

Twice Primed: Cyclin E Is Phosphorylated and Isomerized Before Being Ubiquitinated

Targeting proteins for irreversible degradation must be under tight control and is often regulated at the level of substrate-receptor binding. But does a protein really need to be marked twice with two different modifications, first phosphorylation and then isomerization, to bind its receptor, as van Drogen et al. (2006) show for cyclin E?

The concentrations of many cellular proteins change rapidly as the result of alterations in physiological conditions and are regulated, to a large extent, by the ubiquitin-proteasome pathway. The critical role of this pathway is perhaps best illustrated by its function in cell cycle control. Sequential activation and inactivation of the cyclin-dependent kinases (CDKs) by the binding of a cyclin and a CDK inhibitor (CKI), respectively, constitute the molecular basis for orderly progression through the cell cycle. Most cyclins and CKIs are characteristically short-lived proteins and are degraded by a ubiquitin-dependent process. As a result of their periodic transcriptional activation and rapid destruction, cyclins and CKIs act as rate-limiting activating and inhibitory factors in driving unidirectional cell cycle progression and connecting other cellular pathways to the cell cycle. Deregulation of this process, such as impaired degradation of cyclin E, leads to an accelerated G1 to S phase progression in cultured cells and predicts poor prognosis for cancer patients.

Ubiquitin-mediated proteolysis requires a cascade of three activities performed by E1 (ubiquitin activating), E2 (ubiquitin conjugating), and E3 (ubiquitin ligase) enzymes. A critical step in this process is how individual protein substrates, such as cyclin E, are recruited to specific E3 ligases at the right time, in the right place, and perhaps in the right form. Currently, two major families of E3 ligases have been described. The HECT family of E3s, about 40 in human, contains a domain homologous to the E6-associated protein (E6AP) carboxyl terminus that can form thioester linkages with ubiquitin. Members of the RING family of E3s either contain an intrinsic RING finger domain (as in MDM2 and BRCA1) or bind in trans with the small RING finger protein Roc1 (also known as Rbx1 and Hrt1) to assemble into cullin-RING dependent ligases (CRLs) (Petroski and Deshaies, 2005). Cyclin E is one of the earliest and better-characterized CRL substrates. It is targeted for degradation by the SCF^{Cdc4} E3 ligase where it is recruited by the receptor F box protein Cdc4 (also known as Fbw7, hSEL10 and Archipelago) and bridged by the linker Skp1 to the CUL1-ROC1 catalytic core for ubiquitination. A key regulatory step of cyclin E ubiquitination by SCF^{Cdc4}, as for many other SCF substrates,

is the binding of substrate and receptor—only a phosphorylated form of cyclin E is recognized by Cdc4. Such substrate “priming” provides a mechanism for coupling multiple inputs and thereby integrating different cellular pathways. Degradation of cyclin E is triggered by phosphorylation by at least two kinases, GSK3 β and Cdk2, potentially linking metabolism and development with entry into the S phase (Clurman et al., 1996; Won and Reed, 1996; Welcker et al., 2003). Receptor Cdc4 exists as three splice variants, designated α , β , and γ , which share ten common 3' exons but each have a unique 5' exon (Spruck et al., 2002). What began by Reed and his colleagues as a simple query into which of these three isoforms is involved in cyclin E turnover led to some unexpected findings on how cyclin E is primed for ubiquitination—that, following phosphorylation, cyclin E must undergo SCF^{Cdc4 α} -dependent prolyl isomerization by Pin1 prior to SCF^{Cdc4 γ} -dependent ubiquitination (van Drogen et al., 2006).

siRNA-mediated knockdown of the individual Cdc4 isoforms showed that loss of either Cdc4 α or Cdc4 γ was sufficient to stabilize cyclin E. Using insect cell-derived SCF^{Cdc4 α} and SCF^{Cdc4 γ} , the authors showed that ubiquitination could only be achieved when cyclin E was incubated first with SCF^{Cdc4 α} followed by SCF^{Cdc4 γ} ; no other sequence of reactions was effective. In the absence of any detectable SCF^{Cdc4 α} -dependent modification of cyclin E, and noting the stabilization of cyclin E in *Pin1*^{-/-} MEFs (Yeh et al., 2006), van Drogen et al. probed for and found Pin1 in their reconstituted ubiquitination assay. Indeed, when added with SCF^{Cdc4 α} to the two-step ubiquitination assay, recombinant Pin1 was able to increase the amount of cyclin E ubiquitination well above that achieved by SCF^{Cdc4 α} and SCF^{Cdc4 γ} when Pin1 was not in excess. The strongest in vivo evidence supporting a regulation of cyclin E by SCF^{Cdc4 α} -potentiated isomerization comes from the characterization of a cancer-derived mutation in exon1 α of Cdc4 α that disrupts its ability to bind with cyclin E-Pin1 complexes and to prime cyclin E for polyubiquitination by SCF^{Cdc4 γ} (van Drogen et al., 2006).

While the current study uncovers some new aspects of SCF and Pin1 activities, it also raises a number of unanswered questions. Topping the list is what property buried within the exon1 α -encoded 167 residues renders to Cdc4 α , but not Cdc4 γ , the specific ability to enable Pin1's catalytic activity? Given that Pin1 can bind with the cyclin E-Cdk2 complex in the absence of SCF (Yeh et al., 2006) and that purified Cdc4 α -Skp1 complex cannot prime cyclin E for subsequent ubiquitination by SCF^{Cdc4 γ} (van Drogen et al., 2006), what other component(s) in the SCF is contributing to Pin1's catalytic activity? What is the significance of isomerization at the atypical Pro-Pro bond instead of the usual pSer/Thr-Pro linkage?

Of greater interest is the physiologic function and biochemical mechanism of Pin1 in promoting cyclin E ubiquitination. The degradation of cyclin E stands as an

exception to Pin1's general role in promoting growth through its association with diverse cellular proteins (c-Jun, NF- κ B, p53, β -catenin, c-Myc, and cyclin D). Even more puzzling is the opposite effect of Pin1 on cyclin D, where a loss of Pin1 function correlates with reduced transcription of the cyclin D1 gene as well as decreased protein stability (Liou et al., 2002). What seems to be more likely is that Pin1 regulates the conformation of many different substrates and thus exhibits pleiotropic effects on different cellular pathways, making it far too complicated to attribute the function of Pin1 to a single cellular pathway. The biochemical function of Pin1 remains much more elusive for most of these interactions. Prolyl *cis/trans* isomerization could, as the authors speculate, properly position the target lysine residue(s) for ubiquitination by the SCF active site. Alternatively, prolyl *cis/trans* isomerization might change the conformation of the so-called Cdc4-phosphodegron to allow or facilitate the binding of cyclin E to its true ubiquitin ligase, SCF^{Cdc4 γ} (Orlicky et al., 2003). The former scenario is an intriguing possibility that may address a long-standing question of ubiquitin ligation by SCF—how a substrate is presented to the active site cysteine on the E2 enzyme when they are so far away from each other (59 Å between the substrate binding region within SKP2 and the cysteine on E2) (Zheng et al., 2002). The function of prolyl *cis/trans* isomerization in causing protein conformational change, or local unfolding, makes one wonder whether Pin1 may function to present cyclin E, and potentially other substrates, closer to E2, thereby orienting the right lysine residue(s) to the active site.

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Selected Reading

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Unweaving the Meanings of Messenger RNA Sequences

In addition to protein-coding information, mRNAs harbor regulatory sequences necessary for appropriate processing of their precursors. Goren et al. (2006) and Wang et al. (2006) explore the diversity of these signals and the rules by which they function.

Great writers, from Dante to Joyce, often weave various meanings into their writings. Uninformed readers get the explicit story but may miss a rich source of metaphors and references to previous myths, history, or literature that so much enriches the content and value of classical books. Classic molecular biology experiments cracked the almost universal genetic code as the three-letter codon script in messenger RNAs that directs sequential addition of amino acids into polypeptide chains. In recent issues of *Molecular Cell*, Goren et al. (2006) and Wang et al. (2006) bring us closer to cracking a second, less explicit code in RNA sequences that allows proper maturation of primary

transcripts to generate mRNAs that can be translated into proteins. These papers illustrate the power of combining comparative computational analyses with experimental work, and they provide insights into the control of gene expression and the molecular basis of some genetic diseases.

Processing of the primary products of transcription includes the removal of internal sequences—introns—and splicing together the remaining pieces—exons—to generate mature functional mRNAs. The splicing process is catalyzed by a complex molecular machinery and is often subject to regulation, leading to alternative splicing pathways, distinct mRNAs, and protein products with different functions (Black, 2003). Accurate—but also flexible—identification of intron/exon boundaries is not a simple task in higher eukaryotes because splice site sequences are rather degenerate and often not better matches to the consensus splice sites than other sequences not involved in the splicing process. The only sensible explanation for this paradox is that a variety of other sequences participate in intron/exon recognition. This is indeed consistent with recent observations indicating that mutations that do not affect the splice sites themselves can still cause splicing defects—e.g., exon skipping—that wreak message havoc and