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Chapter

Ubiquitin Signaling in Regulation of the Start of the Cell Cycle

Michael James Emanuele and Taylor Paige Enrico

Abstract

The small protein ubiquitin plays a vital role in virtually all aspects of cellular life. Among the diverse signaling outcomes associated with ubiquitination, the most well-established is the targeted degradation of substrates via the proteasome. During cell growth and proliferation, ubiquitin plays an outsized role in promoting progression through the cell cycle. In particular, ubiquitin-mediated degradation is critically important at transition points where it provides directionality and irreversibility to the cell cycle, which is essential for maintaining genome integrity. Specifically, the boundary between G1 and S-phase is tightly regulated by the ubiquitin proteasome system. Notably, the G1/S boundary represents a major barrier to cell proliferation and is universally dysfunctional in cancer cells, allowing for the unbridled proliferation observed in malignancy. Numerous E3 ubiquitin ligases, which facilitate the ubiquitination of specific substrates, have been shown to control G1/S. In this chapter, we will discuss components in the ubiquitin proteasome system that are implicated in G1/S control, how these enzymes are interconnected, gaps in our current knowledge, and the potential role of these pathways in the cancer cycle and disease proliferation.

Keywords: cell cycle, ubiquitin, cullin RING ligase, anaphase promoting complex/cyclosome (APC/C), G1, S-phase, SCF

1. Introduction

Progression through the cell cycle is driven by the oscillating activity of Cyclin Dependent Kinases (CDKs). The activity of CDKs is controlled by their binding to coactivator subunits termed Cyclins, as well as by CDK inhibitory proteins termed CKIs. The accumulation of both Cyclin and CKI proteins is tightly regulated at the level of transcription. In addition, Cyclin and CKI proteins are controlled at the level of their destruction. Remarkably, during each and every passage through the cell cycle, Cyclins, CKIs, and hundreds of other proteins, accumulate and are subsequently destroyed via a highly regulated process of programmed degradation. This degradation is controlled by ubiquitin.

Ubiquitin is conjugated to substrate lysines, and because ubiquitin itself contains seven lysine residues to which ubiquitin can be added, the repetitive addition of ubiquitin can result in the formation of polyubiquitin chains on substrates. These chains can be formed through each of the different lysines in ubiquitin, as well as through the amino-terminal methionine, leading to chain formations that adopt distinct topological features [1, 2]. The most well-characterized of these are chains

linked through lysine 48 in ubiquitin, so-called K48-linked ubiquitin chains, which target substrates to the proteasome for destruction. More recently, K11-linked chains were also shown to target substrates to the proteasome [3, 4]. Alternatively, ubiquitin chains linked through other lysines (or through methionine 1) lead to diverse signaling outputs by altering protein-protein interactions, protein localization, enzyme activity, etc. This already complex picture is further complicated by the recent discovery of branched ubiquitin chains, which contain non-homogeneous lysine linkages. For example, branched K11/K48 chains likely represent remarkably strong degradative signals [5, 6].

Protein degradation through the ubiquitin proteasome system (UPS) is the major regulator of programmed protein destruction in human cells and plays an outsized role in controlling cell cycle progression [7]. Importantly, the targeted degradation and/or stabilization of specific proteins at transition points (e.g. mitosis/G1 and G1/S boundaries) promotes cell cycle progression, provides directionality and irreversibility to the cell cycle and maintains genome integrity [8]. Accordingly, numerous enzymes in the ubiquitin system have been implicated in these transition points.

The start of DNA replication represents a tightly controlled barrier to proliferation in normal cells. As such, nearly all of the non-dividing cells in the human body are arrested prior to the start of S-phase, in either G1, or in quiescence (G0), where they maintain the equivalent of G1-phase (2C) DNA content. In diseases of uncontrolled proliferation, and most notably in cancer, the S-phase boundary is perturbed. Thus, cancer cells are able to aberrantly enter S-phase due to a weakening of the G1/S border [9]. The retinoblastoma tumor suppressor pathway plays a key role in controlling G1/S. However, the ubiquitin system is also tightly linked to G1/S regulation in normal and cancer cells. Below, we will discuss the particular enzymes and pathways associated with ubiquitin signaling that have been implicated in regulating the start of S-phase.

2. Introduction to cell cycle ubiquitin ligases

2.1 Cullin RING E3 ubiquitin ligases

The RING domain family of E3 ubiquitin ligases is the largest family of E3s in higher eukaryotes, and in humans it is represented by several hundred unique enzymes and/or enzyme complexes. The cullin RING ligases (CRLs) are the largest subfamily of RING E3s, encoding nearly 300 unique enzymes. The CRL E3s all share a common molecular architecture [10]. CRLs utilize a cullin protein backbone, which simultaneously binds to both an E2 ubiquitin conjugating enzyme and substrate, positioning E2 and substrate in close proximity, and enabling the rapid transfer of ubiquitin onto substrates (**Figure 1A**).

The human genome encodes several cullin proteins, including Cul1, Cul2, Cul3, Cul4A, Cul4B, Cul5, Cul7, Cul9 and the related cullin-like protein APC2. With the exception of APC2, each cullin is thought to assemble into a ligase with a similar architecture, where the amino terminus of the cullin engages targets and functions as a substrate targeting module, and the carboxy terminus engages the E2, functioning as a ubiquitin transfer module (**Figure 1B**). Cullin binding to substrates and E2-ubiquitin conjugating enzymes is indirect. Most cullins first bind to an adaptor protein which in turn binds to a family of substrate receptors that then recruit substrates for ubiquitination (**Figure 1**). Similarly, cullin proteins indirectly interact with one of two RING domain containing proteins (Roc1/Rbx1 or Roc2/Rbx2)
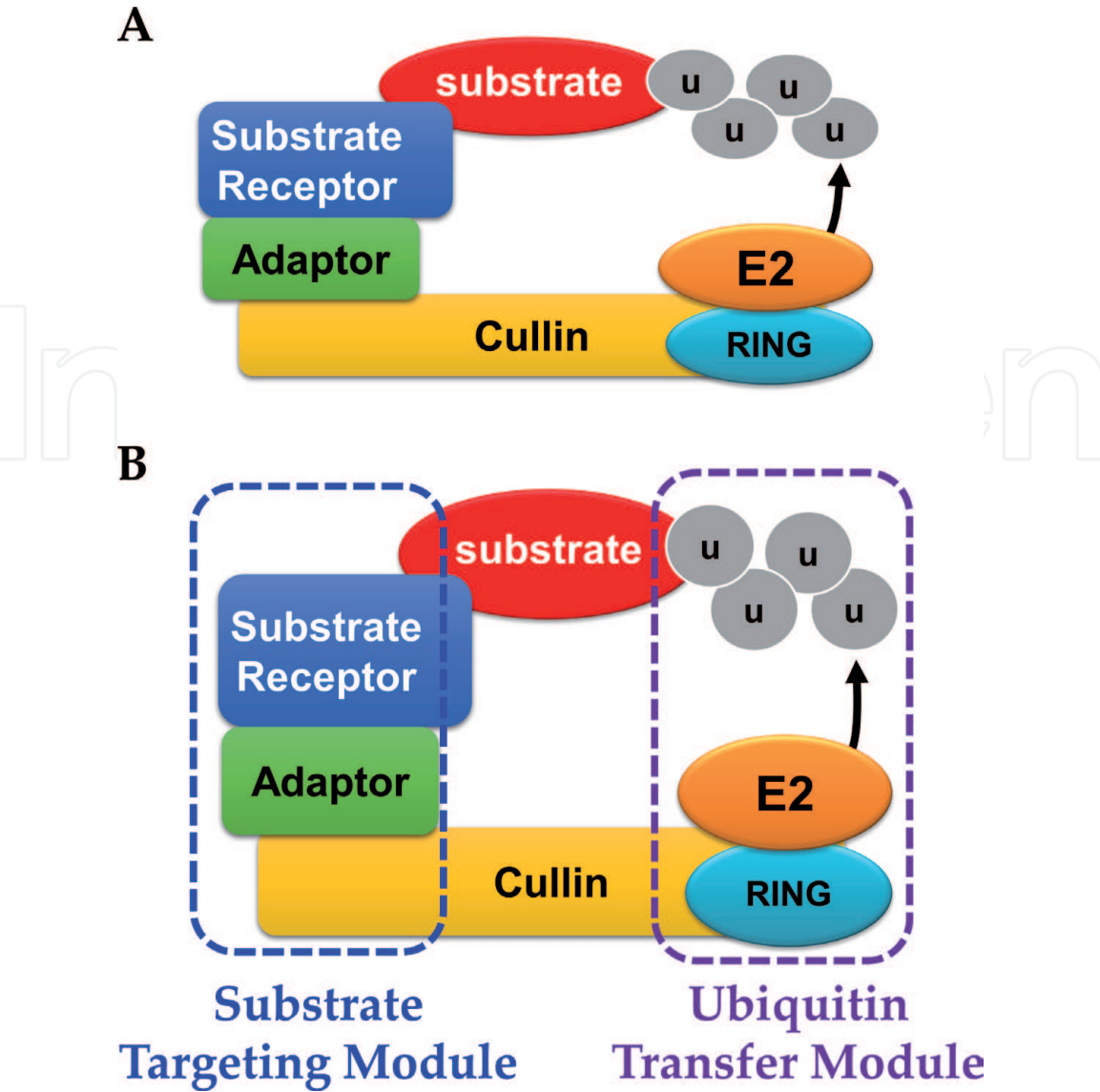
Figure 1.
Architecture of the cullin RING E3 ubiquitin ligases. (A) Architecture of a canonical CRL E3 ligase. (B) Boxes highlighting the substrate targeting (dark blue) and ubiquitin transfer (purple) modules.

which in turn bind to E2 ubiquitin conjugating enzymes. This architecture is shared among all known CRL complexes.

The archetypical CRL sub-family, and one which will be discussed in greater detail, is the Skp1-Cul1-F-box family of CRLs. These ligases, commonly referred to as SCF or CRL1 ligases, utilize a family of 69 interchangeable substrate receptor proteins, termed F-box proteins, which designate substrates for ubiquitination and degradation. F-box proteins rely on an F-box domain to interact with an adaptor protein termed Skp1, which bridges F-box proteins to Cul1 (**Figure 2**). The CRL nomenclature dictates that specific ligase complexes are depicted with the F-Box protein as a superscript, following the name of the cullin complex. Thus, Cul1-based CRLs, in complex with the F-box substrate receptor Skp2, are designated as SCF[Skp2] or CRL1[Skp2] (hereafter, Cul1-based CRL complexes will be referred to as SCF).

Importantly, substrate receptors recognize proteins for degradation based on short, linear sequence motifs, called degrons. Degron sequences are shared among the substrates of a specific E3. In addition, degrons are transferrable, and the addition of degron sequences to non-substrates is often sufficient to trigger their recognition by the E3 and subsequent ubiquitination and degradation. Also, many substrate receptors, although not all, require post-translation modification (e.g.
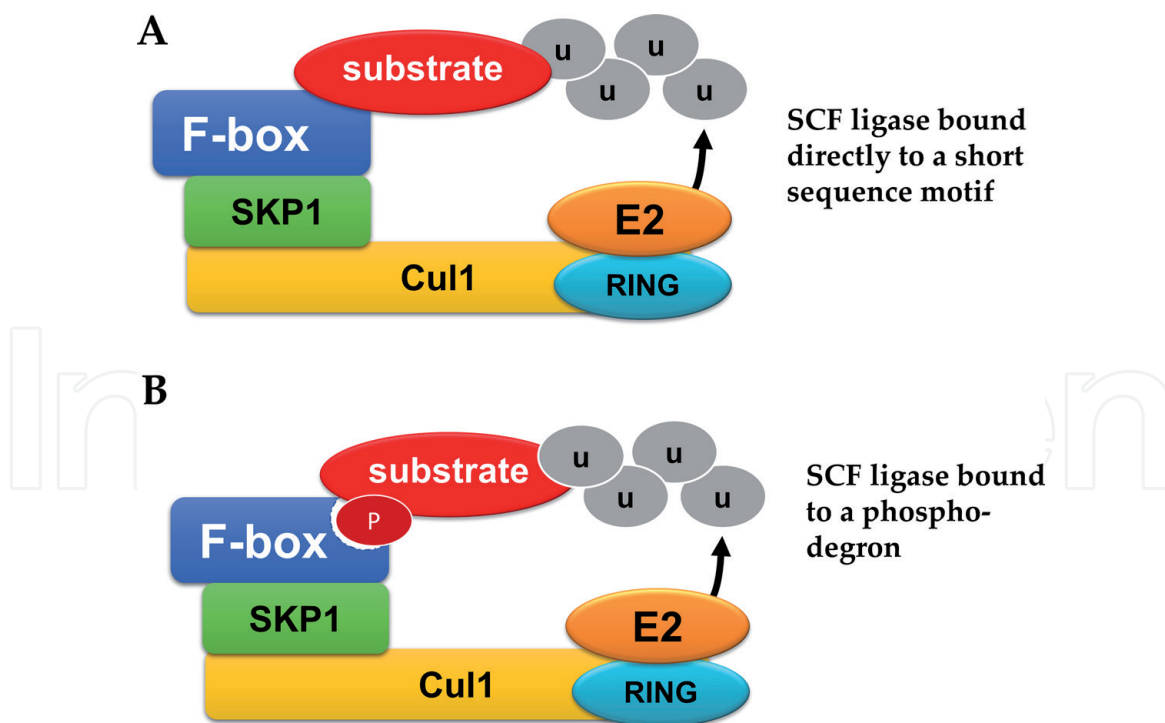
Figure 2.
Architecture of the SCF ligase. (A) Example of an SCF-type ligase bound to a short linear degron sequence motif in a substrate. (B) Example of an SCF-type ligase bound to phosphorylation-dependent degron in a substrate.

phosphorylation) of the substrate within the degron for the substrate to be recognized, ubiquitinated, and degraded. Thus, the degradation of many SCF substrates is regulated at the level of the substrate and is a two-step process. First, the substrate must be present and modified, and second, the ligase must also be available, thereby enabling substrate recognition and degradation. It is important to note that each substrate receptor can have many substrates. Furthermore, individual substrates can be controlled by multiple ligases. Finally, distinct substrate adaptors can themselves be targeted for degradation by other E3 ligases.

The Cul1-based SCF ligases are the founding members of the CRL family. They were first discovered in yeast based on their role in controlling cell cycle progression. Their discovery grew out of gain-of-function screens performed by Elledge and colleagues, which identified suppressors of the yeast cell cycle mutant Cdc4. This screen uncovered a new protein, whose mRNA and protein levels oscillated during the cell cycle. Moreover, the amino acid sequence of this new protein included a Cyclin homology domain, similar to that found in the previously identified Cyclins A, B, D, and E. Thus, this new protein was named Cyclin F [11]. Significantly, Cyclin F contained a domain with sequence similarity to Cdc4, which they named the F-box domain. They found that the F-box domains in Cyclin F and Cdc4 were essential for tethering both proteins to the ubiquitin machinery via binding to Skp1 [12]. Shortly thereafter, the Harper lab, in collaboration with Elledge, as well as the Deshaies lab, showed that SCF complexes could trigger the ubiquitination and degradation of the yeast CDK inhibitor Sic1. Moreover, these studies demonstrated that the F-box protein Cdc4 preferentially bound to the phosphorylated version of Sic1, thereby triggering its ubiquitination and degradation [13, 14].

2.2 The Anaphase Promoting Complex/Cyclosome

Like other E3 ubiquitin ligases, the Anaphase Promoting Complex/Cyclosome (APC/C) plays an important role in protein degradation. APC/C regulates the

ubiquitination and degradation of the CDK activator proteins Cyclin A and Cyclin B, in addition to many other cell cycle regulated proteins. As such, it is a core component of the cell cycle oscillator. As its name suggests, the APC/C is activated in metaphase of mitosis, during which time it triggers the ubiquitination and degradation of numerous proteins including two critical substrates, Cyclin B and securin, thereby "promoting anaphase" and mitotic exit. In addition to its essential function in mitosis, APC/C also plays an evolutionarily conserved role in G1-phase. The APC/C remains active throughout G1, where in contrast to its role in promoting progression through mitosis, the APC/C restrains progression through G1-phase into S-phase [17], and is not turned off until immediately prior to the start of DNA replication [15, 16]. Significantly, APC/C inactivation at the G1/S boundary is required for the start of S-phase.

Similar to the CRLs discussed above, the APC/C has both a cullin-like subunit (APC2) and a RING subunit (APC11). However, the APC/C is significantly different than the CRL ligases discussed above. Notably, the APC/C is composed of 18 polypeptide subunits and is a remarkable 1.2 mDa in size (**Figure 3**). The cullin subunit, APC2, is the most divergent of the cullins, and lacks features that are common among other cullin proteins. For example, while other cullin proteins are post-translationally modified and activated by the small, ubiquitin-like protein Nedd8, this process is not thought to be involved in APC/C activity.

The APC/C utilizes either of two substrate receptors during somatic cell cycles. First, during mitosis, the APC/C binds to the substrate receptor/coactivator Cdc20, which brings Cyclin B and Securin to the APC/C for ubiquitination. Immediately following mitotic exit, APC/C shifts to using a second substrate adaptor, the Cdc20-related protein Cdh1/Fzr1 (hereafter referred to as Cdh1). The Cdh1-bound form of APC/C remains active throughout G1-phase and targets a myriad of cell cycle regulators for degradation, including proteins involved in transcription, nucleotide metabolism, and CDK activation. Thus, it is APC/C^{Cdh1} that must be inactivated prior to the beginning of S-phase. Both Cdc20 and Cdh1 recognize substrates via short, linear degron motifs in substrates. The most well-characterized and widespread of these degron motifs among APC/C substrates are the D-box (amino acid sequence R-X-X-L, where X is any amino acid) and the KEN box (amino acid sequence K-E-N). Thus, the ability of Cdc20 or Cdh1 to recruit substrate proteins harboring D- or KEN-box motifs to the APC/C is required for the subsequent ubiquitination and destruction of APC/C targets.

Like the SCF, the APC/C was identified by virtue of its key role in cell cycle. It had been known that the key CDK activator Cyclin B is controlled by degradation,
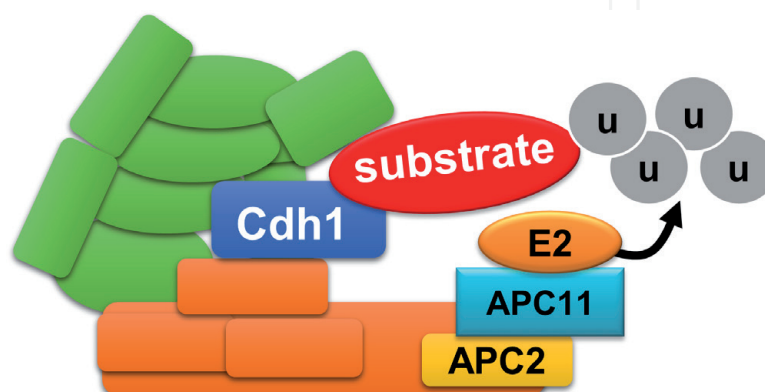

Figure 3.
Architecture of the APC/C ubiquitin ligase. The color scheme is the same as above for SCF ligases. Several proteins are specifically shown, including the cullin subunit APC2, the RING subunit APC11, and the substrate receptor Cdh1. Note that there are many more components.

and that both the accumulation and degradation of Cyclin B play a vital role in cell cycles, particularly in early frog embryos [18]. In 1995, the regulator of Cyclin B was discovered by the Kirschner and Hershko labs, who named it the Anaphase Promoting Complex and Cyclosome, respectively [19, 20].

3. Role and regulation of SCF ligases in G1/S control

The SCF complexes can assemble from any one of 69 well-established substrate receptor F-box proteins in humans. A subset of SCF ubiquitin ligase complexes have been directly implicated in G1/S control. Here we will discuss the role of each of these distinct complexes and/or substrate receptors, aspects of their regulation and function, and their contribution to G1 progression and S-phase initiation.

3.1 CDC4

The yeast specific Cell Division Control gene/protein 4, called Cdc4, was one of the original cell cycle mutants identified by Hartwell and colleagues, who later received the Nobel Prize for the analysis of cell cycle in budding yeast. They showed that Cdc4 mutant yeast arrest at the G1/S boundary, prior to the start of DNA replication [21]. However, it took another 20 years for the essential molecular function of Cdc4 in promoting cell cycle progression to become clear, and in doing so, laid the foundation for the discovery of CRL ligases.

As the analysis of cell cycle control became increasingly popular in the late 1980s and early 1990s, researchers revisited the role of Cdc4. Nasmyth and colleagues showed that the budding yeast Cdc4 mutants, which arrest before the start of DNA replication when grown at their restrictive temperature, lack appreciable CDK activity [22]. Interestingly, cell cycle arrest is caused by an inability of Cdc4 mutant cells to downregulate the yeast CKI Sic1, which normally decreases at the end of G1. The decrease in Sic1 allows the increase in CDK activity needed to enter S-phase. Thus, yeast cells cannot enter S-phase when Cdc4 is inactivated [11].

As discussed above, Cdc4 is an F-box protein that binds to Sic1, promoting its ubiquitination by the SCF^{Cdc4} complex. The mechanism by which Cdc4 recognizes Sic1 to promote its degradation provides a clear example of the interplay between phosphorylation and ubiquitination cascades. Interestingly, Sic1 must first be phosphorylated by Cyclin-CDK complexes, and this phosphorylation enables the binding of Cdc4 to Sic1 [13, 14]. Once phosphorylated and bound to Cdc4, Sic1 is recruited to the SCF complex for ubiquitination (**Figure 4**). Thus, CDKs promote their own activity at the G1/S boundary by triggering the degradation of their inhibitor, Sic1 (**Figure 4B**). This implies a positive feedback loop in control of S-phase entry. While the mechanism by which Cdc4 controls G1/S is largely attributed to its role in destroying Sic1, Cdc4 has also been linked to other cell cycle regulators and proteins involved in proliferative control. Cdc4 substrates include numerous proteins involved in MAPK signaling that mediate cell cycle arrest in response to pheromone [23–26], the replication regulator Cdc6 [27], the sirtuin deacetylase Hst3 [28], as well as proteins involved in sister chromatid cohesion [29], regulation of calcineurin [30], and mating-type switching [31]. Because Cdc4 has many substrates, it plays a complex and multi-faceted role in yeast cell cycle, among other processes.

3.2 Skp2

The F-box protein Skp2 has been well-characterized in human cells and plays an important role in the G1/S transition. Similar to Cdc4, Skp2 plays a key role
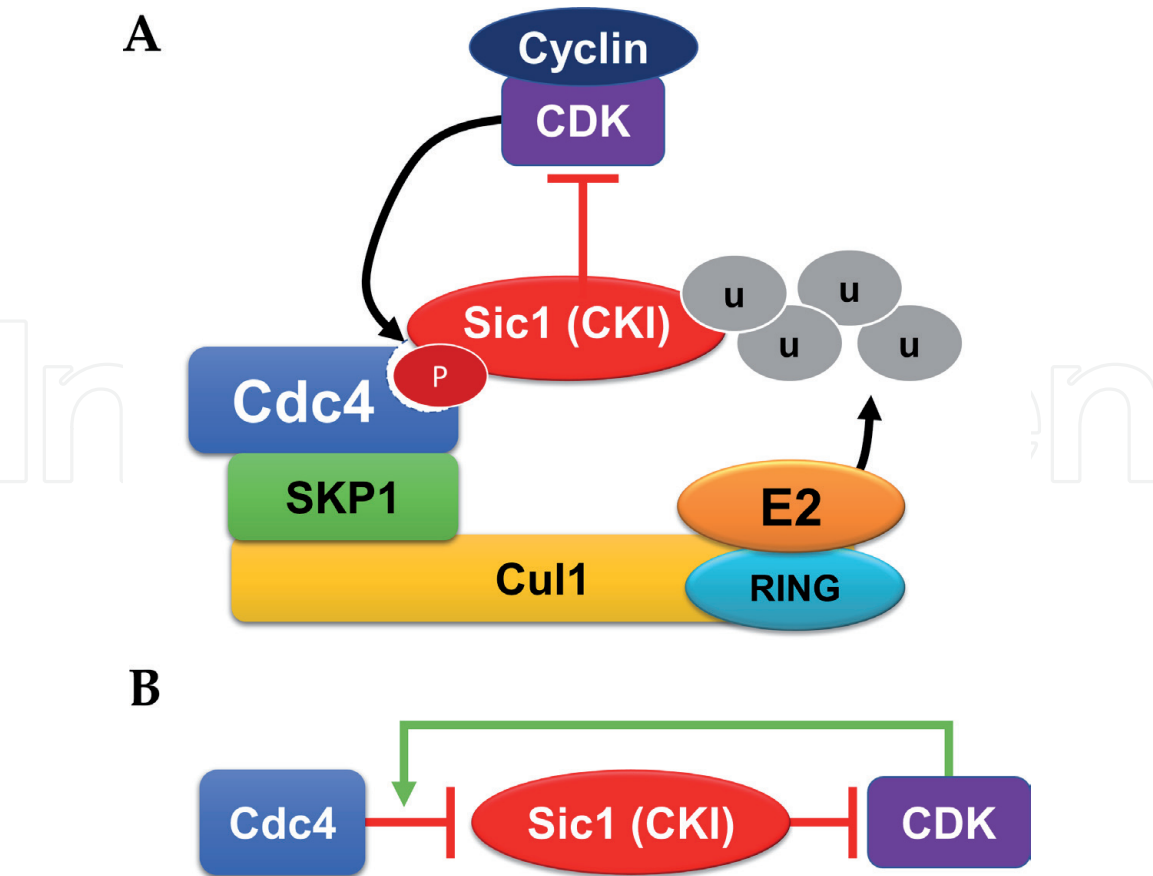
Figure 4.
SCF^{Cdc4} promotes S-phase entry in yeast by triggering the degradation of the CKI Sic1. (A) Binding between Sic1 and Cdc4 is triggered by phosphorylation of Sic1, which then promotes Sic1 ubiquitination and degradation. (B) A positive feedback loop between Cdc4, Sic1 and CDK promotes S-phase entry.

in regulating CDKs by promoting the destruction of CKI proteins. In particular, Skp2 plays an important role in promoting the destruction of the human CKI p27 [32, 33]. Moreover, the ubiquitination of p27 by SCF^{Skp2} requires that it first be phosphorylated by CDK, and this subsequently targets p27 for destruction, suggesting a similar positive feedback loop in G1/S regulation (**Figure 5**) [34]. Similarly, SCF^{Skp2} can target two other CKI proteins for degradation. These are p21 and p57, both of which are degraded in proliferating cells going through the cell cycle [35, 36], although p21 is also degraded by a second Cul4-based CRL ligase once DNA replication has begun [37]. Finally, Skp2 has been linked to the degradation of the retinoblastoma related protein RBL2/p130 [38, 39]. Like RB, RBL2/p130 restrains the activity of a cell cycle E2F transcription factor that promotes proliferation and cell cycle progression.

As might be expected, Due to its role in promoting S-phase via the degradation of CKIs, Skp2 is often overexpressed in cancers, which likely contributes to cancer cell proliferation [40]. Chemical approaches aimed at identifying Skp2 inhibitors have been undertaken, with some success [41, 42].

In addition to its role in regulating several target proteins, including the CKIs discussed above, Skp2 plays a complex and more paradoxical role in regulating proliferation. The Myc transcription factor is a potent oncogene, that is activated in many cancers and which drives proliferation through myriad mechanisms [43]. Myc is ubiquitinated by Skp2 [44, 45]. However, remarkably, the ubiquitination and degradation of Myc catalyzed by SCF^{Skp2} triggers an *increase* in Myc activity. This is consistent with prior work implicating proteolysis in the activation of several transcription factors in both yeast and humans [46]. Accordingly, a stable allele of Myc that cannot be ubiquitinated is more abundant, localized to target promoters,
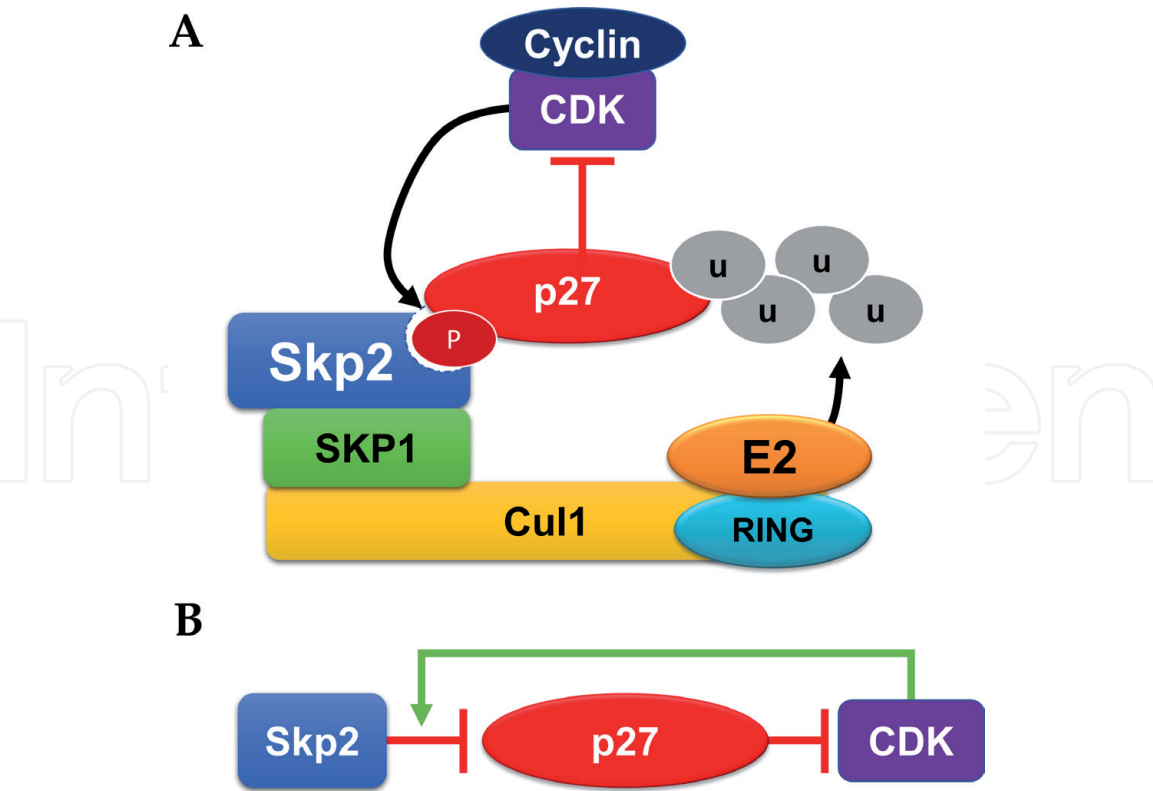
Figure 5.
SCF^Skp2 promotes S-phase entry in humans by triggering the degradation of the CKI p27. (A) Binding between p27 and Skp2 is triggered by phosphorylation of p27, which then promotes p27 ubiquitination and degradation. (B) A positive feedback loop between Skp2, p27 and CDK promotes S-phase entry.

but it is less active [47]. Taken together, these studies paint a complex picture of the role of Skp2 in cell cycle progression but suggest an important role in proliferation and likely in the pathogenesis of cancer.

Interestingly, Skp2 is itself regulated by ubiquitin mediated proteolysis. Skp2 is targeted for degradation by the Anaphase Promoting Complex/Cyclosome during G1-phase of the cell cycle [48, 49]. The degradation of p27 requires the upregulation of Skp2. This degradation would presumably occur after Skp2 levels accumulate, following the inactivation of APC/C, which occurs in late G1. That is, APC/C inactivation should lead to an increase in Skp2 levels, since Skp2 would no longer be degraded. Only then could Skp2 promote the degradation of p27. However, this complex order of events remains unclear and has not yet been tested directly. Since the abundance of CKIs, like p27, should prevent the activation of G1/S CDKs, this also implies that APC/C inactivation precedes CDK activation. As discussed below, this too remains unknown, and recent evidence suggests, in fact, that APC/C inactivation occurs after CDK activation in G1 [16].

In addition to its regulation by ubiquitination, Skp2 is also regulated by phosphorylation. This phosphorylation is mediated, in part, by the oncogenic kinase AKT [50]. Notably, AKT kinase activity is cell cycle regulated, and begins to increase in late G1-phase [51]. Skp2 phosphorylation by AKT increases Skp2 stability and alters its localization. Surprisingly, SCF^Skp2 also ubiquitinates AKT, and enhances AKT activation [52]. The degradation of p27 and activation of AKT and Myc, by Skp2, are likely to play an important role in tumor biology and treatment. The degradation of p27, a negative cell cycle regulator, creates an environment more permissive to proliferation because cells lacking p27 can progress through the cell cycle more rapidly. In addition, the activation of AKT and Myc could contribute significantly to cancer cell cycles.

3.3 Cyclin F/FBX01

The eponymous Cyclin F is the founding member of the F-box family of E3 ubiquitin ligases [11, 12, 53]. Cyclin F is unique among F-box proteins in that it contains a Cyclin homology domain, similar to canonical Cyclins that bind and activate CDKs. However, unlike those other Cyclins, Cyclin F neither binds nor activates a CDK [53]. In addition, Cyclin F levels oscillate strongly throughout the cell cycle, and this is the result of both changes in its transcription and degradation. Notably, Cyclin F is the only F-box protein that was identified as cell cycle regulated in all global studies of human cell cycle transcriptional dynamics [54]. Accordingly, Cyclin F knockout mouse embryonic fibroblasts showed a strong defect in cell cycle entry following synchronization in quiescence [55]. Nevertheless, despite this strong cell cycle phenotype and being the first described F-box protein in higher eukaryotes, Cyclin F went a long time without having a bona fide substrate.

The first two substrates described for Cyclin F were the centrosome protein CP110 and the spindle associated, mitotic phospho-protein NUSAP1 [56, 57], further supporting a role in cell cycle, and pointing to a function in organizing the microtubule cytoskeleton. In addition, Cyclin F regulates the RRM2 subunit of ribonucleotide reductase [58], histone mRNA stem loop binding protein SLBP [59], and the DNA replication protein Cdc6 [60], highlighting a role in S-phase progression and genome stability.

Importantly, Cyclin F regulates the degradation of Cdh1, the substrate receptor for the APC/C ubiquitin ligase (**Figure 6**). APC/C^{Cdh1} is activated throughout G1-phase and its inactivation is critical for S-phase entry. Thus, Cyclin F-mediated degradation of Cdh1 was shown to play a critical role in entry into S-phase [61]. Interestingly, in addition to targeting the APC/C substrate receptor Cdh1 for degradation, Cyclin F is also a substrate of APC/C in mitosis and early G1-phase [61]. Thus, Cyclin F exists in a double-negative feedback loop with APC/C, where it is a substrate in mitosis and early G1, and then the regulator of Cdh1 degradation in late G1 and S-phase (**Figure 6**).

Like Skp2, Cyclin F is also phosphorylated by the oncogenic kinase AKT [62]. Similar to Skp2, the phosphorylation of Cyclin F by AKT leads to a significant increase in Cyclin F stability. Phosphorylation by AKT enhances Cyclin F assembly into SCF ligase complexes. Thus, phosphorylation contributes to the switch in Cyclin F, from being an APC/C substrate to being capable of targeting for Cdh1 degradation in late G1-phase [62]. The tight regulation of Cyclin F throughout the cell cycle, its substrates, phosphorylation by AKT, and regulation by other E3s, point to its critical role in cell cycle progression. Moreover, these results suggest that Cyclin F is a key regulatory node mediating the interaction between AKT-dependent growth factor signaling and the core cell cycle machinery.

3.4 FBXW7/FBW7/FBXO30

The SCF^{Fbxw7} ubiquitin ligase (also called SCF^{FBW7} or SCF^{FBXO30}) is the most tightly linked to cancer proliferation of all SCF-type E3s [63]. Fbxw7, is highly mutated in human cancers, and exhibits both truncating mutations throughout its gene body, as well as "hotspot" point mutations in its substrate binding motif. Interestingly, while Fbxw7 is generally considered a tumor suppressor [64], "hotspot" mutations are more commonly found in oncogenes, such as the common G12V mutation recurrently observed in oncogenic K-Ras in many human malignancies. SCF^{Fbxw7} promotes cell cycle progression by regulating the degradation of
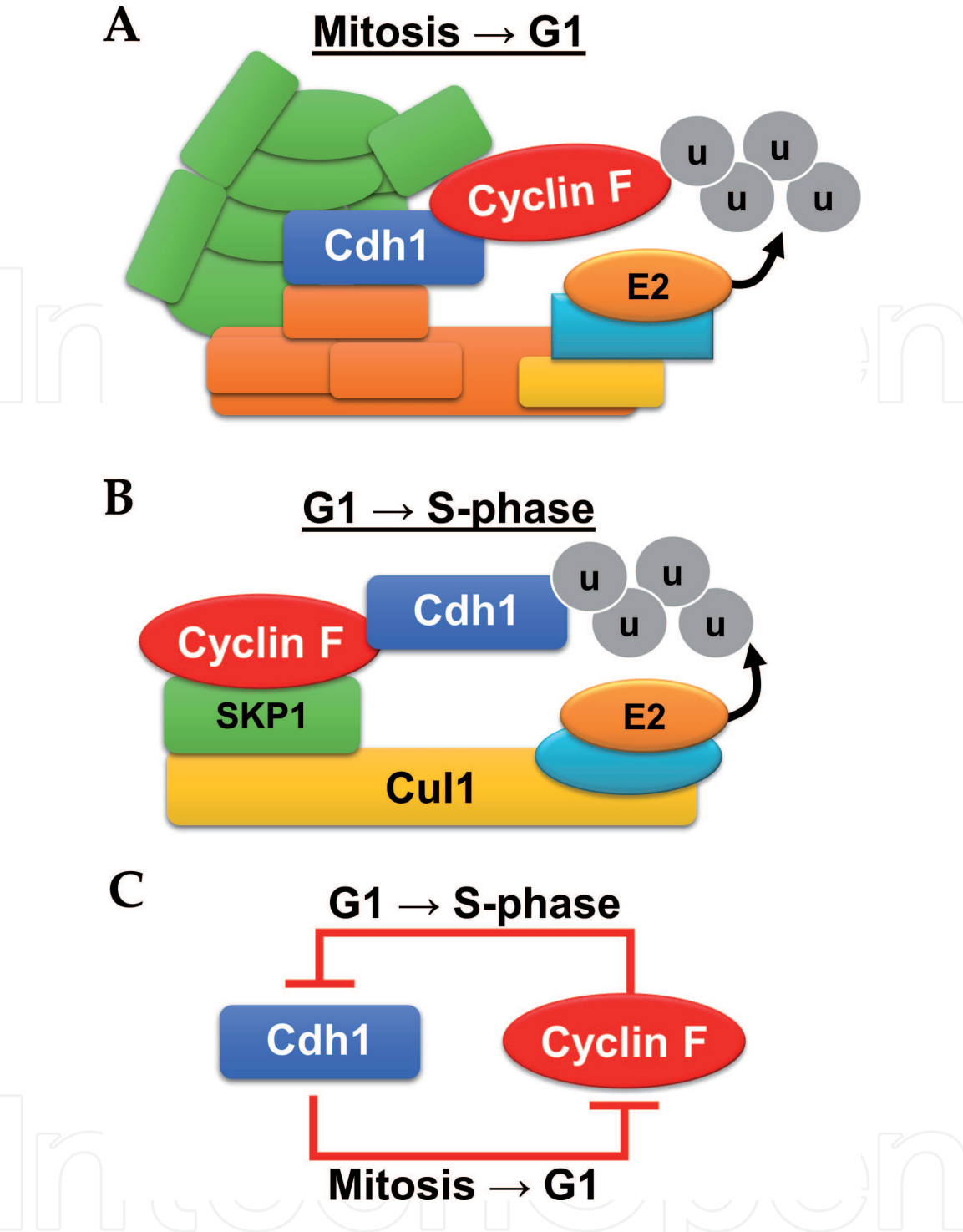
Figure 6.
SCF^{Cyclin F} and APC/C constitute a double-negative feedback loop. (A) APC/C^{Cdh1} targets Cyclin F for degradation in late mitosis and early G1. (B) SCF^{Cyclin F} targets Cdh1 for degradation in late G1 and S-phase. (C) Together, this suggests a temporally ordered, double negative feedback loop that promotes S-phase entry.

Cyclin E, the key activator of CDK2 at the G1/S boundary [63, 65–67]. In addition, Fbxw7 regulates the ubiquitination and destruction of numerous other pro-proliferative and cancer associated proteins, including Myc [68, 69], Notch [70, 71] and Jun [72].

Similar to other SCF ligases, the SCF^{Fbxw7} ligase recognizes substrates through phospho-degron motifs, with the most well characterized being that on Cyclin E. The phosphorylation of Cyclin E, by CDK2 or GSK3, can promote the degradation of Cyclin E by enhancing its binding to Fbxw7 [64, 66, 67, 73]. In addition, Fbxw7 homo-dimerizes, and this dimerization plays an important role in its ability to target substrates for degradation [74].

3.5 EMI1/Fbx05

Emi1 is a cell cycle regulated F-box domain-containing protein. However, Emi1 is unique among F-box proteins in that it has no known substrates, despite the fact that it binds tightly to the SCF adaptor Skp1. Emi1 is instead a key regulator of the cell cycle E3 ligase APC/C [75].

Many studies have demonstrated the potent and extensive role that Emi1 plays in inhibiting APC/C. Emi1 acts as a pseudo-substrate for APC/C, blocking the binding and ubiquitination of substrates [76]. In addition, Emi1 can alter the binding of the APC/C E2 ubiquitin conjugating enzymes, providing additional layers of regulation [77–79].

The association of Emi1 with S-phase entry is complex. Based largely on gain-of-function approaches, Emi1 was shown capable of inhibiting APC/C at the G1/S boundary and promoting S-phase entry [80]. This was fitting, since Emi1 abundance is controlled by the E2F family of transcription factors, which are activated in mid G1 and promote G1/S [80]. However, loss of Rca1, the fly version of Emi1, leads to an accumulation of cells in later stages of the cell cycle, not at G1/S [81]. Similarly, the loss of Emi1 in human cells was reported to induce the reactivation of APC/C during S and G2-phase, and to induce DNA re-replication as a result of the degradation of proteins which normally restrain licensing of replication origins [82, 83]. However, consistent with early gain-of-function studies, recent single cell approaches suggest that Emi1 contributes to the kinetics of APC/C inactivation at G1/S, and that Emi1 locks APC/C in an off state once S-phase begins [16]. Surprisingly, Emi1 might also be a substrate of the APC/C [84]. If Emi1 is a substrate of APC/C, this implies that Emi1 could be ubiquitinated by APC/C in early G1, and that it later accumulates as an inhibitor to inactivate APC/C and promote S-phase entry, much like Cyclin F [84]. This adds to our understanding of Emi1 degradation, wherein previous studies had shown it was degraded in mitosis by the SCF^{bTRCP} ubiquitin ligase [85, 86]. It will be important in the future to determine if altering the ubiquitination and degradation of Emi1 by APC/C accelerates progression through G1/S and to determine how this is coordinated with other SCF ligases that regulate G1/S.

4. Involvement of APC/C in G1/S

An extensive body of evidence has defined the role of APC/C^{Cdh1} in G1/S control [17]. Early studies in yeast showed that Cyclin proteolysis starts in late mitosis but then persists as cells continue through G1-phase [87]. In addition, yeast cells lacking Cdh1 are defective at arresting in G1-phase. Similar results have been observed across all eukaryotes in which loss of Cdh1 has been studied, including worms [88, 89], flies [90], chickens [91], mice [92] and humans [16, 61, 93]. The loss of Cdh1 accelerates progression through G0/G1 and promotes the start of S-phase. In addition, cells lacking Cdh1 are universally defective in G0/G1 arrest [17]. Accordingly, single allelic loss of Cdh1, the APC/C substrate receptor/coactivator in G0/G1-phase, is sufficient to cause tumors in mice [94]. Since the APC/C controls the stability of many dozens of substrates, it is unlikely that any one provides the basis for how cells enter S-phase in the absence of G1 APC/C function. Instead, it is more likely that the concerted upregulation of many cell cycle drivers together provides an explanation for the vital role of APC/C in restraining G1/S. Nevertheless, the APC/C is among a small group of key signaling molecules that prevent entry into S-phase of the cell cycle. These regulators include the retinoblastoma tumor suppressor and its related proteins p107 and p130, as well as the CDK inhibitors p21, p27 and p57.

Myriad mechanisms account for the inactivation of APC/C at the G1/S boundary, some of which were discussed above. This includes the degradation of Cdh1 by SCF$^{Cyclin F}$ and perhaps by the APC/C itself [61, 95]. The APC/C E2 enzymes, Ube2S and Ube2C, are unstable proteins and are also APC/C substrates [4, 96]. The substrate receptor Cdh1 is subject to CDK dependent phosphorylation, preventing its association with the APC/C and likely affecting its localization [89, 97–101]. Finally, accumulation of Emi1 is controlled by E2F, contributing to APC/C inhibition [16, 80, 84].

5. Distilling the complexity of ubiquitination in G1/S

The interconnected web of enzymes, substrates, and pathways discussed above paints a complicated picture of G1/S control. Remarkably, our understanding of the role of ubiquitin ligases in S-phase entry pales in comparison to studies performed on parallel kinase signaling cascades that converge on the E2F transcription factor. In quiescent and early G1 cells, E2F activity is repressed by the retinoblastoma tumor suppressor (RB), as well as the RB-like proteins P130 and P107. The phosphorylation of RB, first by Cyclin D-CDK4/6, and then by Cyclin E-Cdk2, inactivates RB and derepresses E2F. This derepression, in turn, triggers the transcriptional upregulation of many genes needed for S-phase entry.

How then do the pathways described above fit together with each other, and with the canonical CDK-RB-E2F pathway? We propose that multiple pathways act coordinately to promote the start of DNA replication. The most well-studied of these is the RB-E2F pathway, which promotes S-phase entry by promoting the expression of numerous cell cycle genes. In parallel, ubiquitin signaling pathways that control the degradation of numerous cell cycle proteins coordinate entry into S-phase. First, SCF^{Skp2} must be active and able to promote the degradation of CKI proteins. Second, SCF^{Fbw7} must be inactive or otherwise unable to ubiquitinate its substrates Cyclin E and Myc, which accumulate to promote cell cycle. Third, SCF$^{Cyclin F}$ must be available to trigger the degradation of Cdh1 and help promote the inactivation of APC/C. And finally, the APC/C must be inactivated, by Cyclin F and other pathways, allowing for the accumulation of cell cycle proteins (many of which are transcribed by E2F), to promote S-phase entry (**Figure** 7). It is notable that Cyclin F and Skp2, as well as many other cell cycle proteins, are downregulated by APC/C. Altogether, this suggests that aberrant APC/C inactivation could promote cancer cell cycles. Accordingly, single allelic loss of Cdh1 causes cancer in mice [94]. How APC/C might be inactivated in cancer remains an open question of significant importance that has only recently begun to be studied [17].

Upstream of these regulators are myriad kinase signaling cascades. These kinase cascades include, for example, the phosphorylation of RB by CDK4/6 and also CDK2; phosphorylation of Cyclin F and Skp2 by AKT; and, phosphorylation of Myc and Cyclin E, thereby marking them for degradation by Fbw7. Significantly, we hypothesize that these pathways control S-phase entry by globally remodeling the protein landscape either through changes in gene expression or protein degradation. The activity of CDK2, CDK4/6 and AKT is dysregulated in many cancers. This suggests that dysregulated cell cycle transcription, as well as dysregulated cell cycle ubiquitination, likely contributes to a weakening of the G1/S boundary and uncontrolled cancer cell cycles.

Testing this hypothesis and determining how these pathways are integrated remains an important question for future study. Determining the order of and
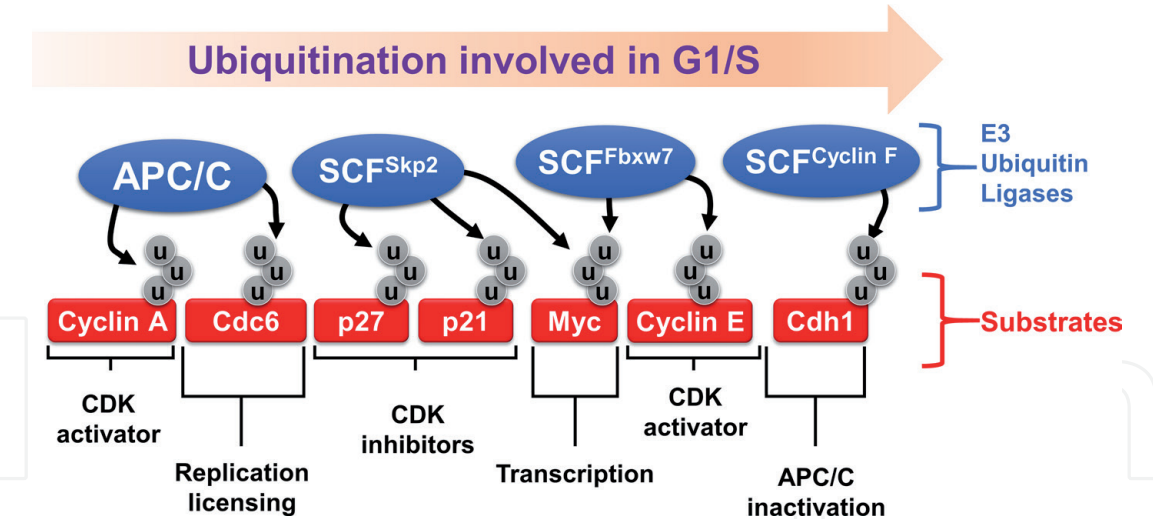
Figure 7.
Overview of ubiquitin signaling pathways involved in G1/S. A subset of substrates are shown. Note that the APC/C controls the stability of several dozen substrate proteins during late mitosis and early G1.

integration between these pathways is also critical. For example, recent live imaging studies demonstrated that CDK2 becomes active in mid-G1, several hours before APC/C is turned off. Moreover, these studies indicate that APC/C inactivation occurs at nearly the same time as DNA replication [16]. What is unclear is how Emi1, Cyclin F, and Skp2 accumulate at this time, as these proteins have never before been studied together in the same experimental system. In addition, the overwhelming majority of studies that have interrogated the kinetics of their accumulation have relied on bulk biochemical measurements (immunoblots) in synchronized cells. While informative, these studies would be better undertaken in asynchronous cells using either immunofluorescence or live cell reporters. Further, CDK2 activity begins to increase many hours before the inactivation of APC/C. It is therefore unknown how APC/C remains active into late G1-phase and is protected from CDK-dependent inactivation. Resolving these important questions will provide insight regarding how cells breach the G1/S boundary during the homeostatic cell cycles that occur during organismal development and growth, or in response to cell damage or wounding. Importantly, the G1/S boundary is universally dysfunctional in cancer and is the target of therapeutic interventions in the treatment of disease. Therefore, unraveling the complex pathways and mechanisms by which the ubiquitin system contributes to G1/S will shed light on both the etiology and treatment of cancer in the future.

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Conflict of interest

The authors declare no competing conflicts of interest.

Author details

Michael James Emanuele* and Taylor Paige Enrico
The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

*Address all correspondence to: emanuele@email.unc.edu

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References

[1] Williamson A, Werner A, Rape M. The colossus of ubiquitylation: Decrypting a cellular code. Molecular Cell. 2013;**49**:591-600

[2] Yau R, Rape M. The increasing complexity of the ubiquitin code. Nature Cell Biology. 2016;**18**:579-586

[3] Matsumoto ML, Wickliffe KE, Dong KC, Yu C, Bosanac I, Bustos D, et al. K11-linked polyubiquitination in cell cycle control revealed by a K11 linkage-specific antibody. Molecular Cell. 2010;**39**:477-484

[4] Williamson A, Wickliffe KE, Mellone BG, Song L, Karpen GH, Rape M. Identification of a physiological E2 module for the human anaphase-promoting complex. Proceedings of the National Academy of Sciences of the United States of America. 2009;**106**:18213-18218

[5] Yau RG, Doerner K, Castellanos ER, Haakonsen DL, Werner A, Wang N, et al. Assembly and function of heterotypic ubiquitin chains in cell-cycle and protein quality control. Cell. 2017;**0**:1-16

[6] Meyer H-J, Rape M. Enhanced protein degradation by branched ubiquitin chains. Cell. 2014;**157**:910-921

[7] Zhang J, Wan L, Dai X, Sun Y, Wei W. Functional characterization of anaphase promoting complex/ Cyclosome (APC/C) E3 ubiquitin ligases in tumorigenesis. Biochimica et Biophysica Acta. 2014;**1845**:277-293

[8] Potapova TA, Daum JR, Pittman BD, Hudson JR, Jones TN, Satinover DL, et al. The reversibility of mitotic exit in vertebrate cells. Nature. 2006;**440**:954-958

[9] Sherr CJ, Bartek J. Cell cycle–targeted Cancer therapies. Annual Review of Cancer Biology. 2017;**1**:41-57

[10] Petroski MD, Deshaies RJ. Function and regulation of cullin-RING ubiquitin ligases. Nature Reviews. Molecular Cell Biology. 2005;**6**:9-20

[11] Bai C, Richman R, Elledge SJ. Human cyclin F. The EMBO Journal. 1994;**13**:6087-6098

[12] Bai C, Sen P, Hofmann K, Ma L, Goebl M, Harper JW, et al. SKP1 connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box. Cell. 1996;**86**:263-274

[13] Skowyra D, Craig KL, Tyers M, Elledge SJ, Harper JW. F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex. Cell. 1997;**91**:209-219

[14] Feldman RM, Correll CC, Kaplan KB, Deshaies RJ. A complex of Cdc4p, Skp1p, and Cdc53p/cullin catalyzes ubiquitination of the phosphorylated CDK inhibitor Sic1p. Cell. 1997;**91**:221-230

[15] Brandeis M, Hunt T. The proteolysis of mitotic cyclins in mammalian cells persists from the end of mitosis until the onset of S phase. The EMBO Journal. 1996;**15**:5280-5289

[16] Cappell SD, Chung M, Jaimovich A, Spencer SL, Meyer T. Irreversible APCCdh1 inactivation underlies the point of No return for cell-cycle entry. Cell. 2016;**166**:167-180

[17] Kernan J, Bonacci T, Emanuele MJ. Who guards the guardian? Mechanisms that restrain APC/C during the cell cycle. Biochimica et Biophysica Acta (BBA)—Molecular Cell Research. 2018

[18] Murray AW, Solomon MJ, Kirschner MW. The role of cyclin synthesis and degradation in the control of maturation

promoting factor activity. Nature. 1989;**339**:280-286

[19] Sudakin V, Ganoth D, Dahan A, Heller H, Hershko J, Luca FC, et al. The cyclosome, a large complex containing cyclin-selective ubiquitin ligase activity, targets cyclins for destruction at the end of mitosis. Molecular Biology of the Cell. 1995;**6**:185-197

[20] King RW, Peters JM, Tugendreich S, Rolfe M, Hieter P, Kirschner MW. A 20S complex containing CDC27 and CDC16 catalyzes the mitosis-specific conjugation of ubiquitin to cyclin B. Cell. 1995;**81**:279-288

[21] Hartwell LH, Culotti J, Pringle JR, Reid BJ. Genetic control of the cell division cycle in yeast. Science. 1974;**183**:46-51

[22] Schwob E, Böhm T, Mendenhall MD, Nasmyth K. The B-type cyclin kinase inhibitor p40SIC1 controls the G1 to S transition in S cerevisiae. Cell. 1994;**79**:233-244

[23] Cappell SD, Baker R, Skowyra D, Dohlman HG. Systematic analysis of essential genes reveals important regulators of G protein signaling. Molecular Cell. 2010

[24] Chou S, Huang L, Liu H. Fus3-regulated Tec1 degradation through SCFCdc4 determines MAPK signaling specificity during mating in yeast. Cell. 2004

[25] Vendrell A, Martánez-Pastor M, González-Novo A, Pascual-Ahuir A, Sinclair DA, Proft M, et al. Sir2 histone deacetylase prevents programmed cell death caused by sustained activation of the Hog1 stress-activated protein kinase. EMBO Reports. 2011

[26] Hurst JH, Dohlman HG. Dynamic ubiquitination of the mitogen-activated protein kinase kinase (MAPKK) Ste7 determines mitogen-activated protein

kinase (MAPK) specificity. The Journal of Biological Chemistry. 2013

[27] Drury LS, Perkins G, Diffley JFX. The Cdc4/34/53 pathway targets Cdc6p for proteolysis in budding yeast. The EMBO Journal. 1997

[28] Edenberg ER, Vashisht AA, Topacio BR, Wohlschlegel JA, Toczyski DP. Hst3 is turned over by a replication stress-responsive SCFCdc4 phospho-degron. Proceedings of the National Academy of Sciences of the United States of America. 2014;**111**:5962-5967

[29] Lyons NA, Morgan DO. Cdk1-dependent destruction of Eco1 prevents cohesion establishment after S phase. Molecular Cell. 2011

[30] Kishi T, Ikeda A, Nagao R, Koyama N. The SCFCdc4 ubiquitin ligase regulates calcineurin signaling through degradation of phosphorylated Rcn1, an inhibitor of calcineurin. Proceedings of the National Academy of Sciences of the United States of America. 2007

[31] Liu Q, Larsen B, Ricicova M, Orlicky S, Tekotte H, Tang X, et al. SCFCdc4 enables mating type switching in yeast by Cyclin-dependent kinase-mediated elimination of the Ash1 transcriptional repressor. Molecular and Cellular Biology. 2011

[32] Pagano M, Tam SW, Theodoras AM, Beer-Romero P, Del Sal G, Chau V, et al. Role of the ubiquitin-proteasome pathway in regulating abundance of the cyclin-dependent kinase inhibitor p27. Science. 1995;**269**:682-685

[33] Carrano AC, Eytan E, Hershko A, Pagano M. SKP2 is required for ubiquitin-mediated degradation of the CDK inhibitor p27. Nature Cell Biology. 1999;**1**:193-199

[34] Montagnoli A, Fiore F, Eytan E, Carrano AC, Draetta GF, Hershko A, et al. Ubiquitination of p27 is regulated

by Cdk-dependent phosphorylation and trimeric complex formation. Genes & Development. 1999

[35] Bornstein G, Bloom J, Sitry-Shevah D, Nakayama K, Pagano M, Hershko A. Role of the SCFSkp2 ubiquitin ligase in the degradation of p21Cip1 in S phase. The Journal of Biological Chemistry. 2003

[36] Kamura T, Hara T, Kotoshiba S, Yada M, Ishida N, Imaki H, et al. Degradation of p57Kip2 mediated by SCFSkp2-dependent ubiquitylation. Proceedings of the National Academy of Sciences of the United States of America. 2003

[37] Abbas T, Sivaprasad U, Terai K, Amador V, Pagano M, Dutta A. PCNA-dependent regulation of p21 ubiquitylation and degradation via the CRL4Cdt2 ubiquitin ligase complex. Genes & Development. 2008;**22**:2496-2506

[38] Tedesco D, Lukas J, Reed SI. The pRb-related protein p130 is regulated by phosphorylation-dependent proteolysis via the protein-ubiquitin ligase SCFSkp2. Genes & Development. 2002;**16**:2946-2957

[39] Bhattacharya S, Garriga J, Calbó J, Yong T, Haines DS, Graña X. SKP2 associates with p130 and accelerates p130 ubiquitylation and degradation in human cells. Oncogene. 2003;**22**:2443-2451

[40] Zheng N, Zhou Q, Wang Z, Wei W. Recent advances in SCF ubiquitin ligase complex: Clinical implications. Biochimica et Biophysica Acta. 2016;**1866**:12-22

[41] Chan C-H, Morrow JK, Li C-F, Gao Y, Jin G, Moten A, et al. Pharmacological inactivation of Skp2 SCF ubiquitin ligase restricts cancer stem cell traits and cancer progression. Cell. 2013;**154**:556-568

[42] Wu L, Grigoryan AV, Li Y, Hao B, Pagano M, Cardozo TJ. Specific small molecule inhibitors of Skp2-mediated p27 degradation. Chemistry & Biology. 2012;**19**:1515-1524

[43] Tansey WP. Mammalian MYC proteins and cancer. New Journal of Science. 2014;**2014**:1-27

[44] Von Der Lehr N, Johansson S, Wu S, Bahram F, Castell A, Cetinkaya C, et al. The F-box protein Skp2 participates in c-Myc proteosomal degradation and acts as a cofactor for c-Myc-regulated transcription. Molecular Cell. 2003;**11**:1189-1200

[45] Kim SY, Herbst A, Tworkowski KA, Salghetti SE, Tansey WP. Skp2 regulates Myc protein stability and activity. Molecular Cell. 2003;**11**:1177-1188

[46] Geng F, Wenzel S, Tansey WP. Ubiquitin and proteasomes in transcription. Annual Review of Biochemistry. 2012;**81**:177-201

[47] Jaenicke LA, von Eyss B, Carstensen A, Wolf E, Xu W, Greifenberg AK, et al. Ubiquitin-dependent turnover of MYC antagonizes MYC/PAF1C complex accumulation to drive transcriptional elongation. Molecular Cell. 2016;**61**:54-67

[48] Bashir T, Dorrello NV, Amador V, Guardavaccaro D, Pagano M. Control of the SCFSkp2–Cks1 ubiquitin ligase by the APC/CCdh1 ubiquitin ligase. Nature. 2004;**428**:190-193

[49] Wei W, Ayad NG, Wan Y, Zhang G-J, Kirschner MW, Kaelin WG. Degradation of the SCF component Skp2 in cell-cycle phase G1 by the anaphase-promoting complex. Nature. 2004;**428**:194-198

[50] Gao D, Inuzuka H, Tseng A, Chin RY, Toker A, Wei W. Phosphorylation by Akt1 promotes cytoplasmic localization of Skp2 and impairs APCCdh1-mediated

Skp2 destruction. Nature Cell Biology. 2009;**11**:397-408

[51] Liu P, Begley M, Michowski W, Inuzuka H, Ginzberg M, Gao D, et al. Cell-cycle-regulated activation of Akt kinase by phosphorylation at its carboxyl terminus. Nature. 2014

[52] Chan C-H, Li C-F, Yang W-L, Gao Y, Lee S-W, Feng Z, et al. The Skp2-SCF E3 ligase regulates Akt ubiquitination, glycolysis, herceptin sensitivity, and tumorigenesis. Cell. 2012;**149**:1098-1111

[53] D'Angiolella V, Esencay M, Pagano M. A cyclin without cyclin-dependent kinases: Cyclin F controls genome stability through ubiquitin-mediated proteolysis. Trends in Cell Biology. 2013;**23**:135-140

[54] Fischer M, Grossmann P, Padi M, DeCaprio JA. Integration of TP53, DREAM, MMB-FOXM1 and RB-E2F target gene analyses identifies cell cycle gene regulatory networks. Nucleic Acids Research. 2016;**44**:6070-6086

[55] Tetzlaff MT, Bai C, Finegold M, Harper JW, Mahon KA, Stephen J, et al. Cyclin F disruption compromises placental development and affects normal cell cycle execution. Molecular and Cellular Biology. 2004;**24**:2487-2498

[56] D'Angiolella V, Donato V, Vijayakumar S, Saraf A, Florens L, Washburn MP, et al. SCF Cyclin F controls centrosome homeostasis and mitotic fidelity through CP110 degradation. Nature. 2010;**466**:138-142

[57] Emanuele MJ, Elia AEH, Xu Q, Thoma CR, Izhar L, Leng Y, et al. Global identification of modular cullin-RING ligase substrates. Cell. 2011;**147**:459-474

[58] D'Angiolella V, Donato V, Forrester FM, Jeong Y, Pellacani C, Kudo Y, et al. Cyclin F-mediated degradation of ribonucleotide reductase M2 controls genome integrity and DNA repair. Cell. 2012;**149**:1023-1034

[59] Dankert JF, Rona G, Clijsters L, Geter P, Skaar JR, Bermudez-Hernandez K, et al. Cyclin F-mediated degradation of SLBP limits H2AX accumulation and apoptosis upon genotoxic stress in G2. Molecular Cell. 2016

[60] Walter D, Hoffmann S, Komseli E-S, Rappsilber J, Gorgoulis V, Sørensen CS. SCFCyclin F-dependent degradation of CDC6 suppresses DNA re-replication. Nature Communications. 2016;**7**:10530

[61] Choudhury R, Bonacci T, Arceci A, Lahiri D, Mills CA, Kernan JL, et al. APC/C and SCF(cyclin F) constitute a reciprocal feedback circuit controlling S-phase entry. Cell Reports. 2016;**16**:3359-3372

[62] Choudhury R, Bonacci T, Wang X, Truong A, Arceci A, Zhang Y, et al. The E3 ubiquitin ligase SCF(Cyclin F) transmits AKT signaling to the cell-cycle machinery. Cell Reports. 2017;**20**:3212-3222

[63] Welcker M, Clurman BE. FBW7 ubiquitin ligase: A tumour suppressor at the crossroads of cell division, growth and differentiation. Nature Reviews. Cancer. 2008;**8**:83-93

[64] Koepp DM, Schaefer LK, Ye X, Keyomarsi K, Chu C, Harper JW, et al. Phosphorylation-dependent ubiquitination of cyclin E by the SCFFbw7 ubiquitin ligase. Science. 2001;**294**:173-177

[65] Strohmaier H, Spruck CH, Kaiser P, Won KA, Strohmaier H, Reed SI. Archipelago regulates Cyclin E levels in Drosophila and is mutated in human cancer cell lines. Nature. 2001

[66] Strohmaier H, Spruck CH, Kaiser P, Won KA, Sangfelt O, Reed SI. Human F-box protein hCdc4 targets cyclin E for

proteolysis and is mutated in a breast cancer cell line. Nature. 2001

[67] Welcker M, Singer J, Loeb KR, Grim J, Bloecher A, Gurien-West M, et al. Multisite phosphorylation by Cdk2 and GSK3 controls cyclin E degradation. Molecular Cell. 2003

[68] Welcker M, Orian A, Jin J, Grim JE, Harper JW, Eisenman RN, et al. The FBW7 tumor supressor regulates glycogen synthase kinase 3 phosphorylation-dependent c-Myc protein degradation. PNAS. 2004

[69] Yada M, Hatakeyama S, Kamura T, Nishiyama M, Tsunematsu R, Imaki H, et al. Phosphorylation-dependent degradation of c-Myc is mediated by the F-box protein Fbw7. The EMBO Journal. 2004

[70] Wu G, Lyapina S, Das I, Li J, Gurney M, Pauley A, et al. SEL-10 is an inhibitor of notch signaling that targets notch for ubiquitin-mediated protein degradation. Molecular and Cellular Biology. 2001

[71] Gupta-Rossi N, Le Bail O, Gonen H, Brou C, Logeat F, Six E, et al. Functional interaction between SEL-10, an F-box protein, and the nuclear form of activated Notch1 receptor. The Journal of Biological Chemistry. 2001

[72] Nateri AS, Riera-Sans L, Da Costa C, Behrens A. The ubiquitin ligase SCFFbw7 antagonizes apoptotic JNK signaling. Science. 2004:80

[73] Won KA, Reed SI. Activation of cyclin E/CDK2 is coupled to site-specific autophosphorylation and ubiquitin-dependent degradation of cyclin E. The EMBO Journal. 1996

[74] Welcker M, Larimore EA, Swanger J, Bengoechea-Alonso MT, Grim JE, Ericsson J, et al. Fbw7 dimerization determines the

specificity and robustness of substrate degradation. Genes & Development. 2013;**27**:2531-2536

[75] Reimann JDR, Freed E, Hsu JY, Kramer ER, Peters JM, Jackson PK. Emi1 is a mitotic regulator that interacts with Cdc20 and inhibits the anaphase promoting complex. Cell. 2001;**105**:645-655

[76] Miller JJ, Summers MK, Hansen DV, Nachury MV, Lehman NL, Loktev A, et al. Emi1 stably binds and inhibits the anaphase-promoting complex/cyclosome as a pseudosubstrate inhibitor. Genes & Development. 2006

[77] Frye JJ, Brown NG, Petzold G, Watson ER, Grace CRR, Nourse A, et al. Electron microscopy structure of human APC/C(CDH1)-EMI1 reveals multimodal mechanism of E3 ligase shutdown. Nature Structural & Molecular Biology. 2013;**20**:827-835

[78] Wang W, Kirschner MW. Emi1 preferentially inhibits ubiquitin chain elongation by the anaphase-promoting complex. Nature Cell Biology. 2013;**15**:797-806

[79] Chang L, Zhang Z, Yang J, McLaughlin SH, Barford D. Atomic structure of the APC/C and its mechanism of protein ubiquitination. Nature. 2015

[80] Hsu JY, Reimann JDR, Sørensen CS, Lukas J, Jackson PK. E2F-dependent accumulation of hEmi1 regulates S phase entry by inhibiting APC(Cdh1). Nature Cell Biology. 2002;**4**:358-366

[81] Grosskortenhaus R, Sprenger F. Rca1 inhibits APC-Cdh1(Fzr) and is required to prevent cyclin degradation in G2, Dev. Cell. 2002;**2**:29-40

[82] Machida YJ, Dutta A. The APC/C inhibitor, Emi1, is essential for

prevention of rereplication. Genes & Development. 2007;**21**:184-194

[83] DiFiore B, Pines J. Defining the role of Emi1 in the DNA replication-segregation cycle. Chromosoma. 2008;**117**:333-338

[84] Cappell SD, Mark KG, Garbett D, Pack LR, Rape M, Meyer T. EMI1 switches from being a substrate to an inhibitor of APC/CCDH1 to start the cell cycle. Nature. 2018

[85] Margottin-Goguet F, Hsu JY, Loktev A, Hsieh HM, Reimann JDR, Jackson PK. Prophase destruction of Emi1 by the SCFβTrCP/Slimbubiquitin ligase activates the anaphase promoting complex to allow progression beyond prometaphase. Developmental Cell. 2003

[86] Guardavaccaro D, Kudo Y, Boulaire J, Barchi M, Busino L, Donzelli M, et al. Control of meiotic and mitotic progression by the F box protein β-Trcp1 in vivo. Developmental Cell. 2003

[87] Amon A, Irniger S, Nasmyth K. Closing the cell cycle circle in yeast: G2 cyclin proteolysis initiated at mitosis persists until the activation of G1 cyclins in the next cycle. Cell. 1994;**77**:1037-1050

[88] Fay DS, Keenan S, Han M. fzr-1 and lin-35/Rb function redundantly to control cell proliferation in C elegans as revealed by a nonbiased synthetic screen. Genes & Development. 2002;**16**:503-517

[89] The I, Ruijtenberg S, Bouchet BP, Cristobal A, Prinsen MBW, van Mourik T, et al. Rb and FZR1/Cdh1 determine CDK4/6-cyclin D requirement in C elegans and human cancer cells. Nature Communications. 2015;**6**:5906

[90] Buttitta LA, Katzaroff AJ, Edgar BA. A robust cell cycle control mechanism limits E2F-induced proliferation of terminally differentiated cells in vivo. The Journal of Cell Biology. 2010;**189**:981-996

[91] Sudo T, Ota Y, Kotani S, Nakao M, Takami Y, Takeda S, et al. Activation of Cdh1-dependent APC is required for G1 cell cycle arrest and DNA damage-induced G2 checkpoint in vertebrate cells. The EMBO Journal. 2001;**20**:6499-6508

[92] Sigl R, Wandke C, Rauch V, Kirk J, Hunt T, Geley S. Loss of the mammalian APC/C activator FZR1 shortens G1 and lengthens S phase but has little effect on exit from mitosis. Journal of Cell Science. 2009;**122**:4208-4217

[93] Yuan X, Srividhya J, De Luca T, Lee J-HE, Pomerening JR. Uncovering the role of APC-Cdh1 in generating the dynamics of S-phase onset. Molecular Biology of the Cell. 2014;**25**:441-456

[94] García-Higuera I, Manchado E, Dubus P, Cañamero M, Méndez J, Moreno S, et al. Genomic stability and tumour suppression by the APC/C cofactor Cdh1. Nature Cell Biology. 2008;**10**:802-811

[95] Listovsky T, Oren YS, Yudkovsky Y, Mahbubani HM, Weiss AM, Lebendiker M, et al. Mammalian Cdh1/Fzr mediates its own degradation. The EMBO Journal. 2004;**23**:1619-1626

[96] Rape M, Kirschner MW. Autonomous regulation of the anaphase-promoting complex couples mitosis to S-phase entry. Nature. 2004;**432**:588-595

[97] Kramer ER, Scheuringer N, Podtelejnikov AV, Mann M, Peters JM. Mitotic regulation of the APC activator proteins CDC20 and CDH1. Molecular Biology of the Cell. 2000;**11**:1555-1569

[98] Hall MC, Warren EN, Borchers CH. Multi-kinase phosphorylation of the APC/C activator Cdh1 revealed

by mass spectrometry. Cell Cycle. 2004;**3**:1278-1284

[99] Höckner S, Neumann-arnold L, Seufert W. Dual control by Cdk1 phosphorylation of the budding yeast APC/C ubiquitin ligase activator Cdh1. Molecular Biology of the Cell. 2016;**27**:2198-2212

[100] Zachariae W, Schwab M, Nasmyth K, Seufert W. Control of cyclin ubiquitination by CDK-regulated binding of Hct1 to the anaphase promoting complex. Science. 1998;**282**:1721-1724

[101] Jaquenoud M, Van Drogen F, Peter M. Cell cycle-dependent nuclear export of Cdh1p may contribute to the inactivation of APC/CCdh1. The EMBO Journal. 2002;**21**:6515-6526