Crashing waves of destruction: The cell cycle and APC^{Cdh1} regulation of SCF^{Skp2}

Coordination of events required for cell cycle progression is orchestrated in large part by the ubiquitin (Ub)-mediated destruction of key regulatory proteins such as cyclins and their inhibitors. Until now, the G1/S and mitotic phases of the cell cycle were thought to be controlled by discrete families of multisubunit Ub-ligases: SCF ligases controlled the G1 to S transition, whereas APC ligases controlled the onset and exit from mitosis. New work, published in the March 11 issue of *Nature*, challenges this concept by revealing that an essential function of APC is to limit SCF activity during the G1 phase of the cell cycle.

Orderly passage through the cell cycle is controlled by the well-timed appearance and disappearance of numerous regulatory proteins, most notably the cyclins and their inhibitors. One of the major forces driving the cell cycle is ubiquitin (Ub)-mediated proteolysis, a process in which covalent attachment of Ub to target proteins signals their destruction by the 26S proteasome. Specificity in proteolysis by the Ub-proteasome system is governed largely by the action of Ub-protein ligases (Ubls), multisubunit complexes that recognize "degrons" in substrate proteins and bring them face-to-face with core components of the ubiquitylation machinery. Two families of Ubls dominate cell duplication and division—SCF and APC—and until now these families have been thought to act independently as "waves of destruction" that regulate discrete phases in the cell cycle (Figure 1).

The SCF (*S*kp-*C*ullin-*F* box) family of Ubls consist of a number of core components—Skp1, Cul1, Roc1—in complex with a unique "F box" protein that confers substrate specificity. F box proteins have at least two functional domains—an ~40 amino acid F box domain that binds Skp1 and tethers the F box protein to the core SCF components, and a protein-protein interaction domain (often a series of leucine-rich repeats or WD40 domains) that specifically binds substrate proteins. A vast collection of F box proteins, each with different substrate specificity, exists in mammals, with proteins such as β-TrCP, hCDC4, and Skp2 being the best characterized (reviewed in Spruck and Strohmaier, 2002).

SCF-type Ubls have been show to regulate a variety of different processes from transcription through to development. Perhaps their most prominent role, however, is control of the G1/S transition of the cell cycle. Skp2-containing SCF complexes (SCF^{Skp2}), in particular, are fundamental to this transition, targeting the destruction of numerous regulatory proteins—including p27, p21, and p130—that inhibit S phase entry (reviewed in Reed, 2003). By destroying these S phase inhibitors, SCF^{Skp2} thus removes the S phase block, allowing DNA replication to proceed. Given its central role in the G1/S transition, it is not surprising that Skp2 behaves like an oncogene. Forced expression of Skp2 drives cellular transformation both in vitro and in vivo, and several human cancers, including carcinomas and lymphomas, display elevated Skp2 protein levels (Gstaiger et al., 2001). Increased expression of Skp2 in cancer patients correlates with reduced levels of the SCF^{Skp2} target protein p27, which in turn is an indicator of poor prognosis in such cancers (reviewed in Bloom and Pagano, 2003).

Like SCF, the APC, or anaphase-promoting complex, is a multisubunit Ubl that consists of a number of core components in complex with a unique substrate specificity factor. For APC, however, the math is a little different; there are 13 core components, but just two specificity factors—Cdc20 and Cdh1 (Reed, 2003). As its name implies, APC is intimately associated with mitosis, and Cdc20 and Cdh1 play distinct roles in various phases of cell division. Cdc20 is synthesized late in G2 and coordinates events required for the onset of mitosis through to anaphase. Cdh1, on the other hand, becomes active toward the end of anaphase and ultimately brings about the end of mitosis by targeting destruction of Cdc20, as well as mitotic cyclins and other regulators.

Unlike SCF^{Skp2}, there have been only a few reports linking APC to cancer. Genetic alterations in two subunits of the APC, CDC16 and CDC23, are found in some colon cancer cells (Wang et al., 2003). And recently it has been suggested that RASSF1A, a tumor suppressor that is frequently silenced in cancers, can act as an inhibitor of Cdc20, thus preventing mitotic progression due to premature APC activation (Song et al., 2004).

Although it has been satisfying to think of SCF and APC as two independent machines that drive distinct phases of the cell cycle, this view will now have to change. Two papers published in the March 11 issue of *Nature* (Bashir et al., 2004; Wei et al., 2004)
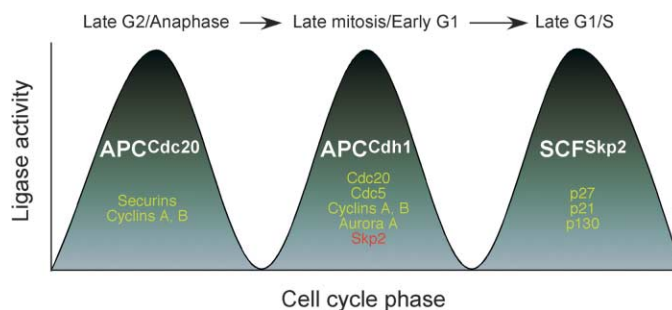

Figure 1. Waves of destruction

SCF and APC ubiquitin-ligases are important regulators of cell cycle transitions. As such, the cell cycle has been described as being driven by "waves" of Ub-dependent protein degradation (Wei et al., 2004). By destroying specific substrates, APC-type ligases regulate entry into and exit from mitosis, whereas SCF^{Skp2} controls entry into S phase. A subset of cell-cycle regulatory substrates for each Ubl is listed. Note that by targeting the SCF-component Skp2 for destruction, APC^{Cdh1} not only regulates exit from mitosis, but also controls the duration of G1. Because of the central role of Skp2 in timing the onset of S phase, loss of Skp2 regulation by APC^{Cdh1} could result in ectopic S phase entry and in the development of cancer.

blur the demarcation between SCF and APC by demonstrating that APC[Cdh1] targets destruction of Skp2 during the G1 phase of the cell cycle. This work is important because it challenges the compartmentalized view of Ubls and the cell cycle, and because it identifies a Ubl for a major G1/S regulator and oncoprotein, Skp2. Critically, however, the work is also profound because it establishes APC[Cdh1] as a major G1 regulator, controlling both entry into G1—by destruction of Cdc20 and mitotic cyclins—and, now, the duration of G1—by destruction of Skp2.

The experiments performed by each group were similar in approach and scope. During an investigation of cell cycle changes in Skp2 protein expression, Bashir et al. found that Skp2 is downregulated in late M and G1, and induced only as cells near S phase. This expression pattern correlated with protein half-life, with Skp2 turning over more rapidly in cells in late M/early G1 than those in prometaphase. Wei et al. provided similar data, demonstrating that lysates prepared from cells synchronized in G1 support rapid turnover of Skp2, whereas lysates prepared from cells synchronized in S phase did not. Both groups noted the similarity of Skp2 expression patterns to that of the mitotic cylins and demonstrated that Skp2 contains a functional "D box," one of two degrons recognized by the APC (Reed, 2003). Importantly, both groups provide strong and compelling data that APC[Cdh1] is indeed a Ub-protein ligase for Skp2; together, their experiments show that forced expression of Cdh1 promotes Skp2 proteolysis, that attenuating Cdh1 expression by siRNA stabilizes Skp2, that Skp2 and Cdh1 physically interact, and that immunopurified APC[Cdh1] can polyubiquitylate Skp2 in vitro.

Perhaps the most striking feature of all this destruction is the effect of the APC-SCF interaction on cell cycle control. Forms of Skp2 that are refractory to APC[Cdh1]-mediated destruction promoted a premature entry into S phase. Suppression of Cdh1 by siRNA (which in turn promotes accumulation of Skp2) increased the rate of p27 degradation and dramatically accelerated S phase entry. Amazingly, the ability of Cdh1 knockdown to stimulate the onset of DNA replication was entirely dependent on Skp2, revealing that Skp2 is an essential target through which APC[Cdh1] regulates the timing of the G1/S transition. These data firmly establish that APC[Cdh1] antagonizes SCF[Skp2] action during G1, and demonstrate convincingly that APC isn't just a regulator of mitosis anymore.

What is the relevance of this mode of regulation to cancer? Given that APC[Cdh1] targets Skp2 for destruction, and that Skp2 itself is very likely to be the product of an oncogene, it is tempting to speculate that Cdh1 may function as a tumor suppressor. As mentioned, evidence suggesting that Cdh1, or indeed any APC subunit, is dysregulated in cancer is scanty. One possible explanation is that APC is a central cog in the cell cycle machinery, and that it is difficult for cancer to select for mutations that accelerate the rate of cell proliferation without simply causing the entire cell cycle to crash. But it is also possible that we simply haven't been looking in the right place. Skp2 *is* overexpressed in some cancers, and although the mechanism is often attributed to gene amplification (Dowen et al., 2003), it seems plausible that stabilization of Skp2 could also be a mechanism of Skp2 activation in cancer. Based on the Bashir et al. and Wei et al. studies, we might predict that Cdh1 itself is disrupted in human malignancy, or at the very least that the APC[Cdh1]-Skp2 D box interaction is targeted by some cancer-relevant process. Thus, in addition to providing food for thought about how Ub-ligases can stretch their influence throughout the cell cycle, the reports by Bashir et al. and Wei et al. might just have given us the heads up on an unexpected mechanism targeted during oncogenesis.

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Selected reading

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