Mechanism of cell-cycle control: ligating the ligase

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The F-box protein SKP2 promotes the G1–S transition by targeting key regulators for proteasomal degradation via its capacity to function as the specificity factor for the SKP1 Cullin F-box SCFSKP2 ubiquitin ligase. SKP2 is a labile protein, the levels of which oscillate in a cell cycle-dependent manner. SKP2 accumulation is often deregulated in cancer, which indicates that temporal control of SKP2 is essential for normal cell proliferation. Two new studies now suggest that SKP2 accumulation is determined by a second ubiquitin ligase, the anaphase-promoting complex or cyclosome, APC/C CDH1. These studies highlight a novel mechanism wherein mitotic machinery communicates with proteins that regulate G1 phase progression.

Regulated protein degradation is achieved by targeting proteins to the 26S proteasome via the covalent attachment of polyubiquitin chains. Ubiquitin conjugation is mediated by the sequential activities of an E1 enzyme or ubiquitin-activating enzyme, an E2 ubiquitin-conjugating enzyme and an E3 ubiquitin ligase; the E2 and E3 enzymes function to coordinate the transfer of ubiquitin to the substrate protein. The E3, or ubiquitin ligase, drives substrate specificity and, thus, has been of intense interest. Two distinct classes of ubiquitin ligases regulate cell-cycle progression (Figure 1): the SKP1 Cullin F-box (SCF) complex, which is implicated in G1–S progression, and the anaphase-promoting complex or cyclosome (APC/C), which is necessary for separation of sister chromatids at anaphase and for exit from M phase into G1 [1,2]. The prototypical SCF complex contains an F-box protein (SKP2 in the SCFSKP2 complex), which serves as an adaptor between the other components of the SCF complex (SKP1, Cullin 1 (CUL1) and Ring-box protein 1 (Rbx1)) and the target protein substrates to be ubiquitinated [3]. By acting as a specificity factor for the SCFSKP2 ubiquitin ligase, SKP2 targets several cell-cycle regulators for proteasomal degradation and promotes entry into S phase. SKP2 begins to accumulate in late G1, with maximal abundance during S and G2 phases [4]. Until recently, the degradation of SKP2 in G0 or G1 was thought to occur via an autocatalytic mechanism, in which SKP2 was auto-ubiquitinated within the SCF complex [5]. Now, two papers add another level of complexity to the mechanism of SKP2 regulation and places APC/C CDH1 (where CDH1 is the specificity subunit of APC/C) as a major regulator of SKP2 levels in G0 and G1 [6,7].

Substrates of the SCFSKP2 ubiquitin ligase
SKP2 was first identified as a protein that interacts with the cyclin A–cyclin-dependent kinase 2 (CDK2) complex [8]. Since then, it has been shown to target several cell-cycle regulators for proteasomal degradation. Substrate recognition by F-box proteins usually depends on specific phosphorylation of the substrate, providing a key level of regulation. Among the phosphorylated substrates that SKP2 recognizes are p27KIP1, p57KIP2, p21WAF1, pocket-protein p130 and cell-division cycle (CDC) 10-dependent transcript 1 (cdc10). The G1–S transition [4,9–12]. Perhaps the most intriguing SKP2 substrate is c-Myc, an oncogenic transcription factor involved in cell proliferation and survival. It is somewhat counterintuitive that an S-phase-promoting factor (SKP2) is responsible for the destruction of another S-phase-promoting protein (c-Myc). However, in addition to inducing its degradation, SKP2 activates c-Myc target genes by acting as a transcriptional cofactor in ubiquitinated c-Myc target promoters [13]. As a result, SKP2 also enhances c-Myc-induced S-phase transition.
Despite the ability of SKP2 to target several substrates for degradation, a recent study by Nakayama et al. [14] suggests that the CDK inhibitor p27KIP1 is the major downstream effector of the SCF\textsuperscript{SKP2} ubiquitin ligase. By generating Skp2\textsuperscript{−/−}p27\textsuperscript{−/−} mice, Nakayama et al. demonstrate that all abnormalities observed in Skp2\textsuperscript{−/−} mice, such as nuclear enlargement and polyploidy, are almost completely rescued in Skp2\textsuperscript{−/−}p27\textsuperscript{−/−} mice, which is consistent with p27\textsuperscript{KIP1} as the principal substrate of SCF\textsuperscript{SKP2}. In addition, Skp2\textsuperscript{−/−}p27\textsuperscript{−/−} mice exhibit phenotypes similar to p27\textsuperscript{−/−} mice (i.e. large body size and multiple organ hyperplasia) [14]. The observation that Skp2\textsuperscript{−/−}p27\textsuperscript{−/−} mice still have a small degree of polyploidy and that they are similar, but not identical, to the p27\textsuperscript{−/−} mice provides evidence that, although p27\textsuperscript{KIP1} is the main target of SCF\textsuperscript{SKP2}, other SCF\textsuperscript{SKP2} substrates might also exist in vivo.

Given that SKP2 regulates the accumulation of growth-regulatory proteins, it is not surprising that SKP2 is overexpressed in a variety of human cancers and is implicated in tumor progression. Transgenic mice with SKP2 expression limited to the prostate gland exhibit low-grade carcinoma [15]. Furthermore, overexpression of SKP2 is also observed in a variety of epithelial cancers, and high levels of SKP2 correlate with low levels of p27\textsuperscript{KIP1} [16]. The simultaneous activation of an oncogene (c-Myc) and destruction of cell-cycle inhibitors such as p27\textsuperscript{KIP1} might underlie the mechanism whereby SKP2 drives the G1–S phase transition and induces tumor progression. What has not been clear is how SKP2 expression is deregulated in human cancers. New work by Wei et al. [6] and Bashir et al. [7] provides clues on how overexpression of SKP2 in tumors might occur.

**SKP2 ubiquitination**

SKP2 expression is cell cycle regulated; expression is low during early G1 and rises during late G1 and early S phase. SKP2 accumulation peaks during S phase and drops as cells proceed through M phase and early G1. Strikingly, this pattern of SKP2 accumulation parallels that of APC/C substrates cyclin A and B, which indicates a potential connection between SKP2 loss and APC/C activity. Consistent with this notion, both groups noted the presence of potential ‘destruction boxes’ (D-box) – a stretch of amino acids (RxxLxxxx[N/D/E]) in the N terminus of SKP2 that is necessary for its degradation by the SCF\textsuperscript{SKP2} [2]. Although mutations within the D-box of SKP2 did not disrupt SKP2–APC/C association, they did prevent APC/C-dependent SKP2 proteolysis.

The activity and substrate specificity of the APC/C E3 ubiquitin ligase are derived from activator proteins CDC20 and CDH1, thus forming APC/C\textsuperscript{CDC20} and APC/C\textsuperscript{CDH1} ubiquitin ligase complexes. APC/C\textsuperscript{CDC20} is active in the G2 and M phases of the cell cycle, whereas APC/C\textsuperscript{CDH1} is active during mitotic exit and early G1 [2]. Wei et al. [6] demonstrate that SKP2 is a direct substrate for APC/C\textsuperscript{CDH1}. In vitro polyubiquitination and destruction of SKP2 were induced by APC/C\textsuperscript{CDH1} and, by contrast, were inhibited by an N-terminal fragment of cyclin B1 – a bona fide APC/C\textsuperscript{CDH1} substrate. In addition, CDH1 siRNA promoted accumulation of SKP2 in G1, downregulation of p27\textsuperscript{KIP1} and earlier S-phase entry as compared with untreated cells. Importantly, accumulation of SKP2 in G1 was dependent on its N-terminal D-box because mutation of this region stabilized SKP2 in G1 and inhibited its in vivo polyubiquitination by APC/C\textsuperscript{CDH1}.

Concurrently, Bashir et al. [7] also noted that SKP2 has five potential D-boxes, but mutation of only one D-box (the one located in the N terminus of SKP2) stabilizes SKP2. Similar to the work of Wei et al. [6], SKP2 polyubiquitination and degradation could be catalyzed by APC/C\textsuperscript{CDH1} in vitro and were dependent on the N-terminal D-box of SKP2. These results were confirmed in vivo using CDH1-specific siRNA.

In addition, Bashir et al. provide clear evidence for APC/C\textsuperscript{CDH1}-dependent regulation of CDC28 kinase subunit 1 (Cks1) proteolysis. Cks1 is an essential accessory factor that is required for p27\textsuperscript{KIP1} ubiquitination and degradation by the SCF\textsuperscript{SKP2} complex [17]. Similar to SKP2, the expression levels of Cks1 oscillate during the cell cycle and are low in G0 and G1 [7]. Bashir and co-workers also note that, as is the case for other APC/C substrates, Cks1 has a D-box in its C terminus. Mutation of this region prevented Cks1 degradation, whereas siRNA against CDH1 promoted Cks1 accumulation in G1. However, Bashir et al. could not demonstrate direct in vitro ubiquitination of Cks1 by the APC/C\textsuperscript{CDH1} complex as they could for SKP2, suggesting the absence of an essential accessory factor in the in vitro ubiquitination reactions, or that Cks1 is not a direct APC/C\textsuperscript{CDH1} substrate.

Previous work by Wirbelauer et al. [5] indicated that SKP2 is regulated by a Cul1-based core ubiquitin ligase complex containing Skp1, Cul1 and Rbx1 (an apo-SCFSKp2) via an autocatalytic mechanism. One important difference is that this study was performed in cells that entered quiescence by serum starvation [5]. By contrast, Wei et al. and Bashir et al. examine SKP2 regulation mostly in cycling cells following release from nocodazole (i.e. mitotic) block. The experiments of Bashir et al. [7] revealed that Cul1 depletion did not inhibit SKP2 destruction in serum-starved cells, whereas CDH1 ablation did promote SKP2 stabilization. By contrast, the experiments by Wirbelauer et al. [5] demonstrated SKP2 accumulation in serum-starved cells treated with Cul1 antisense oligonucleotides. Wei et al. [6] also found that a SKP2 mutant that cannot form SCF complexes is a better substrate for APC/C\textsuperscript{CDH1} than wild-type SKP2, hinting that regulation of SKP2 involves competition between APC/C\textsuperscript{CDH1} and apo-SCFSKp2 complexes. However, data indicating that free SKP2 is a better substrate for APC/C\textsuperscript{CDH1} are scant. In light of these results, if competition between apo-SCFSKp2 and APC/C\textsuperscript{CDH1} truly exists, more work will be needed to address which mechanism of SKP2 regulation is dominant in vivo.

**Concluding remarks and future perspectives**

Questions regarding the regulation of SKP2 by APC/C\textsuperscript{CDH1} still remain unanswered. A confounding fact is that APC/C\textsuperscript{CDH1} seems to be active in early G1, a period
of low cyclin-dependent kinase activity, and becomes inactive in S and G2 phases, a period of high CDK activity [2]. What, then, is the upstream signal that deactivates APC/C<sup>CDH1</sup> in G1 to enable SKP2 accumulation in late G1 and S phases? Identification of novel cyclin D–CDK4, cyclin E–CDK2 and cyclin A–CDK2 kinase substrates that modulate APC/C<sup>CDH1</sup> complex activity might provide insights for the answer. Finally, is this mechanism responsible for SKP2 overexpression in human cancers? The answer awaits the identification of SKP2 mutations from patient-derived tumors that impair SKP2 ubiquitination and destruction by APC/C<sup>CDH1</sup> (i.e. a SKP2 D-box or an APC/C<sup>CDH1</sup> binding-deficient mutant). Generation of transgenic mice in which SKP2 mutant versions that cannot be ubiquitinated by APC/C<sup>CDH1</sup> are expressed in specific epithelial organs, or are knocked in place of wild-type SKP2, could also reveal the role of this mechanism in tumor progression.

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Coiled coils meet the chaperone world

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Coiled coils are versatile structural modules that engage in a variety of cellular activities. Recent studies illuminate their role as substrate-binding elements in the chaperone cofactor prefoldin and in the AAA + ATPases involved in protein (un)folding processes. The use of coiled coils to mediate the binding of non-native proteins represents a novel strategy in chaperone design and a new function for coiled coils.

Coiled coils are widespread protein motifs that engage in a broad array of functions, primarily through the formation of large, mechanically rigid structures such as hair (keratin), blood clots (fibrin), extracellular and intracellular matrices (laminin and intermediate filaments), levers (myosin), spacers (murein lipoprotein) and protective surface layers (non-fimbrial adhesins). They are frequently encountered as oligomerization domains (leucine zippers) and their ability to expose extended surfaces with amphiphilic properties enables them to act as scaffolds for protein assemblies (e.g. tropomyosin and tetrabracion). They can undergo dynamic rearrangements either by refolding into an alternate structure (e.g. membrane fusion proteins) or by rigid-body motion around a hinge (e.g. the ‘thumbs’ and ‘arms’ of polymerases and topoisomerases). Built upon an arrangement of intertwined α helices, their exact properties depend on a combination of core (hydrophobic) and surface (hydrophilic) residue characteristics [1] (Figure 1). Recent studies have revealed a new function for coiled coils; in

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