Cks1, a subunit of cyclin-dependent kinases, has now been identified as an essential cofactor in the ubiquitination of the Cdk inhibitor p27 by the SCF^Skp2 ubiquitin ligase. This activity, which can be independent of Cdk binding, links Cks to positive growth control pathways regulating the G1/S transition and to cancer.

How do Cks proteins control Cdk function? Much of our understanding of Cks function has come from analysis of mutants in yeast and from biochemical experiments in *Xenopus* extracts. Cks proteins, when associated with Cdks, do not act as inhibitors or activators in the classic sense, but seem to modulate substrate choice or the extent of phosphorylation. Certain Cks1 mutations lead to cell-cycle arrest in G1, and this appears to reflect defects in G1 cyclin-Cdk1 activity towards important substrates, such as the Cdk inhibitors Sic1 and Far1 [9,10].

Removal of Cks function, either in yeast or *Xenopus* extracts, can also lead to defects in the entry into, passage through and exit from mitosis [9,11]. In general, this seems to reflect the fact that Cks1 enhances phosphorylation of various mitotic Cdk1 substrates, including Cdc25, Wee1 and components of the anaphase-promoting complex. Precisely how Cks1 facilitates phosphorylation of Cdk targets is not known, but it is conceivable that certain phosphorylated proteins can interact with a putative phosphate-binding surface on Cks1 [8], and in this context, such target proteins are phosphorylated more efficiently by the associated Cdk. Finally, inactivation of mitosis-promoting factor (MPF) via destruction of cyclin B is rate limiting for exit from mitosis. Here, Cks1 also plays a role by helping target ubiquitinated cyclin B to the proteasome for degradation, as demonstrated in budding yeast [12]. Consistent with this, recent work in *Caenorhabditis elegans* [13] indicates that one of the two Cks proteins in this organism, Cks1, is also required for mitotic exit, as well as for completion of maternal meiotic divisions.

Now, just when we thought we were beginning to understand how Cks1 functions in mitosis, new data have emerged that link Cks1 to the destruction of the Cdk inhibitor p27 by the SCF^Skp2 ubiquitin ligase. p27 acts as a negative regulator of the G1–S transition by binding to cyclin E–Cdk2 and to cyclin A–Cdk2. These kinases control the S-phase program, and cyclin E–Cdk2 also controls centrosome duplication, although the molecular details of these activities are sketchy. Two mechanisms combine to eliminate p27 activity during G1 (reviewed in [14]; Figure 1). First, the assembly of cyclin D–Cdk4 in response to mitogens requires p27 or its relatives, p21 and p57. Accumulation of cyclin D–Cdk4–p27 occurs at the expense of a portion of the cyclin E–Cdk2–p27 complexes present in G1. Thus, partial activation of Cdk2 is achieved by sequestration of p27 by cyclin D–Cdk4. Partial Cdk2 activation then facilitates phosphorylation of p27 on threonine residue T187 [14]. This event targets p27 for ubiquitin-mediated proteolysis [15] through the SCF^Skp2 ubiquitin ligase [16–18], thereby eliminating Cdk2-associated p27.
SCF complexes are composed of Skp1, Cul1, Rbx1, and a member of the F-box family of proteins [18–21]. These complexes act as E3 ubiquitin ligases in conjunction with E2 ubiquitin conjugating enzymes and an E1 ubiquitin activating enzyme. F-box proteins are modular substrate specific receptors of ubiquitination targets. They interact with the core ubiquitin ligase through the F-box motif and with substrates via carboxy-terminal protein–protein interaction domains, including a leucine-rich repeat (LRR) domain in the case of Skp2 (Figure 2a). SCF substrates typically interact with their specific F-box proteins in a phosphorylation dependent manner. Several lines of evidence indicate a role for SCFSkp2 in p27 destruction. First, cell extracts lacking Skp2 — either derived from G1 cells or generated by Skp2 immunodepletion — are deficient in p27 ubiquitination activity, but addition of recombinant Skp2 reconstitutes phosphorylation-dependent ubiquitination [16,18]. Second, Skp2 interacts specifically with the T187 phosphorylated form of p27 in crude cell or reticulocyte extracts. Third, cells from Skp2−/− mice accumulate p27 in the T187 phosphorylated form, and this is reversed by expression of Skp2 [22]. Fourth, Skp2 immune complexes supplemented with E1 and E2 activity catalyze p27 ubiquitination [16].

Although these data support a role for Skp2 in p27 turnover, several questions have remained unanswered. Most notable was the fact that insect-cell-derived SCFSkp2 is inefficient at catalyzing p27 ubiquitination, unlike its yeast cousin SCFCdc4, which is responsible for ubiquitination of the Sic1 Cdk inhibitor [19,20]. Moreover, although T187-phosphorylated p27 can interact with Skp2 in crude extracts, it cannot do so with purified Skp2, indicating the existence of another factor or modification important for p27 ubiquitination. To solve this problem, Hershko, Pagano and co-workers [3] searched for proteins in crude cell extracts that activate p27 ubiquitination by recombinant SCFSkp2, and found none other than Cks1. An independent solution to this puzzle came from the work of Reed and co-workers [2], who found that deletion of Cks1 in the mouse leads to viable animals that are smaller than heterozygous littermates, a phenotype reminiscent of mice lacking Skp2 [22]. Analysis of Cks1−/− cells revealed dramatic accumulation of T187-phosphorylated p27. This suggested that p27 ubiquitination was being blocked at a step after its phosphorylation. Consistent with this, Cks1−/− extracts were defective in p27 ubiquitination, but the reaction could be reconstituted by addition of recombinant Cks1.

Previous work had demonstrated that ubiquitination of p27 by SCFSkp2 requires that it be assembled with either cyclin E–Cdk2 or cyclin A–Cdk2 [23,24]. As Cdk2 interacts with Cks1, one could imagine a scenario in which association of Cks1 with Cdk2 is required to assemble the p27–cyclin E–Cdk2 complex onto SCFSkp2. To examine this question, Spruck et al. [2] employed a mutant Cks1 protein that does not bind Cdk2, Cks1(E63Q). Surprisingly, this mutant protein supported p27 ubiquitination to
a level almost as high as wild-type Cks1, suggesting that the mechanism of activation can be Cdk2 independent.

How then does Cks1 participate? Work from both groups indicates that Cks1 enhances the interaction of phosphorylated p27 with Skp2. However, precisely how Cks does this is not clear. One possibility is that binding of Cks to Skp2 promotes a structural change that reveals the LRR surface, allowing p27 to bind to Skp2 in a phosphorylation-dependent manner. In model B, Cks1 interacts simultaneously with Cdk2 and Skp2, thereby facilitating the interaction of phosphorylated p27 with the LRRs. In model C, Cks1 interacts simultaneously with Cdk2, Skp2, and p27. The interaction with phosphorylated p27 occurs through a phosphate-binding site located on the surface of Cks1.

Alternatively, Cks1 might physically bridge p27 and Skp2. Here, two extremes are possible. In one case, Cks1 interacts in a phosphorylation-dependent manner with p27 while simultaneously binding Skp2 (Figure 2a, model A). This model is suggested by the finding that Cks1 can interact with Skp2 [2,3] independent of its association with Cdk2. The presumptive substrate-binding surface in Skp2, the LRRs, are occluded by a carboxy-terminal strand of Skp2 [25]. Given the precedent of other LRR interactions, this strand would need to be nudged away in order for p27 recognition and Cks1 might fulfill this task. One distinguishing feature of this model is that Cks1 functions independently of both Cdk2 and p27.

While, in principle, both bridging models could be Cdk independent, we cannot yet exclude the possibility that whether Cks1 is simply recognizing the phosphate group or whether there are additional specific interactions, as would be anticipated if this is a physiologically relevant interaction. In this regard, analysis of the interaction of Cks2 with phospho-p27 might be informative since this protein retains the phosphate binding site (Figure 2b) but does not support p27 ubiquitination or bind Skp2 [2,3]. In the second case, Cks1 may interact simultaneously with Skp2 and p27 and allow specificity recognition of phospho-T187 by Skp2 (Figure 2a, model B).
the form of Cks1 used in p27 ubiquitination in vivo is actually the Cdk bound form (Figure 2a, models B and C). In its complex with cyclin E-Cdk2-p27, Cks1 could simultaneously contact p27 and Skp2, thereby holding one or both proteins in a conformation appropriate for interaction. Moreover, both cyclins A and E have been demonstrated to interact with Skp2, so in principle, addition contacts may exist in the complex. The use of multiple interaction sites provides the basis for the exquisite specificity seen in ubiquitination reactions and provides a simple biophysical explanation for why free p27 is a relatively poor ubiquitination substrate.

It is also likely that the precise docking of the p27 complex with SCFSkp2 is important for orienting the relevant lysine residues in p27 for ubiquitin transfer. Thus, the role of Cks1 may go beyond simply facilitating binding of substrate and SCF. Obviously, structural analysis of the complex will be required to determine the overall contributions of these various interactions and whether Cks1 function is Cdk independent or not. In addition, it is clear that many targets of ubiquitin ligases are components of multiprotein complexes. It seems likely that much of the specificity in these cases reflect multisubunit interactions, many of which do not involve the protein that is targeted for ubiquitination.

Mammals and C. elegans both have two Cks homologs. As mentioned above, Cks2 is unable to support p27 ubiquitini
tion, despite the fact that it is 87% similar to Cks1. But the fact that Cks1 mice are viable indicates that Cks2 may be largely redundant with Cks1, especially for progression through mitosis. The Cdk-binding and phosphate-binding surfaces, which are derived from the four-stranded β sheet region, are conserved in Cks proteins (Figure 2b). In contrast, the major structural differences are located on the two α helices and also at the carboxyl terminus, which generate a largely contiguous surface (Figure 2b). It is likely that these regions perform Cks-specific functions, such as Skp2 binding in the case of Cks1. Given the selectivity of Cks1 in promoting p27 ubiquitination, it seems likely that Cks2 may also function in one or more pathways that are non-redundant with Cks1. Thus, an analysis of mice lacking Cks2 and both Cks proteins is anxiously awaited.

The implications of this new work go well beyond mechanistic details of p27 ubiquitination. It is now widely appreciated that p27 functions to control cell-cycle exit during development, and as such it is a target for inactivation during transformation. Although mutations in the p27 structural gene are rare, p27 levels and localization are nevertheless altered in several classes of human tumors, including breast and prostate cancer. It is currently believed that alterations in p27 proteolysis are central to its removal during sporadic transformation. Overexpression of Skp2 can promote S-phase entry in quiescent fibroblasts in a manner that is blocked by non-phosphorylatable p27 [17] and Skp2 can collaborate with oncogenes such as Ras to transform cells in the lymphoid compartment [26]. Moreover, it has recently been demonstrated that Skp2 is induced in multiple tumor types and this correlates with reduced levels of p27 in such tumors [26].

Like Skp2, Cks1 expression is growth regulated; its mRNA is absent during G1 but present during S phase [7]. Thus, one would imagine that inactivation of p27 in cancer via the ubiquitin pathway would require, not only the induction of Skp2, but of Cks1 as well. In this regard, epithelial cells shut-off Cks1 transcription in response to the growth inhibitor TGF-β [27]. Given this link, it is tempting to speculate that other negative growth regulatory pathways will function by limiting the levels of Skp2 and/or Cks1 (Figure 1). Conversely, it is plausible that the Skp2 and Cks1 genes are coordinately activated in order to ensure that the two proteins are both present at the appropriate time in the cell cycle to catalyze p27 destruction. Understanding how these pathways are altered during transformation will be a challenge for the future.

References


