Extra Views

Don’t skip the G₁ phase
How APC/C<sup>Cdh₁</sup> Keeps SCF<sup>Skp₂</sup> in Check

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Received 05/12/04; Accepted 05/14/04

This manuscript has been published online, prior to printing for Cell Cycle, Volume 3, Issue 7. Definitive page numbers have not been assigned. The current citation is:

This manuscript has been published online, prior to printing for Cell Cycle, Volume 3, Issue 7. Definitive page numbers have not been assigned. The current citation is:

Cell Cycle 2004; Vol. 3 Issue 7

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Keywords
Skp2, Cks1, p21, p27, APC/C, Cdh1, Cdc20, Ems1, SCF, cell cycle

ACKNOWLEDGEMENTS
We thank T. Cardozo for critically reading the manuscript. We apologize to colleagues whose work could not be mentioned due to space limitations.

Work in the Pagano lab is supported by grants from the NIH (R01-CA76584 and R01-GM57587).

ABSTRACT

By keeping the levels of Skp2 and Cks1 low during G₁ progression, APC/C<sup>Cdh₁</sup> prevents unscheduled degradation of SCF<sup>Skp₂</sup> substrates and premature entry into S phase. Thus, APC/C<sup>Cdh₁</sup>, a ubiquitin ligase involved in mitotic exit and maintenance of G₀/G₁ phase, directly controls SCF<sup>Skp₂</sup>, a ubiquitin ligase involved in the regulation of S phase entry.

The cell cycle is controlled by a family of protein kinases known as cyclin-dependent kinases or CDKs. Timely destruction of CDK subunits and their upstream regulators is an essential means to ensure proper progression through the cell division cycle. Protein degradation is irreversible, and thus constitutes a well suited mechanism to promote the unidirectional passage from one cell cycle phase to the next.

In mammalian cells, the transition from G₁ to S phase is controlled by the activity of the CDK inhibitors (CKIs) p27 and, to a lesser extent, p21. Both CKIs act as negative cell cycle regulators that prevent entry into S phase by inhibiting the activity of Cdk2/Cyclin E, Cdk2/Cyclin A and likely other CDKs. Progression into S phase therefore necessitates the ubiquitin-mediated degradation of the inhibitory proteins. The triggering signal is the CDK-mediated phosphorylation of p27 and p21, allowing the SCF<sup>Skp₂</sup> (Skp1, Cul1, F box protein Skp2) ubiquitin ligase to specifically recognize the two CKIs as substrates. SCF<sup>Skp₂</sup> subsequently catalyzes the polyubiquitination of p27 and p21, thus earmarking both proteins for destruction by the 26S proteasome. The recognition and efficient ubiquitination of p27 and p21 requires the small accessory protein Cks1 as an indispensable factor in the SCF<sup>Skp₂</sup> complex. Through its interaction with Skp2, Cks1 may function as an allosteric effector that induces conformational changes in the F box protein to increase its affinity for the substrate, or, more likely, contributes to the binding of phosphorylated p27 by forming a bimolecular interface with Skp2.

As negative regulators of cell proliferation, p27 and p21 intrinsically possess tumor suppressive properties. Hence, the elimination of these impediments to unrestrained proliferation, particularly p27, is a hallmark of many types of cancer. Diminished p27 protein levels are frequently found in human malignancies and are often associated with high aggressiveness and poor prognosis. As the antagonist of p27, Skp2 acts as an oncoprotein, and Skp2 expression was found to inversely correlate with p27 in various carcinomas and lymphomas. Until recently, the investigation of Cks1 protein levels in cancer has been hampered by the fact that specific antibodies (i.e., antibodies that do not recognize the closely related Cks2 protein) have been unavailable. However, mRNA data and the current limited immunohistochemical data indicate that in some types of cancer overexpression of Cks1 is indeed found, correlating with low levels of p27. Although more data are needed to evaluate the role of Cks1 in cancer development and progression, it would not be surprising to find that Cks1 has oncogenic properties similar to Skp2 or at least contributes to Skp2 tumorigenicity. This would highlight the need for tight regulation of both Skp2 and Cks1 to avoid unscheduled degradation of p27 and p21.

How are Skp2 and Cks1 regulated during an unperturbed cell cycle? It has been previously shown that Skp2 mRNA is present in all phases of the cell cycle, displaying only minor fluctuations. Skp2 protein, however, is absent in G₀ and G₁ cells, accumulates during the G₁-S transition and persists through S phase until mitosis. The discrepancy in Skp2 levels between G₀/G₁ and S phase is due to differences in protein stability; Skp2 protein is very unstable in G₀/G₁, but upon entry into S phase it is stabilized significantly. However, the mechanism that controls Skp2 stability had not been well understood. Our study and that of Wei et al. have now provided evidence that the degradation of Skp2 in...
G₀/G₁ is mediated by the APC/C_CMdh₁ (anaphase-promoting complex/cyclosome in complex with its activator Cdh₁) ubiquitin ligase.¹⁷,¹⁸
1. Overexpression of Cdh₁ (but not its homolog Cdc20) in cells leads to a considerable destabilization of Skp₂.
2. Depletion of Cdh₁ by RNA interference results in the stabilization and accumulation of Skp₂ in G₀/G₁.
3. Cdh₁ interacts physically with Skp₂.
4. APC/C supplemented with recombinant Cdh₁ is capable of ubiquitinating Skp₂ in vitro.

The APC/C_CMdh₁-mediated degradation of Skp₂ is dependent on an N-terminal destruction box (D box) motif (RXXL). Mutation of this motif generates a stable Skp₂ protein that is fully active in G₁ cells. Intriguingly, Skp₂ mutants harboring an inactive D box are still able to interact with Cdh₁, suggesting that the D box sequence itself does not function as a landing pad for the APC/C moiety of the APC/C. A more detailed analysis of the Skp₂ N-terminus revealed that the actual binding site for Cdh₁ resides within a stretch of 50 amino acids that is located approximately 40 amino acids downstream from the D box (amino acids 45–94). In line with recent suggestions, the D box may thus interact with components of the APC/C other than Cdh₁, or it may function as a signalling sequence that orients the multi-subunit APC/C complex to the binding site.¹⁹

The oscillations and protein stability of Cks₁ during the cell cycle parallel those of Skp₂.¹⁷ Cks₁ was also shown to bind Cdh₁, and overexpression or depletion of Cdh₁ in cells have an effect on Cks₁ levels similar to Skp₂. Although many findings point to APC/C_CMdh₁ as a direct regulator of Cks₁ protein stability, we were unable to ubiquitinate Cks₁ in vitro using APC/C_CMdh₁. This leaves open the possibility that APC/C_CMdh₁ does not target Cks₁ directly, but may instead trigger another, APC/C_CMdh₁-dependent, ubiquitin ligase. Alternatively, an as yet to be identified factor necessary for Cks₁ ubiquitination is missing in the purified preparation of APC/C_CMdh₁.

Several control mechanisms ensure that the activity of APC/C_CMdh₁ itself is restricted to late M and G₀/G₁ phase.²⁰ The complex is activated through the binding of dephosphorylated Cdh₁ to APC/C in late M phase, and the continued absence of Cdk₁ and Cdk₂ kinase activity keeps APC/C_CMdh₁ functional throughout G₁ (or G₀). At the G₁/S transition APC/C_CMdh₁ function is blocked through both the interaction of Cdh₁ with the inhibitory protein Emi₁ and Cdk₄-mediated phosphorylation of Cdh₁.²¹,²² The inactivation of APC/C_CMdh₁ permits the accumulation of Skp₂ and Cks₁, the assembly of an active SCFSkp₂ complex and the targeting of p27 and p21 for ubiquitin-mediated degradation.

What are the consequences of defective Skp₂ degradation in G₁ cells? Our experiments have shown that a nondegradable Skp₂ mutant expressed at physiological levels in G₁ cells promotes the unscheduled degradation of p27 and p21. As a consequence, these cells progressed significantly faster into S phase than control cells. In this context, it should be noted that the expression of the stable Skp₂ mutant concomitantly stabilized endogenous Cks₁ protein. It is thus conceivable that the interaction between Skp₂ and Cks₁ shields the latter from ubiquitination and destruction, suggesting that the regulation of Skp₂ and Cks₁ protein stability is closely linked. The APC/C_CMdh₁-mediated elimination of Skp₂ and Cks₁ is therefore important to maintain the G₁ state. In contrast, continuous aberrant removal of p27 and p21 is likely to be a cause of uncontrolled cell proliferation and may ultimately contribute to the development of cancer. The frequently observed overexpression of Skp₂ in cancer tissues has in some cases been attributed to gene amplification,²³,²⁴ however, other potential causes have not been investigated yet. There is accumulating evidence that aberrant protein degradation of cell cycle regulators is an important factor contributing to tumorigenesis,²⁵ and this may hold true for Skp₂ (and Cks₁) as well (see Fig. 1). Indeed, Skp₂ stabilization has been observed in aggressive oral cancers (Kudo Y, Pagano M, Takata T, unpublished results). Increased protein levels of Skp₂ in cancer cells may be a consequence of inactive Cdh₁ (e.g., due to mutations or overexpression of Emi₁) or reduced Cdh₁ expression. No data concerning the Cdh₁ status in cancer cells are available at present, but downregulation of Cdh₁ has been shown to accelerate cell cycle progression, a potential cause of genetic instability.¹⁷,¹⁸,²⁶ It is also possible that alterations in other components of the APC/C_CMdh₁ complex contribute to Skp₂-Cks₁ stabilization. Indeed, it has been shown that in human colon cancer cells two subunits of the APC/C, Apc6/Cdc16 and Apc8/Cdc23,

![Figure 1. The potential effects of defective Skp₂/Cks₁ degradation. Normal cell (left panel): APC/C_CMdh₁ is activated in late M/early G₁ phase and induces the degradation of the SCF subunits Skp₂ and Cks₁. Consequently, the CKIs p27 and p21 accumulate, bind to CDK/cyclin complexes and block their activity, thereby ensuring that G₁ phase is established and maintained. During the G₁/S transition, APC/C_CMdh₁ is inactivated through association with Emi₁ and CDK-mediated inhibitory phosphorylation of Cdh₁. Skp₂ and Cks₁ are stabilized, become part of an active SCFSkp₂ complex and target p27 and p21 for degradation. CDK/cyclin complexes become active and initiate progression into S phase. Cancer cell (right panel): In certain cancer cells APC/C_CMdh₁ may be unable to interact with Skp₂/Cks₁ in G₁ phase, hence the ubiquitination and subsequent degradation of both proteins is compromised. As a result, p27 and p21 are targeted for unscheduled degradation, rendering CDK/cyclin complexes active. The cell progresses faster into S phase and DNA replication is prematurely initiated, which can be a cause of genetic instability.](image-url)
which are known to be key function elements in the complex, often display inactivating mutations. Alternatively, Skp2 may harbor mutations that obstruct any physical interaction between Skp2 and APC/C Cdh1. Conceivably, such mutations would primarily affect the N-terminal D box or the Cdh1 binding site, rendering Skp2 refractory to APC/C Cdh1-mediated ubiquitination and destruction. Future studies will therefore have to unravel how APC/C Cdh1 targets Skp2 and Cks1 mechanistically to fully understand the role of defective Skp2/Cks1 degradation in cancer.

References