 732-736.

Sowa, M. E., Bennett, E. J., Gygi, S. P. and Harper, J. W. (2009). Defining the human deubiquitinating enzyme interaction landscape. *Cell* **138**, 389-403.

Stegmeier, F., Rape, M., Draviam, V. M., Nalepa, G., Sowa, M. E., Ang, X. L., McDonald, E. R., 3rd, Li, M. Z., Hannon, G. J., Sorger, P. K. et al. (2007). Anaphase initiation is regulated by antagonistic ubiquitination and deubiquitination activities. *Nature* **446**, 876-881.

Stewart, S. and Fang, G. (2005a). Anaphase-promoting complex/cyclosome controls the stability of TPX2 during mitotic exit. *Mol. Cell. Biol*. **25**, 10516-10527.

Stewart, S. and Fang, G. (2005b). Destruction box-dependent degradation of aurora B is mediated by the anaphase-promoting complex/cyclosome and Cdh1. *Cancer Res*. **65**, 8730-8735.

Strnad, P., Leidel, S., Vinogradova, T., Euteneuer, U., Khodjakov, A. and Gonczy, P. (2007). Regulated HsSAS-6 levels ensure formation of a single procentriole per centriole during the centrosome duplication cycle. *Dev. Cell* **13**, 203-213.

Sullivan, M. and Morgan. D. O. (2007). Finishing mitosis, one step at a time. *Nat. Rev. Mol. Cell. Biol*. **8**, 894-903.

Sumara, I., Quadroni, M., Frei, C., Olma, M. H., Sumara, G., Ricci, R. and Peter, M. (2007). A Cul3-based E3 ligase removes Aurora B from mitotic chromosomes, regulating mitotic progression and completion of cytokinesis in human cells. *Dev. Cell* **12**, 887-900.

Summers, M. K., Pan, B., Mukhyala, K. and Jackson, P. K. (2008). The unique N terminus of the UbcH10 E2 enzyme controls the threshold for APC activation and enhances checkpoint regulation of the APC. *Mol. Cell* **31**, 544-556.

Tardat, M., Brustel, J., Kirsh, O., Lefevbre, C., Callanan, M., Sardet, C. and Julien, E. (2010). The histone H4 Lys 20 methyltransferase PR-Set7 regulates replication origins in mammalian cells. *Nat. Cell Biol*. **12**, 1086-1093.

Tedesco, D., Zhang, J., Trinh, L., Lalehzadeh, G., Meisner, R., Yamaguchi, K. D., Ruderman, D. L., Dinter, H. and Zajchowski, D. A. (2007). The ubiquitin-conjugating enzyme E2-EPF is overexpressed in primary breast cancer and modulates sensitivity to topoisomerase II inhibition. *Neoplasia* **9**, 601-613.

Teichner, A., Eytan, E., Sitry-Shevah, D., Miniowitz-Shemtov, S., Dumin, E., Gromis, J. and Hershko, A. (2011). p31comet promotes disassembly of the mitotic checkpoint complex in an ATP-dependent process. *Proc. Natl. Acad. Sci. USA* **108**, 3187-3192.

Thrower, J. S., Hoffman, L., Rechsteiner, M. and Pickart, C. M. (2000). Recognition of the polyubiquitin proteolytic signal. *EMBO J*. **19**, 94-102.

Tokunaga, F., Sakata, S., Saeki, Y., Satomi, Y., Kirisako, T., Kamei, K., Nakagawa, T., Kato, M., Murata, S., Yamaoka, S. et al. (2009). Involvement of linear polyubiquitylation of NEMO in NF-kappaB activation. *Nat. Cell Biol*. **11**, 123-132.

Tokunaga, F., Nakagawa, T., Nakahara, M., Saeki, Y., Taniguchi, M., Sakata, S., Tanaka, K., Nakano, H. and Iwai, K. (2011). SHARPIN is a component of the NF-kB activating linear ubiquitin chain assembly complex. *Nature* **471**, 633-636.

van Ree, J. H., Jeganathan, K. B., Malureanu, L. and van Deursen, J. M. (2010). Overexpression of the E2 ubiquitin-conjugating enzyme UbcH10 causes chromosome missegregation and tumor formation. *J. Cell Biol*. **188**, 83-100.

Wagner, K. W., Sapinoso, L. M., El-Rifai, W., Frierson, H. F., Butz, N., Mestan, J., Hofmann, F., Deveraux, Q. L. and Hampton, G. M. (2004). Overexpression, genomic amplification and therapeutic potential of inhibiting the UbcH10 ubiquitin conjugase in human carcinomas of diverse anatomic origin. *Oncogene* **23**, 6621-6629.

Wei, W., Ayad, N. G., Wan, Y., Zhang, G. J., Kirschner, M. W. and Kaelin, W. G., Jr (2004). Degradation of the SCF component Skp2 in cell-cycle phase G1 by the anaphase-promoting complex. *Nature* **428**, 194-198.

Wertz, I. E., Kusam, S., Lam, C., Okamoto, T., Sandoval, W., Anderson, D. J., Helgason, E., Ernst, J. A., Eby, M., Liu, J. et al. (2011). Sensitivity to antitubulin chemotherapeutics is regulated by MCL1 and FBW7. *Nature* **471**, 110-114.

Whitfield, M. L., Sherlock, G., Saldanha, A. J., Murray, J. I., Ball, C. A., Alexander, K. E., Matese, J. C., Perou, C. M., Hurt, M. M., Brown, P. O. et al. (2002). Identification of genes periodically expressed in the human cell cycle and their expression in tumors. *Mol. Biol. Cell* **13**, 1977-2000.

Wickliffe, K. E., Lorenz, S., Wemmer, D. E., Kuriyan, J. and Rape, M. (2011). The mechanism of linkage-specific ubiquitin chain elongation by a single-subunit e2. *Cell* **144**, 769-781.

Williamson, A., Wickliffe, K. E., Mellone, B. G., Song, L., Karpen, G. H. and Rape, M. (2009). Identification of a physiological E2 module for the human anaphase-promoting complex. *Proc. Natl. Acad. Sci. USA* **106**, 18213-18218.

Williamson, A., Banerjee, S., Zhu, X., Philipp, I., Iavarone, A. T. and Rape, M. (2011). Regulation of ubiquitin chain initiation to control the timing of substrate degradation. *Mol. Cell* **42**, 744-757.

Wu, T., Merbl, Y., Huo, Y., Gallop, J. L., Tzur, A. and Kirschner, M. W. (2010). UBE2S drives elongation of K11-linked ubiquitin chains by the anaphase-promoting complex. *Proc. Natl. Acad. Sci. USA* **107**, 1355-1360.

Wu, W. K., Cho, C. H., Lee, C. W., Wu, K., Fan, D., Yu, J. and Sung, J. J. (2010). Proteasome inhibition: a new therapeutic strategy to cancer treatment. *Cancer Lett*. **293**, 15-22.

Xia, G., Luo, X., Habu, T., Rizo, J., Matsumoto, T. and Yu, H. (2004). Conformation-specific binding of p31(comet) antagonizes the function of Mad2 in the spindle checkpoint. *EMBO J*. **23**, 3133-3143.

Xu, P., Duong, D. M., Seyfried, N. T., Cheng, D., Xie, Y., Robert, J., Rush, J., Hochstrasser, M., Finley, D. and Peng, J. (2009). Quantitative proteomics reveals the function of unconventional ubiquitin chains in proteasomal degradation. *Cell* **137**, 133-145.

Ye, Y. and Rape, M. (2009). Building ubiquitin chains: E2 enzymes at work. *Nat. Rev Mol. Cell. Biol*. **10**, 755-764.

Young, L. M. and Pagano, M. (2010). Cdc25 phosphatases: differential regulation by ubiquitin-mediated proteolysis. *Cell Cycle* **9**, 4613-4614.

Zeng, X., Sigoillot, F., Gaur, S., Choi, S., Pfaff, K. L., Oh, D. C., Hathaway, N., Dimova, N., Cuny, G. D. and King, R. W. (2010). Pharmacologic inhibition of the anaphase-promoting complex induces a spindle checkpoint-dependent mitotic arrest in the absence of spindle damage. *Cancer Cell* **18**, 382-395.

Zhang, Y., van Deursen, J. and Galardy, P. J. (2011). Overexpression of Ubiquitin Specific Protease 44 (USP44) induces chromosomal instability and is frequently observed in human T-cell leukemia. *PLoS ONE* **6**, e23389.

Zhong, W., Feng, H., Santiago, F. E. and Kipreos, E. T. (2003). CUL-4 ubiquitin ligase maintains genome stability by restraining DNA-replication licensing. *Nature* **423**, 885-889.

Ziv, I., Matiuhin, Y., Kirkpatrick, D. S., Erpapazoglou, Z., Leon, S., Pantazopoulou, M., Kim, W., Gygi, S. P., Haguenauer-Tsapis, R., Reis, N. et al. (2011). A perturbed ubiquitin landscape distinguishes between ubiquitin in trafficking and in proteolysis. *Mol. Cell. Proteomics* **10**, M111 009753.

Journal of Cell Science