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Biochim Biophys Acta. Author manuscript; available in PMC 2014 July 01.

Published in final edited form as:
Biochim Biophys Acta. 2014 January ; 1843(1): . doi:10.1016/j.bbamcr.2013.02.028.

The ubiquitin proteasome system – Implications for cell cycle control and the targeted treatment of cancer

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Abstract

Two families of E3 ubiquitin ligases are prominent in cell cycle regulation and mediate the timely and precise ubiquitin-proteasome-dependent degradation of key cell cycle proteins: the SCF (Skp1/Cul1/F-box protein) complex and the APC/C (Anaphase Promoting Complex or Cyclosome). While certain SCF ligases drive cell cycle progression throughout the cell cycle, APC/C (in complex with either of two substrate recruiting proteins: Cdc20 and Cdh1) orchestrates exit from mitosis (APC/C^{Cdc20}) and establishes a stable G1 phase (APC/C^{Cdh1}). Upon DNA damage or perturbation of the normal cell cycle, both ligases are involved in checkpoint activation. Mechanistic insight into these processes has significantly improved over the last ten years, largely due to a better understanding of APC/C and the functional characterization of multiple F-box proteins, the variable substrate recruiting components of SCF ligases. Here, we review the role of SCF- and APC/C-mediated ubiquitylation in the normal and perturbed cell cycle and discuss potential clinical implications of SCF and APC/C functions.

Keywords

Ubiquitin proteasome system; cell cycle; SCF complex; F-box; APC/C; cancer

1. Introduction

The mammalian cell cycle is a strictly regulated process controlled by the oscillating activities of cyclin-dependent kinases (CDKs), which are activated by cyclins and inhibited by CDK inhibitors (CKIs). Many cyclins and CDKs have been described, with each cyclin associating with one or more CDKs, and most CDKs interacting with one or more cyclins [1]. The prototypical cyclins driving cell cycle progression are cyclin A, B, and E, which are expressed in a cell cycle dependent manner and associate with Cdk1 or Cdk2 to mediate downstream events, such as DNA replication and mitosis. Specifically, cyclin E-Cdk2 is activated in late G_1 phase to promote S-phase entry and DNA replication, cyclin A-Cdk2

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and cyclin A-Cdk1 are involved in S-phase progression and the G_2/M transition respectively, while cyclin B-Cdk1 is activated to promote mitotic entry.

This oscillating activity of CDKs is regulated by diverse mechanisms, starting from the transcriptional and translational level, to posttranslational modifications (phosphorylation in particular), and the periodic degradation of cyclins and CKIs by the ubiquitin-proteasome system [2, 3]. Contrary to reversible modifications, such as phosphorylation or association with CKIs, ubiquitin-mediated proteasomal degradation is an irreversible mechanism that assures the strict unidirectionality of the cell cycle, and it plays a key role in cell cycle regulation by mediating the precise spatial and temporal proteolysis of the main components of the cell cycle machinery.

Ubiquitylation, the covalent attachment of the small 76-amino-acid polypeptide ubiquitin to a target protein, occurs through a three-step enzymatic cascade. Ubiquitin is first bound and activated by the E1 ubiquitin activating enzyme in an ATP dependent manner. It is subsequently transferred to the E2 ubiquitin conjugating enzyme, before the E3 ubiquitin ligase enzyme specifically binds the substrate protein to mediate the transfer of ubiquitin to a lysine residue in the target [4]. Several rounds of ubiquitin conjugation produce long ubiquitin chains in which each ubiquitin molecule is covalently bound to a specific lysine of the previous ubiquitin moiety (polyubiquitylation). In the case of polyubiquitylation via lysine 48 or lysine 11, the polyubiquitylated substrate is committed to association with, and subsequent degradation by, the 26S proteasome. However, there are different types of ubiquitylation, which can lead to other molecular consequences, depending on the nature of the E2. Indeed, substrates can be monoubiquitylated or polyubiquitylated through five other internal lysine residues (K6, K27, K29, K33, and K63) or the N-terminus of ubiqutin [5–7]. While monoubiquitylation or K63-linked polyubiquitylation specify non-proteolytic fates for a substrate, the implications of other chain linkages are less understood [5, 7, 8]. Ubiquitylation itself can be reversed by specific deubiquitinating enzymes (DUBs) leading to a model in which ubiquitylation is controlled by the balance between an E3 ligase and a DUB [9].

To allow for high specificity in the ubiquitylation of target proteins, more than 600 different E3 ligases exist in the human genome [10]. Depending on their homology domains, these ligases can be subdivided in two major classes, the HECT (Homologous to E6-AP C-Terminus) family E3 ligases and the RING (Really Interesting New Gene) family E3 ligases [11]. The approximately 30 different HECT ligases form transient covalent linkages with ubiquitin during the ubiquitylation process, while RING ligases only mediate the transfer of ubiquitin from the E2 directly to the substrate. RING E3 ligases can be further subcategorized into those in which one protein contains both the RING and the substrate adaptor domain or multi-subunit complexes, in which these domains are part of distinct proteins within the complex [11]. One of the best described E3 families within the multi-subunit RING ligases is the cullin RING ligase (CRL) superfamily [12], which includes the CRL1 (better known as SCF, standing for Skp1-Cul1-F-box protein complex) and the APC/C (anaphase promoting complex/cyclosome, which is also referred to as a CRL-like ligase) E3 ligases.

The SCF complex consists of Cul1, the scaffold of the complex, Rbx1, the RING protein, which eventually recruits the E2, and Skp1 (S-phase kinase associated protein 1), which serves as an adaptor to bind the F-box protein, the substrate-binding subunit (Fig. 1) [12–14]. F-box proteins are named after the F-box domain, a 40-amino-acid motif initially identified in cyclin F (aka Fbxo1), which binds Skp1 [15]. They contain an additional motif, which constitutes the substrate binding domain. Depending on these domains, they are classified into three groups. F-box proteins containing WD-40 domains are called Fbxws,

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while those with leucine-rich repeats are called Fbxls. The remaining F-box proteins, with diverse motifs (*e.g.*, tetratrico peptide repeats, kelch repeats, proline-rich motifs) are named Fbxos [13, 16, 17].

Sixty-nine different F-box proteins are encoded by the human genome, and individual F-box proteins recognize different substrates. Therefore, these proteins determine the broad functional bandwidth of SCF complexes. Importantly, specific substrates or biological activities have so far been assigned to only a few F-box proteins, and each of these play a key role in essential cellular processes, such as cell cycle control, apoptosis, DNA damage responses, gene transcription, or translation [3, 18].

The APC/C is a prominent E3 ubiquitin ligase involved in cell cycle regulation. It consists of thirteen different subunits and, in somatic cells, has two co-activators: Cdc20 and Cdh1 (also known as Fzr1), which define substrate specificity and associate with the APC/C core at defined stages of the cell cycle (Fig. 1) [19]. While SCF ligases can be active throughout the cell cycle, APC/C activity is restricted to the time between metaphase and the end of the next G_1, and during this time, APC/C^{Cdc20} initiates anaphase and mitotic exit, while APC/C^{Cdh1} contributes to mitotic exit and establishment of a stable G_1 state [3, 20–22].

In the following sections, we outline the current understanding of how SCF and APC/C E3 ligases regulate cell cycle progression, their response to environmental cues in each phase of the cell cycle, and potential clinical and therapeutic implications of SCF and APC/C biology.

2. The G_0 and G_1 phases

2.1 Quiescence and G_1

In response to mitogen withdrawal (or after exit from mitosis), a stable G_0 or G_1 state has to be established. The APC/C^{Cdh1} complex is the central player in this task, and it is activated in late mitosis to complete mitotic exit and reset the cell cycle by targeting a variety of proteins involved in DNA replication, cycle progression, and mitosis. Among the targets that promote DNA replication is Cdc6, which binds to the origin recognition complex (ORC) to form pre-replication complexes (preRCs). Cdc6 is kept at low levels during early G_1 through APC/C^{Cdh1}-mediated degradation (Fig. 2A) [23]. APC/C^{Cdh1} also negatively regulates pro-proliferative signal transduction by targeting Ets2 (Fig. 2A), a transcription factor involved in Ras-Raf-MAPK signaling that induces the expression of cyclin D1 [24, 25]. APC/C^{Cdh1} activity also precludes early accumulation of positive cell cycle regulators [21, 26]. Specifically, Skp2 (S phase kinase-associated protein, aka Fbl1) is targeted for proteolysis to avert premature formation of the SCF^{Skp2} complex, which promotes cell cycle progression by mediating the degradation of CKIs (see below and Fig. 2A) [26–28]. Importantly, the Cdc25A phosphatase, which promotes S-phase entry and mitosis by dephosphorylating and thus activating Cdk2 and Cdk1, is also kept at low levels during early G by APC/C^{Cdh1} (Fig. 2A) [29]. Finally, APC/C^{Cdh1} targets mitotic proteins for proteasomal degradation [19–21, 30]. Among the most prominent targets are cyclin A and cyclin B (Fig. 2A), the main drivers of mitotic entry [31, 32]. Furthermore, Aurora A, Aurora B, and polo-like kinase 1 (Plk1), important mitotic kinases involved in centrosome maturation, spindle assembly, and chromosome separation, are additional targets of APC/C^{Cdh1} (Fig. 2A) [33–36].

Hence, by targeting protooncogenic proteins, such as Skp2 and Ets2, APC/C^{Cdh1} exerts an anti-proliferative activity. In fact, APC/C^{Cdh1} acts as a tumor suppressor in mice, where *Fzr1* heterozygous mice have elevated rates of spontaneous tumor formation [37].

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In response to mitogen withdrawal, cells must also switch to a state of nutritional saving and restrict protein translation and cell growth to sustain survival. SCF^{Fbxo9} contributes to this process via differential regulation of mTORC1 and mTORC2 signaling. Specifically, the Tel2 (telomere maintenance 2) and Tti1 (Tel2 interacting protein 1) proteins, integral components of both the mTORC1 and mTORC2 complexes, are phosphorylated by CKII to target them for degradation by SCF^{Fbxo9} upon growth factor withdrawal. This pathway specifically targets Tel2 and Tti1 in the mTORC1 complex (Fig. 2B) [38]. As a consequence, mTORC1 signaling is attenuated to restrain protein translation and cell growth, but via relief of mTORC1-mediated feedback inhibition, mTORC2 signaling is sustained to promote survival [38].

2.2 The growth factor response

If growth factors and nutrients are available, cells respond with a number of events that lead to growth and, eventually, cell division. Certain SCF ligases are acutely involved in the orchestration of growth factor responses during G_1 phase. For example, the tumor suppressor PDCD4 (programmed cell death protein 4), which inhibits the translation initiation factor eIF4A, is degraded in response to mitogens in a two-step mechanism. First, PDCD4 is phosphorylated on serine 67 (and possibly adjacent serine residues) by S6K1, and it is subsequently ubiquitylated by $SCF^{\beta TrCP}$ (Fig. 2B), resulting in increased protein synthesis and cell growth [39]. In response to mitogens and survival signals, $SCF^{\beta TrCP}$ furthermore promotes survival by targeting the proapoptotic BH3-only protein BimEL for degradation in an Rsk1/2- and Erk1/2-dependent manner (Fig. 2B) [40]. Another important target of $SCF^{\beta TrCP}$ in growth factor responses is the mTOR inhibitor DEPTOR (Fig. 2B). After mitogen stimulation, DEPTOR is phosphorylated by mTORC1 or mTORC2 and CK1α, which directs ubiquitylation and degradation, relieving mTOR inhibition [41, 42]. Thus, in cooperation with $SCF^{\beta TrCP}$, mTOR signaling promotes further mTOR activation via this auto-amplification loop.

2.3 Post-restriction point G_1 and the G_1 to S phase transition

With sufficient mitogen stimulation and cell growth, cells commit to cell division, passing a point of G_1 known as “restriction point”, after which cells will proceed through a round of cell division even if mitogens are removed. The transition from G_1 to S phase results from decreasing APC/C^{Cdh1} activity, decreasing CKIs levels, rising cyclin expression, increasing CDK activity, phosphorylation and inactivation of Rb (retinoblastoma) protein family members, and activation of E2F transcription factor family members.

Inactivation of APC/C^{Cdh1} during progression through the late G_1 phase occurs via different mechanisms. First, UbcH10, the APC/C specific E2 ubiquitin conjugating enzyme, is ubiquitylated by APC/C^{Cdh1} (Fig. 2C), thereby providing a negative feedback loop limiting APC/C^{Cdh1} activity [43]. Likewise, Cdh1 initiates autoubiquitylation within APC/C (Fig. 2C), thus limiting its activity [44]. Furthermore, rising levels of CDK activity lead to phosphorylation of Cdh1, disrupting its binding to APC/C (Fig. 2C) [3, 45]. Later, in S-Phase, an unidentified SCF complex contributes to Cdh1 degradation (Fig. 2C), either directly or indirectly, and this process may depend on previous Cdh1-phosphorylation [46]. Finally, at the G_1/S transition, the transcription factor E2F induces expression of Emi1 (an F-box protein also known as Fbxo5), which binds to APC/C^{Cdh1} as a pseudosubstrate inhibitor, inhibiting APC/C^{Cdh1} throughout S and G_2 (Fig. 2C) [47, 48].

Rising CDK activity during G_1 phase occurs through several different mechanisms. Primarily, the expression of cyclins is induced on a transcriptional level, with cyclin E and cyclin A expression being initiated by E2F (Fig. 2C) [49, 50]. However, the majority of cyclin-Cdk complexes are inhibited via association with CDK inhibitors (CKIs), such as p21

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or p27. In late G_1, Cdk4 and Cdk6 bound to cyclin D1 catalyze the phosphorylation (and consequent inactivation) of Rb family proteins (pRb, p107, and p130), which bind and inhibit E2F transcription factors, preventing their promotion of cell cycle progression. With decreasing APC/C^{Cdh1} activity in late G_1, Skp2 accumulates and forms the SCF^{Skp2} complex (Fig. 2C), which targets cell cycle inhibitors for degradation, promoting cell cycle progression throughout S, G_2, and M [22]. Importantly, SCF^{Skp2} binds p27 and mediates its proteasomal degradation, liberating cyclin E-Cdk2 from p27 inhibition (Fig. 2C) [51–54]. Ubiquitylation of p27 depends on phosphorylation of threonine 187 by cyclin E-Cdk2 and the co-factor Cks1 [55–59]. Other CKIs ubiquitylated by SCF^{Skp2} include p21 and p57, whose degradation further reinforces cyclin-Cdk1/2 activation [60, 61]. By analogy to p27, phosphorylation by cyclinECdk2 and binding of Cks1 are likely prerequisites for SCF^{Skp2} dependent ubiquitiylation of p21 and p57 [60, 61]. These functions distinguish Skp2 as an E3 ligase with oncogenic properties, which is further supported by the frequent overexpression of Skp2 in many tumors [62].

3. S and G_2 Phases of the cell cycle

3.1 S phase

Cyclin E-Cdk2 and (later) cyclin A-Cdk2 are the two main CDK complexes in S phase and mediate the initiation of DNA and centrosome duplication. At the same time, cyclin A-Cdk2 further inhibits APC/C^{Cdh1} via Cdh1 phosphorylation to ensure cyclin stability (Fig. 3A) [43]. Upon successful S-phase entry, cyclin D1 is phosphorylated by GSK3β, targeting it for degradation by an SCF ligase containing Fbxo4 and the small heat-shock protein α/B-crystallin (the latter being a substrate adaptor) [63]. As cells progress through S and approach G_2, the levels of S phase cyclins decrease again. Notably, cyclin E abundance is regulated via a negative feedback loop. Specifically, as Cdk2-activity increases, Cdk2-bound cyclin E is phosphorylated on threonine 380 in a Cdk2-dependent manner, whereupon phosphorylated cyclin E is targeted for degradation by the SCF^{Fbw7} complex (Figs. 2C and 3A) [64, 65].

3.2 DNA damage checkpoint

During S and G_2 phases, it is crucial for cells to assure accurate and complete DNA replication. Therefore, DNA damage detection mechanisms and checkpoints provide precise surveillance. Upon DNA damage, ATM activates the checkpoint kinase Chk2 and ATR activates Chk1 in a Claspin-dependent process (Fig. 4). Chk1 and Chk2 then mediate inhibitory phosphorylation of Cdc25A on serine 216 [66, 67]. Cdc25A promotes cell cycle activation by virtue of its ability to remove inhibitory phosphorylations from Cdk1 and Cdk2, but Cdc25A phosphorylation by Chk1/2 results in ubiquitylation by SCF$^{\beta TrCP}$ and degradation, attenuating CDK activity (Fig. 4) [68]. Degradation of Cdc25A leads to a pause in cell cycle progression during S or G_2, depending on the timing of the DNA damage.

Another target of the DNA damage checkpoint in G_2 phase is Plk1 [69, 70]. In response to DNA damage, the phosphatase Cdc14B is released from the nucleolus to the nucleoplasm, where it dephosphorylates Cdh1 (Fig. 4). As a consequence, APC/C^{Cdh1} becomes reactivated to target Plk1 for degradation (Fig. 4). In this way, Claspin and Wee1 are stabilized to activate the checkpoint and inhibit cell cycle progression (Fig. 4) [70]. Notably, other known targets of APC/C^{Cdh1} are likely protected during this process by DUBs, particularly USP28 [70, 71].

SCF$^{\beta TrCP}$ also plays a role during recovery from the checkpoint. Claspin, which mediates ATR-induced activation of Chk1 upon DNA damage, is phosphorylated by Plk1 during checkpoint recovery and subsequently degraded in an SCF$^{\beta TrCP}$ dependent manner [72, 73]. As a consequence, activation of Chk1 declines, and the cell recovers from the checkpoint.

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Moreover, $SCF^{\beta TrCP}$ also regulates translation during the silencing of the G_2 DNA damage checkpoint. In response to genotoxic stress, AMPK (Adenosine Monophosphate-activated Protein Kinase) phosphorylates and activates eEF2K (eukaryotic Elongation Factor 2 Kinase), which, in turn, inhibits elongation by phosphorylating and inhibiting eEF2, a protein that mediates movement of the ribosome along mRNA [74]. During checkpoint silencing, $SCF^{\beta TrCP}$ targets eEF2K for degradation to enable rapid resumption of translation elongation [74].

3.3 G_2 phase and G_2/M transition

After successful and accurate completion of DNA replication, production of deoxyribonucleotides (dNTPs) must be avoided. To this end, RRM2 (Ribonucleotide Reductase family Member 2), which converts ribonucleotides to dNTPs for DNA synthesis, is phosphorylated on threonine 33 by CDKs and ubiquitylated by the $SCF^{CyclinF}$ complex, reducing the availability of dNTPs (Fig. 3A) [75]. In contrast, cyclin F is downregulated in response to DNA damage in an ATR-dependent manner to stabilize RRM2 (Fig. 4). By this means, production of dNTPs is enhanced to allow for efficient DNA repair [75].

Beyond DNA replication, $SCF^{CyclinF}$ also regulates centrosome duplication. Cyclin F and CP110, a protein essential for centrosome duplication, physically associate on the centrioles during G_2 [76]. By targeting CP110 for proteasomal degradation (Fig. 3A), $SCF^{CyclinF}$ assures that centrosomes are replicated only once during the cell cycle, preventing centrosome overduplication, which could lead to multipolar or asymmetric mitotic spindles and subsequent chromosome aberrations [76].

With progression through G_2 phase, the cell prepares for mitotic entry, which is orchestrated by cyclin B-Cdk1. Therefore, cyclin B-Cdk1 is a strictly regulated kinase complex. In late G_2 phase, $SCF^{\beta\text{-}TrCP}$ promotes activation of Cdk1 by mediating the degradation of Wee1, a Cdk1 inhibitory kinase (Fig. 3B). A prerequisite for $SCF^{\beta\text{-}TrCP}$ binding to Wee1 is phosphorylation of serine 53 and serine 123 by Plk1 and Cdk1, respectively [77]. Thus, a positive feedback loop between Cdk1 activation and Wee1 degradation assures rapid activation of Cdk1 upon mitotic entry (Fig. 3B). The activity of Plk1 itself is also tightly controlled through the synergistic actions of Bora and Aurora A kinase. Accumulation of Bora during G_2 leads to Aurora A-mediated activation of Plk1, which contributes to Wee1 degradation and subsequent activation of Cdk1 (Fig. 3B) [78].

To preclude premature mitotic entry, early activation of cyclin B-Cdk1 must be prevented. To this end, SCF^{NIPA} targets nuclear cyclin B1 during S and G_2 (Fig. 3A) [79]. However, in late G_2, NIPA is phosphorylated at serine 395 by cyclin B-Cdk1, after initial phosphorylation on serine 354 and serine 359 by Erk2 (Fig. 3B) [80, 81]. Phosphorylation inhibits assembly of a functional SCF^{NIPA} complex, allowing nuclear accumulation of cyclin B1 and subsequent mitotic entry. In this context, cyclin B1 regulates its own abundance through a positive feedback loop (Fig. 3B).

4. Mitosis

4.1 Prometaphase and spindle assembly checkpoint

Cyclin B-Cdk1 orchestrates essential steps of early mitosis, including the assembly of the mitotic spindle, breakdown of the nuclear envelope, cessation of gene transcription, and condensation of chromosomes. These processes drive the cell through prophase until metaphase.

Starting from metaphase, the APC/C becomes a key player in promoting mitotic progression and, eventually, mitotic exit. Thus, inhibitory mechanisms that keep the APC/C in check

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throughout S- and G_2 phases must be removed. In this regard, $SCF^{\beta TrCP}$ mediates ubiquitylation and degradation of the APC/C inhibitor Emi1 following phosphorylation by cyclin B-Cdk1 and Plk1 in early mitosis (Fig. 5A) [82, 83]. However, until all kinetochores are correctly attached to spindle fibers and the chromosomes are properly aligned on the metaphase plate, the Spindle Assembly Checkpoint (SAC) inhibits APC/C activation, blocking the onset of anaphase [84]. In the presence of unattached kinetochores, the mitotic checkpoint proteins, BubR1, Bub3, and Mad2, bind to Cdc20 to form the Mitotic Checkpoint Complex (MCC), which sequesters Cdc20 and directly inhibits activation of the APC/C^{Cdc20} (Fig. 5A) [84–86]. Another model suggests that Cdc20 is targeted for degradation by APC/C in an MCC-dependent manner during SAC activation [87, 88]. Furthermore, inhibitory phosphorylation of Cdc20 by protein kinases, such as Cdk1 (Fig. 5A), MAPK, or Bub1, contributes to Cdc20 inhibition during SAC activation [89–91]. Interestingly, the transcriptional activation of Mad2 is also regulated by $SCF^{\beta TrCP}$. During late G_2, $SCF^{\beta TrCP}$ targets REST (Repressor-Element-1-Silencing Transcription factor) for degradation, allowing transcriptional derepression of Mad2 (Fig. 5A) [92].

Despite the many mechanisms keeping APC/C^{Cdc20} in check, its activity is not completely inhibited; a minor fraction remains active even during SAC activation. This subpopulation sustains cyclin B-Cdk1 activity during prometaphase by targeting p21 for degradation (Fig. 5A) [93]. Although p21 is degraded via SCF^{Skp2} at the G_1/S transition, it re-accumulates during G_2 and interacts with cyclin B-Cdk1 before it is ubiquitylated by APC/C^{Cdc20}. Interestingly, APC/C^{Cdc20} and APC/C^{Cdh1} have opposing effects on p21 levels. While p21 is degraded via APC/C^{Cdc20} in early mitosis, APC/C^{Cdh1} indirectly stabilizes p21 in G_1 phase by targeting Skp2 and Cdc20 for degradation [27, 94, 95]. In addition, APC/C^{Cdc20} targets two other substrates for degradation in early mitosis, cyclin A and Nek2A, even when the spindle checkpoint is active. How the checkpoint-resistant activity of the APC/C^{Cdc20} is regulated remains unclear. Potential mechanisms include Cdc20-independent binding of substrates to APC10 or an increase in substrate affinity for Cdc20, which allows substrates to outcompete SAC proteins [96–98].

4.2 Metaphase to anaphase transition and mitotic exit

Upon proper attachment of all spindle microtubules to chromosome kinetochores, the SAC is rapidly inactivated, and APC/C^{Cdc20} is released from inhibition via an uncertain mechanism (Fig. 5B). Checkpoint inactivation depends on Cdc20-dependent ubiquitylation. According to some, this leads to dissociation of the MCC from APC/C^{Cdc20}, but others suggest that it causes degradation of Cdc20 [99, 100]. An additional, ATP-dependent step is also required for both dissociation of the MCC from APC/C and disassembly of the free MCC [101, 102]. This step has been shown to involve β–γ bond cleavage of ATP, suggesting the presence of an additional ubiquitin independent process, which may be kinase- or chaperone-related [102]. The understanding of SAC inactivation is further complicated by the fact that Cdc20 proteolysis is also stimulated by Mad2 and BubR1 to keep levels of Cdc20 low in order to avoid premature activation of APC/C and maintain the checkpoint [87, 88]. Eventually, after kinetochore attachment, activated APC/C^{Cdc20} initiates anaphase by targeting Securin for degradation (Fig. 5B) [20, 103, 104]. During metaphase, sister chromatids are held together by Cohesin. Securin is a chaperone that binds and inhibits Separase, an enzyme capable of cleaving Cohesin. With Securin degradation, Separase is released, and centromeric Cohesin is cleaved, which leads to sister chromatid segregation (Fig. 5B) [105]. Moreover, Shugoshin, a protector of centromeric sister chromatid cohesion, is targeted for degradation by APC/C^{Cdh1}; however, this step is not indispensable for chromatid segregation and seems more important during meiosis [106, 107].

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Both mitotic cyclins, cyclin A and cyclin B, are targeted for degradation by the APC/C (Fig. 5B) [108]. Whereas cyclin A degradation starts in prometaphase, cyclin B ubiquitylation starts at the metaphase/anaphase transition, mediated first by APC/C^{Cdc20} and later by APC/C^{Cdh1} [109–111]. Notably, APC/C^{Cdc20} has different effects on Cdk1 activity depending on the mitotic phase. While it augments cyclin B-Cdk1 activity during prometaphase via degradation of p21 (Fig. 5A), it suppresses cyclin B-Cdk1 via degradation of cyclin B in late mitosis (Fig. 5B) [93]. Mitotic cyclin-CDK activity inhibits Separase, so the degradation of cyclin A and cyclin B by APC/C^{Cdc20} is directly involved in sister chromatid separation (Fig. 5B) [20]. Furthermore, drastically reduced CDK activity in anaphase allows for disassembly of the mitotic spindle, chromosome decondensation, cytokinesis, and reconstitution of the nuclear envelope [20].

Although APC/C is the most prominent E3 ubiquitin ligase orchestrating the metaphase to anaphase transition, SCF ubiquitin ligases are also involved in the regulation of anaphase onset. For instance, Bora, which works together with Plk1 and Aurora A to regulate microtubule polymerization, spindle stability, and tension between kinetochores, is targeted for degradation by $SCF^{\beta TrCP}$ at the metaphase to anaphase transition (Fig. 5A) [112, 113]. Interestingly, a prerequisite for Bora ubiquitylation is phosphorylation by Plk1, which itself is activated in a Bora-dependent manner at the G_2/M transition [78, 112]. Timely activation and degradation of Bora assures regulated cell cycle progression, as inhibition of Bora degradation delays anaphase onset and knockdown of Bora activates the spindle checkpoint [112, 113].

In late mitosis, Aurora A and Plk1 are eventually targeted for degradation via the APC/C^{Cdh1} to promote mitotic exit (Fig. 5B) [33, 34, 36]. Another important substrate of APC/C in late mitosis is Geminin (Fig. 5B), which inhibits DNA re-replication by precluding formation of the pre-replication complex (pre-RC) after S-phase, rendering replication possible during the subsequent cell cycle [114].

Overall, the APC/C is the central E3 ligase that coordinates mitotic progression, mitotic exit, and the subsequent establishment of a G_1 state. While anaphase onset is induced by APC/C^{Cdc20}, both APC/C^{Cdc20} and APC/C^{Cdh1} orchestrate mitotic exit, and APC/C^{Cdh1} contributes to establishment and maintenance of the G_1 phase of the next cell cycle. This distinct activation profile is the result of different regulatory steps. As mentioned earlier, Cdh1 is kept inactivated via phosphorylation by CDKs during S, G_2 and part of mitosis [115]. Degradation of cyclin B reduces CDK activity at the metaphase to anaphase transition, so the levels of dephosphorylated Cdh1 rise. Dephosphorylated Cdh1 binds APC/C and negatively regulates APC/C^{Cdc20} by targeting Cdc20 for degradation (Fig. 5B) [94, 95]. Moreover, APC/C dependent autoubiquitylation of Cdc20 in anaphase has been proposed to further contribute to the inactivation of APC/C^{Cdc20} (Fig. 5B) [99, 100]. The oscillation between Cdc20 and Cdh1 expression partly explains how the APC/C times the degradation of its various substrates. Depending on their destruction box and their affinity for Cdc20 or Cdh1, APC/C substrates will be targeted earlier or later during mitosis or G_1 for proteasomal degradation [116, 117].

5. Other CRLs involved in cell cycle control

Other CRLs are also implicated in cell cycle control and the DNA damage response. CRL3 complexes appear to control mitosis via proteolytic and non-proteolytic mechanisms. The best understood cell cycle-related CRL3 substrate is Aurora B. K63-type ubiquitylation of Aurora B targets it to mitotic chromosomes to control their alignment [118]. The CRL4 ligases also control features of the cell cycle. In addition to Cul4 and Rbx1, CRL4 ligases contain the adaptor protein DDB1 (DNA Damage Binding protein 1), which recruits

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members of the DCAF (DDB1–Cul4 Associated Factors) family to dictate the specificity of substrate degradation [119–121]. $CRL4^{Cdt2}$ is a prominent member of this family implicated in DNA replication and the DNA damage response. Notably, $CRL4^{Cdt2}$ interacts with different UBCs (UBCH8, UBE2G1, and UBE2G2) to mediate ubiquitylation of its various substrates [122]. An important target of $CRL4^{Cdt2}$ is Cdt1, a replication licensing factor and pre-replication complex (pre-RC) component that is tightly regulated to ensure that DNA replication only occurs once per cell cycle. After S phase, Cdt1 activity is attenuated both by Geminin binding and by proteasomal degradation. Importantly, $CRL4^{Cdt2}$-mediated degradation of Cdt1 is initiated shortly after S phase onset [123–126]. This event requires Cdt1 binding to PCNA via a PIP (PCNA Interacting Protein)-box motif present in Cdt1. Upon DNA damage, Cdt1 is degraded in the same way to preclude DNA replication [127]. (Interestingly, Cdt1 is also targeted for degradation by SCF^{Skp2} in response to CDK-dependent phosphorylation [123, 128].)

An additional target of $CRL4^{Cdt2}$ is p21, which is degraded both in response to UV-induced DNA damage and during the unperturbed cell cycle. In the later context, $CRL4^{Cdt2}$ collaborates with SCF^{Skp2} to mediate timely degradation of p21. Like Cdt1, p21 degradation by $CRL4^{Cdt2}$ requires interaction with PCNA via a PIP-box [129]. $CRL4^{Cdt2}$ is further involved in cell cycle control via regulation of histone methylation by ubiquitylation of Set8. Set8 mono-methylates Lys 20 of histone H4 (H4K20me1) during G_2 to promote chromatin compaction. During S phase and upon DNA damage, Set8 is degraded by $CRL4^{Cdt2}$ in a PCNA-dependent manner [130–132]. Perturbation of this mechanism leads to accumulation of H4K20me1, premature chromatin compaction, and activation of the G_2/M checkpoint [130, 131].

Interestingly, Cdt2 levels are regulated via SCF^{Fbxo11} during the cell cycle [133, 134] and inhibition of Cdt2 degradation delays cell cycle exit. In contrast to most other SCF substrates, whose phosphorylation promotes binding to the F-box protein, CDK-dependent phosphorylation of the Cdt2 degron inhibits binding to and degradation via Fbxo11 [134]. Thus, crosstalk between an SCF ligase, CDKs, and a CRL4 complex controls the abundance of Cdt2 to regulate the timing of cell cycle exit.

6. Clinical implications

6.1 SCF complexes as targets in the therapy of cancer and other diseases

The SCF complexes and the APC/C are key players in cell cycle control and the DNA damage response. As such, they govern cell proliferation and genome stability, which, when deregulated, contribute to tumorigenesis. Among the SCF ligases controlling cell growth and proliferation (discussed above), this situation has been described in particular for SCF^{Skp2}, SCF^{TrCP}, SCF^{Fbxw7}, and SCF^{Fbxo9}. The recent therapeutic success of the proteasome inhibitor bortezomib in multiple myeloma and certain types of B-cell Non-Hodgkin's lymphoma has expedited the efforts to develop inhibitors of ubiquitin ligases or its components [135, 136]. While bortezomib exhibits a certain degree of selectivity towards cancer cells and has a noticeable therapeutic index, it non-specifically blocks the ubiquitin-proteasome-dependent degradation of all cellular proteins, resulting in considerable side effects and the development of resistance. Therapies directed against individual E3 ligases or ligase families known to be deregulated in human cancers may thus prove more efficacious. To qualify as a target for therapeutic inhibition, a ligase should be an oncoprotein (based on the activity of its substrates - *e.g.*, tumor suppressors) and deregulated in tumors.

6.1.1 Skp2—Skp2 is an oncoprotein by virtue of its function in degrading negative cell cycle regulators, including p27, p21, p130, and p57 [137, 138]. Skp2 is overexpressed in

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various malignancies, such as gastric cancer [139, 140], colon cancer [141], and breast cancer [142], with an associated decrease in p27 levels and indicating a poor prognosis. The oncogenic function of Skp2 is further underscored by mouse genetic studies. Targeted expression of Skp2 in the T-lymphoid lineage cooperates with activated N-Ras to induce T-cell lymphomas [143], while tissue specific expression of Skp2 in the prostate induces hyperplasia and low grade carcinoma [144]. In addition, a critical role for Skp2-dependent degradation of p27 in colon adenoma-carcinoma development was demonstrated using a Skp2 knock-in model [145]. Similarly, in *Skp2*-null cells, aberrant oncogenic signaling or inactivation of tumor suppressor genes trigger a potent, tumor-suppressive senescence response, although Skp2 inactivation alone does not induce senescence [146]. Finally, RNAi-mediated knockdown of Skp2 inhibits the growth of tumor cells, including cell lines derived from glioblastoma [147], melanoma [148], and oral cancer [149]. Specific inhibition of Skp2 would thus be expected to be effective in the treatment of cancers with activated Skp2 signaling.

Interestingly, Skp2 also interacts with a number of proteins produced by pathogenic viruses, such as X-protein (HBV), EBNA3C (EBV), and E7 (HPV16/18) [150], suggesting that Skp2 inhibitors may be also utilized in certain viral infections.

6.1.2 βTrCP—Given the diversity of its substrates (reviewed in [151]), βTrCP might be expected to exert both oncogenic and tumor suppressor activities. However, in certain tissues, βTrCP is clearly an oncoprotein based on its ubiquitylation activity against tumor suppressors, such as IκB, a negative regulator of NFκB [152], PDCD4, an inhibitor of eIF4A-mediated protein translation [39], and BimEL, a potent proapoptotic protein [40]. Indeed, βTrCP is overexpressed in breast cancer [153], and forced overexpression of βTrCP induces transformation in breast epithelium [154]. Likewise, upregulation of βTrCP has been shown in colon cancer, where it correlates with elevated NFκB activity and poor prognosis [155] and in pancreatic cancer cells, where it impacts chemoresistance [156]. Further evidence demonstrating the oncogenic potential of βTrCP stems from transgenic mouse models, in which tumor formation was observed in the mammary gland, liver, and kidney when βTrCP expression was directed to these tissues [154, 157]. Interestingly, no obvious phenotype was observed upon targeted expression of βTrCP in lymphoid organs, suggesting that βTrCP-dependent tumorigenesis is tissue specific [154]. Therefore, disrupting the interaction of βTrCP and its tumor suppressive substrates may be an attractive therapeutic strategy in defined tumor entities.

Similarly to Skp2, βTrCP interacts with a number of proteins produced by pathogenic viruses, such as Tax (HTLV1). Moreover, the HIV-1 protein Vpu targets $SCF^{\beta TrCP}$ to CD4 [158]. This finding, together with the evidence that other HIV-1 proteins (i.e. Vif and Vpr) bind CRLs to eliminate cellular proteins with antiviral activity (e.g., APOBEC3), indicates that $SCF^{\beta TrCP}$ and other CRLs are involved in the HIV life cycle and represent potential targets in the fight against this virus.

6.1.3 Fbxo9 and Fbxw7—The rationale for targeting the ubiquitin-proteasome system is particularly evident in multiple myeloma, due to the high efficacy of proteasome inhibitors in this disease. Indeed, Bortezomib, a reversible proteasome inhibitor, has been approved as the first line treatment for multiple myeloma. In addition, Carfilzomib, a second generation irreversible proteasome inhibitor has recently been approved by the Food and Drug Administration (FDA) for the treatment of patients with relapsed and refractory multiple myeloma, and demonstrates increased potency and an improved therapeutic index [159, 160]. Two ubiquitin ligases of the SCF family have recently been reported to contribute to the pathogenesis of multiple myeloma, SCF^{Fbxo9} and $SCF^{Fbxw7\alpha}$.

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As outlined above, Fbxo9 targets Tel2/Tti1 proteins in a CK2-dependent manner to adjust mTOR signaling to the availability of growth factors. Significantly, Fbxo9 is overexpressed in multiple myeloma and drives constitutive activation of the PI3K/mTORC2/Akt pathway to promote survival. In multiple myelomas with elevated Fbxo9 expression, inhibition of Fbxo9 represents an attractive approach to inhibit Akt signaling, one of the most important mediators of cell survival in this disease [159]. Inhibition of CK2, the kinase that promotes Fbxo9-mediated ubiquitylation of Tel/Tti1, may also be an interesting therapeutic approach in multiple myeloma, particularly since CK2 inhibitors are readily available [161].

Although Fbxw7 behaves as a tumor suppressor due to its ubiquitylation activity against mitogenic substrates (*e.g*., Notch, c-Myc, cyclin E), Fbxw7 functions as a pro-survival gene in multiple myeloma by constitutively targeting the NFκB inhibitor p100 in a GSK3-dependent manner [162]. While a number of cancers, including T-ALL, breast cancers, and gastric adenocarcinoma often carry mutations in the Fbxw7 gene, leading to an accumulation of mitogenic substrates [163–165], these mutations do not occur in B-cell malignancies like multiple myeloma [166]. Fbxw7 and GSK3 may thus serve as promising targets for the treatment of multiple myelomas with constitutive activation of the NFκB pathway.

In a short term setting, acute delivery of an Fbxw7 inhibitor could also be effective in tumors where this F-box protein plays a tumor suppressive role by inducing cancer stem cells to proliferate and, therefore, become susceptible to traditional chemotherapies. However, this hypothesis remains to be proved experimentally.

6.1.3 Other F-box proteins—Fbxo11 mutations are present in human cancers, such as diffuse large B cell lymphomas (DLBCLs), colon, lung, ovary, and head and neck tumors [167–171] (and Staudt L., personal communication). At least in DLCBLs, these mutations inhibit SCF^{Fbxo11}. The presence of inactivating mutations suggests that Fbxo11 may function as a tumor suppressor, whose loss of function contributes to the pathogenesis of DLBCL (via BCL6 accumulation) and other cancers (through the stabilization of unidentified oncogenic substrates). Furthermore, inactivating mutations have been reported for Fbxo4 in esophageal carcinoma, and these mutations are associated with an increase in cyclin D1 levels. The mutations occur in the N-terminal regulatory regions of Fbxo4 and disrupt dimerization and activation [172]. Moreover, there is evidence for the loss of α/B crystallin in breast cancer and a corresponding increase in cyclin D1 levels. α/B crystallin is the substrate adaptor for cyclin D1 together with Fbxo4 [63, 173]. These data suggest a tumor suppressor role for the $SCF^{Fbxo4\text{-}\alpha/B\ crystallin}$ ligase in human tumors based on its ability to destabilize cyclin D1. Finally, cyclin F has been reported to be downregulated in hepatocelular carcinoma (HCC). In this context, low cyclin F expression was an independent poor prognostic marker for overall survival and correlated with tumor size and clinical stage [174]. Given the role of cyclin F in regulating centrosome homeostasis and the balanced abundance of dNTPs, these findings hint at a function for cyclin F as a tumor suppressor in HCC by maintaining genomic stabilty [75, 76].

6.2 Strategies to target CRLs

In theory, targets for inhibiting substrate degradation by the ubiquitin proteasome system can involve any of the three enzymes involved in ubiquitin transfer, including the E1 activating, the E2 conjugating, and the E3 ligase enzymes. These approaches would be expected to inhibit most or certain ubiquitin ligase families, when targeting E1 or E2 enzymes, respectively, and become highly specific when targeting single E3 ligases.

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6.2.1 Targeting E1 ubiquitin activating enzymes—E1s (of which there are two in mammals) catalyze the initial step in ubiquitin conjugation, which involves an ATP-dependent covalent attachment of ubiquitin to its active cysteine site [4]. Efforts have been undertaken to target E1. These studies have yielded candidate compounds like PYR-41, an irreversible ubiquitin E1 inhibitor, but further investigations will be necessary to assess if they are clinical candidates [175]. Additional efforts have been directed to the E1s for ubiquitin like proteins (such as NEDD8, SUMO, *etc*.). Recently, MLN4924, an adenosine sulfamate analog, was reported to inhibit the E1 responsible for NEDDylation, the covalent addition of NEDD8 to substrates [176]. The cullin family of proteins are the most important substrates for neddylation, and the activity of SCF and other CRLs requires cullin neddylation [12, 177, 178]. In contrast to an inhibitor for the ubiquitin E1s, a small molecule inhibitor of the NEDD8 E1 would only inhibit CRLs. Currently, MLN4924 is in phase I/II clinical trials for the treatment of multiple myeloma and non-Hodgkins lymphoma, and the preclinical data suggest high efficacy in AML [179, 180].

6.2.2 Targeting E2 ubiquitin conjugating enzymes—Together with the E3 ligase, E2s mediate the transfer of ubiquitin to the target protein and govern the type and extent of ubiquitin linkage [181]. The human genome encodes at least 38 E2 enzymes, and Cdc34 (aka Ubc3) appears to be the major E2 for SCF ligases in promoting K48-linked polyubiquitylation and proteasomal degradation [12]. Recently, CC0651, a small molecule allosteric inhibitor of Cdc34 was reported [182]. CC0651 inhibits p27 ubiquitylation and degradation, and it demonstrates convincing specificity for human Cdc34, as evidenced by a lack of reactivity against any other E2/E3 pairs tested [182]. While current data on other E2 inhibitors is limited, the development of CC0651 indicates the feasibility of developing highly selective inhibitors of E2s and encourages efforts to target other E2s in a similar manner.

6.2.3 Targeting E3 ubiquitin ligases—E3 ligases determine which target protein becomes ubiquitylated. Inhibition of a single E3 ligase would be the most selective targeting approach, since it would affect a limited number of proteins, potentially translating into a better therapeutic ratio and fewer side effects. Most E3 ligases do not feature a canonical active site, and instead, the active site is a protein-protein interaction with a substrate. Inhibition of these protein-protein interactions is typically considered more difficult than inhibition of a catalytic site. However, this task appears increasingly feasible with advances in the structural understanding of these interactions [183]. MDM2 is an extensively studied ubiquitin E3 ligase with strong clinical relevance by virtue of its ability to regulate the abundance of the tumor suppressor p53. Previously, the Nutlin class of imidazoline chemotypes was identified, and they specifically disrupt the protein-protein binding interface between MDM2 and p53, stabilizing p53 [184]. Nutlin-3a is the most promising candidate and has favorable preclinical characteristics in terms of pharmacological properties and toxicity [185, 186]. Currently, Nutlin-3a is being investigated in early phase clinical trials for several solid and hematological tumor entities, setting a precedent for the specific inhibition of a ligase-substrate pair as a promising clinical approach.

In the case of SCF complexes, inhibition of either the F-box protein-substrate interface or the recruitment of the F-box protein to the SCF core are attractive strategies to selectively inhibit individual ubiquitylation events. In this regard, the small molecule inhibitor CpdA has previously been found to prevent the binding of Skp2 to the SCF ligase complex, inducing G1/S arrest and apoptosis by stabilizing p27, p21, and other Skp2 target proteins. In addition, CpdA sensitized multiple myeloma cells to cytostatic agents and bortezomib, and it was active against both myeloid and lymphoblastoid leukemia blasts [187].

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Independent studies have recently demonstrated the feasibility of direct inhibition of the interfaces between the F-box protein and either the substrate or SCF core. SCF-I2 is an allosteric inhibitor of substrate recognition by the yeast F-box protein Cdc4 and inhibits binding and ubiquitylation of substrates. SCF-I2 inserts itself between the β-strands of the WD-40 propeller domain of Cdc4, impairing recognition of Cdc4-specific phosphodegrons [188]. In addition, a recent study screened for inhibitors that selectively target the p27-binding interface formed by Skp2-Cks1. This approach yielded four compounds that stabilize the expression of p27 in different human cancer cell lines and induce cell cycle arrest in G1 [189]. Finally, a chemical genetics screen for enhancers of rapamycin identified SMER3, an inhibitor of the SCF^{Met30} ligase. SMER3 specifically inhibits binding of the F-box protein subunit Met30 to the SCF core, stabilizing substrates such as Met4 [190].

Another approach to prevent substrate degradation at the E3 ligase level is to inhibit the kinase that phosphorylates a substrate to mark it for degradation. This approach particularly applies to SCF ubiquitin ligases, as it is well established that substrate phosphorylation is frequently a prerequisite for F-box protein binding [13, 151, 191]. Indeed, kinases like Plk1, which control the degradation of tumor suppressors, are potential targets, and inhibitors have already entered early clinical trials [192]. Notably, this approach could quickly impact the clinic, as inhibitors to numerous kinases are readily available, but broader application will require further investigations.

Together, SCF ligases are promising therapeutic target structures, whose pharmacological modulation would offer highly specific therapeutic approaches to a wide variety of malignancies. The success of this undertaking will largely depend on the pharmacological progress in developing specific inhibitors of F-box proteins and a better mechanistic understanding of the many uncharacterized F-box proteins, both with regard to their substrates and the posttranslational modifications that prime them for SCF-dependent ubiquitylation. Finally, it will be critical to further define tumor entities in which distinct SCF ligases are deregulated. This data will help define distinct F-box proteins as biomarkers and allow the selection of patient subpopulations that would profit from a targeted approach against SCF ligases.

Acknowledgments

We thank Jeffrey Skaar for critically reading the manuscript. We apologize to colleagues whose work could not be mentioned owing to space limitations. Work in the Bassermann laboratory is supported by grants from the German Research Foundation (Emmy Noether Program, BA 2851/3-1) and the German Cancer Aid (#109543), and in the Pagano laboratory by grants from the NIH (R01-GM057587, R37-CA076584, and R21-CA161108). MP is an investigator with the Howard Hughes Medical Institute.

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Highlights

- Role of Cullin RING ligases (CRLs) in the normal cell cycle
- Involvement of CRLs in the cellular response to different environmental cues
- Particular focus on ligases of the SCF and APC/C families
- Clinical implications in targeted cancer therapies

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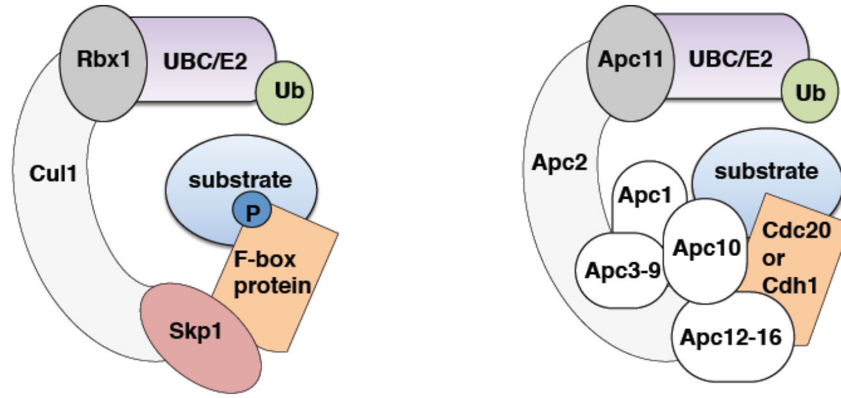

Figure 1. Structure of the SCF and the APC/C ubiquitin ligases

The SCF and APC/C E3 ligases are both members of the Cullin-RING ligase (CRL) superfamily. Cul1 and Apc2 (light grey) are the scaffold proteins of SCF and APC/C, respectively. On one end, they bind to a RING finger protein, Rbx1 or Apc11 (dark grey), which recruits the E2 ubiquitin conjugating enzyme (UBC). On their other end, they connect to the substrate specific unit via an adaptor molecule (red). In the case of SCF, the F-box proteins are the variable substrate binding components (orange), while Cdh1 and Cdc20 (in somatic cells), together with Apc10, recruit substrates to the APC/C. The scheme illustrates the relationship between APC/C and SCF components and does not represent the topology of APC/C subunits.

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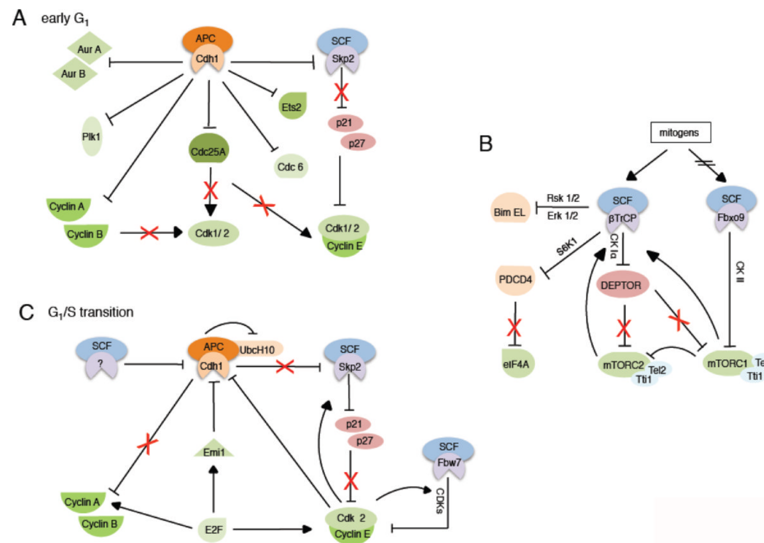

Figure 2. SCF and APC/C mediated degradation processes in G_1 phase and the response to mitogen stimulation

A. APC/C^{Cdh1} maintains a stable G_1 phase by targeting mitotic proteins like Aurora A, Aurora B, Plk1, cyclin A, and cyclin B for degradation. In addition, APC/C^{Cdh1} prevents premature accumulation of positive cell cycle regulators, such as Ets2, Cdc6, and Cdc25A. The CDK inhibitors p21 and p27 are stabilized via APC/C^{Cdh1}-dependent degradation of Skp2 to keep CDK activity low during G1.

B. Upon mitogen stimulation, $SCF^{\beta TrCP}$ mediates the degradation of inhibitory or proapoptotic proteins, such as PCDC4, DEPTOR, and BimEL, promoting cell growth. Upon mitogen withdrawal, SCF^{Fbxo9} targets Tel2 and Tti1 for degradation within mTORC1 to attenuate mTORC1 activity and sustain mTORC2 signaling.

C. At the G_1/S transition, APC/C^{Cdh1} activity is inhibited through several mechanisms, such as inhibitory phosphorylation by CDKs and binding to Emi1. Skp2-dependent degradation of p21 and p27 leads to activation of cyclin E-Cdk2, which initiates S phase before being degraded via SCF^{Fbw7}. See main text for more details.

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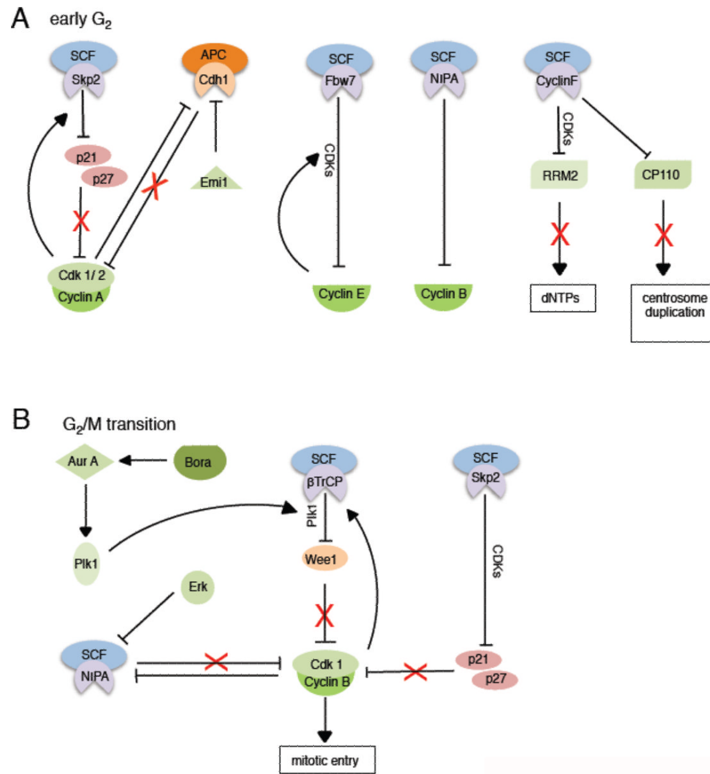

Figure 3. SCF and APC/C mediated degradation processes in S and G_2 phases of the cell cycle

A. In early G_2, APC/C^{Cdh1} remains inactivated, SCF^{Skp2} maintains cyclin A-Cdk1/2 activity via degradation of p21 and p27, SCF^{Fbw7} promotes cyclin E degradation, and SCF^{NIPA} prevents premature accumulation of nuclear cyclin B. Other S phase proteins (*e.g.*, RRM2 and CP110) are also degraded via cyclin F to stop dNTP production and assure centrosomes are only replicated once per cell cycle.

B. At the G_2/M transition, SCF^{Skp2} continues to target p21 and p27, while Wee1 is degraded in a Plk1- and SCF$^{\beta TrCP}$-dependent manner to release cyclin B-Cdk1 from inhibition. See main text for more details.

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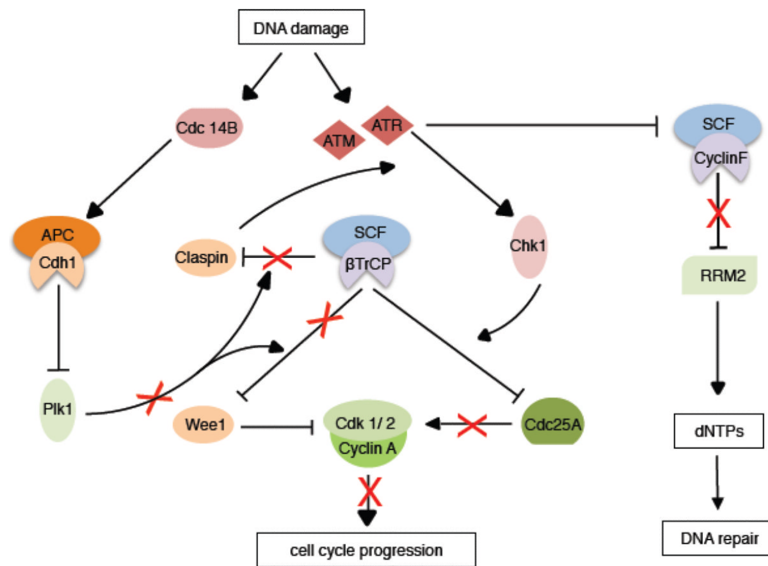

Figure 4. Role of SCF and APC/C ligases in the response to DNA damage

Upon DNA damage, SCF and APC/C activate S and G2 checkpoints to halt cell cycle progression. Chk1 (which is activated by ATR) promotes the $SCF^{\beta TrCP}$-dependent of Cdc25A to inhibit CDK activity, while dephosphorylation of Cdh1 by Cdc14B allows for Plk1 degradation and subsequent stabilization of Claspin and Wee1. See main text for more details.

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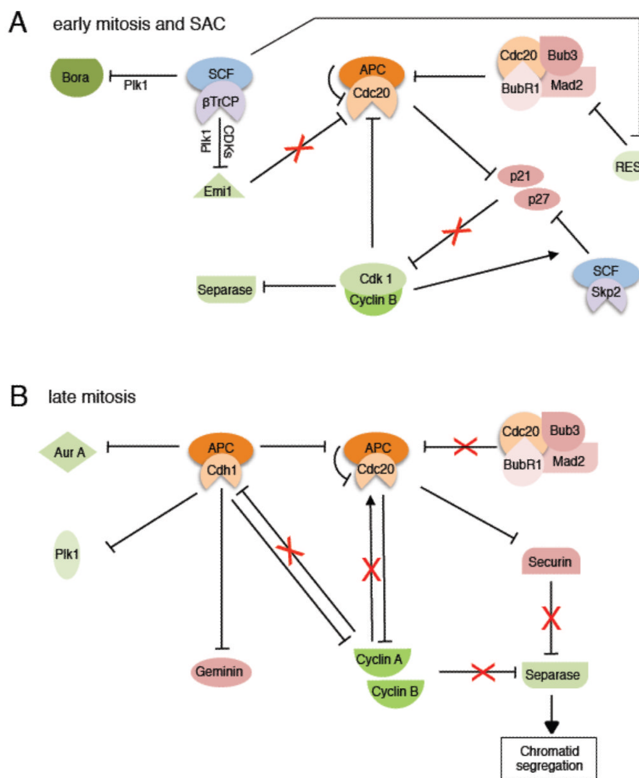

Figure 5. SCF and APC/C mediated degradation processes during mitosis

A. In early mitotis, $SCF^{\beta TrCP}$ ubiquitylates Emi1 to eliminate the repression of APC/C^{Cdh1} (which remains inactive because of Cdh1 phosphorylation by CDKs) and, possibly, APC/C^{Cdc20}. However, during activation of the Spindle Assembly Checkpoint (SAC), APC/C^{Cdc20} activity is repressed, with cyclin B-Cdk1 contributing to its inhibition.

B. After fulfillment of the SAC, APC/C^{Cdc20} inhibition is relieved, allowing it to target mitotic cyclins and Securin for degradation, the latter leading to Separase activation and sister chromatid separation. With mitotic progression, APC/C^{Cdc20} activity decreases via several mechanisms, and APC/C^{Cdh1} assembles to target cell cycle proteins for degradation. See main text for more details.