SCF E3 Ligase Substrates Switch from CAN-D to Can-ubiquitylate

Daniel C. Scott[1] and Brenda A. Schulman[1,2,*]
[1]Department of Structural Biology, St. Jude Children's Research Hospital, Memphis, TN 38105, USA
[2]Department of Molecular Machines and Signaling, Max Planck Institute of Biochemistry, 82152 Martinsried bei München, Germany
*Correspondence: schulman@biochem.mpg.de
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Liu et al. (2018) report a mathematical model predicting how the cellular repertoire of SCF E3 ligases is assembled by "adaptive exchange on demand," with the limited pool of CUL1 scanning the vast sea of F-box proteins for those with substrates demanding ubiquitylation.

Cullin-RING E3 ubiquitin ligases (CRLs) are major regulators of eukaryotic cell biology, controlling the half-lives, activities, assemblies, and localizations of thousands of proteins. This depends on hundreds of distinct CRLs in humans—and even more in plants and other organisms—assembling from structurally related modules, whereby a cullin-RING complex is a scaffold bridging a variable cullin-binding substrate receptor module with a RING-binding E2 or E3 enzyme that delivers ubiquitin to the receptor-bound substrate (Lydeard et al., 2013). The first discovered and prototypical CRLs comprise the SCF subfamily, in which the scaffold is CUL1-RBX1, and substrate receptor module consists of SKP1 and an associated F-box protein (FBP). The sheer number of FBPs (69 in humans), combined with a ~4-fold higher concentration of SKP1:CUL1 in human cells, poses an interesting question: how does the limited pool of CUL1 select among a sea of FBPs while, at the same time, managing to not exclude others when needed for expedient substrate ubiquitylation? If affinity toward CUL1 were the sole determinant of SCF assembly, tightest binding SKP1-FBP pairs would dominate. Yet different signaling pathways, cell types, and developmental programs depend on altering the expression patterns of FBPs to cope with ever-changing needs to ubiquitylate different substrates (Reitsma et al., 2017). What then determines how a given SKP1-FBP module is assembled into active an SCF E3 ligase? In this issue, Liu et al. (2018) report the development of a mathematical model that provides new insights and ways to investigate the rules governing the repertoire of SCFs assembled as demanded for cellular regulation.

A key premise of the new work is that SCF assembly is coordinated with continuous cycles of CUL1 conjugation to/deconjugation from the ubiquitin-like protein NEDD8 ("neddylation," Figure 1, reviewed in Lydeard et al., 2013). In the absence of NEDD8, different SKP1-FBP modules continuously sample CUL1 due to CAND1 or CAND2 (referred to here collectively as "CAND") expelling SKP1-FBPs from unmodified CUL1-RBX1 and vice-versa (Pierce et al., 2013; Wu et al., 2013; Zemla et al., 2013). CAND binding to CUL1 is structurally incompatible with neddylation (Goldenberg et al., 2004). Although SKP1-FBP-bound/CAND-free CUL1 is readily neddylated, NEDD8 is rapidly deconjugated from CUL1 by the COP9 signalosome (CSN). The deneddylated SCF can then undergo additional cycles of CAND-catalyzed SKP1-FBP exchange. Substrates are capable of putting an abrupt halt to this cycling by sterically blocking CSN-dependent deneddylation. By preventing deneddylation, an FBP-bound substrate indirectly preserves NEDD8 on its associated SCF, thereby preventing disassembly and maintaining ubiquitylation activity (Bornstein et al., 2006). While this attractive model explains some aspects of SCF assembly, it remains unclear to what extent substrate ubiquitylation relies on CAND-driven FBP exchange, or why cells developed such a seemingly complex regulatory system. The vast number of players competing for SCF assembly and disassembly, and their ever changing levels as cells respond to various cues (Reitsma et al., 2017), has presented a significant challenge for predicting system-wide changes upon perturbation.

To predict levels of SCF assembly, Liu et al. (2018) first quantified the remaining necessary kinetic parameters and derived a model in combination with known functions and concentrations of components. The model agrees astonishingly well with many experimental observables. In terms of SCF assembly, the model correlates with the ratios of SKP1-FBP-associated CUL1 upon inhibiting neddylation or doubly knocking out (DKO) CAND1 and CAND2. The model also predicted how the perturbed system in DKO cells affects levels of the $SCF^{\beta\text{-}TrCP}$ substrate phosphorylated IκBα (Liu et al., 2018). A perplexing result not explored is that the half-life of phosphorylated IκBα is 15 times shorter than the time it would take for half the cellular complexes between this substrate and $SCF^{\beta\text{-}TrCP}$ to dissociate. It is possible that the dissociation rate for this substrate-FBP complex is accelerated by ubiquitylation, as this value has not yet been measured. Alternatively, this may imply that an unknown exchange factor actively removes substrate from FBPs or that the proteasome potentially degrades ubiquitylated substrates bound to a CUL1-SKP1-FBP complex.

Unexpectedly, Liu et al. (2018) uncovered that the CAND1-bound population of CUL1 is dramatically biased for binding to DCN1, a neddylation co-E3 that helps recruit the NEDD8 conjugating enzyme UBE2M (Kurz et al., 2005; Scott et al., 2014). While the underlying mechanism is unclear, the authors speculate that this primes CUL1 to be neddylated concurrently with binding a SKP1-FBP module and displacement of CAND1. It

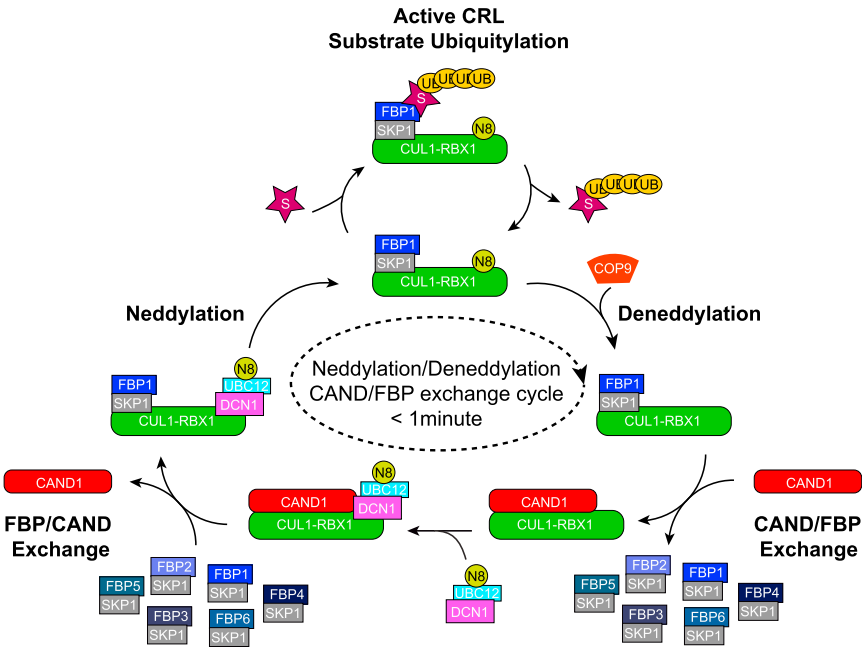

Figure 1. Simplified Scheme for "On Demand" SCF Assembly by Adaptive F-box Protein Exchange

In the absence of an SCF substrate, the neddylation/deneddylation and CAND/FBP exchange cycle allows a single CUL1-RBX1 complex to rapidly scan a vast array of different SKP1-F-box protein (FBP) complexes. A substrate "demands" stability of its cognate NEDD8 (N8)-modified SCF by removing it from the cycle, allowing ubiquitylation.

will be interesting to see how inhibition of UBE2M binding to DCN1 could alter the landscape of SCF assembly.

One extraordinary parameter that emerged from the modeling is that in the absence of bound substrate, CUL1 undergoes an entire exchange cycle in less than 1 min. Even more astonishingly, if all FPBs have equal access to CUL1, the entire pool of FPBs would sample CUL1 in less than 4 min in 293T cells (Liu et al., 2018). Such rapid and indiscriminate cycling could safeguard the SCF system from bias against FBPs that are expressed at low levels or that display weak affinity for CUL1. In a grander sense, the implications of these numbers are profound and suggest that CUL1-RBX1 and, by inference, other cullin-RING complexes are on an endless search-and-rescue mission continuously on the hunt for substrate-bound FBPs, ensuring active SCF assembly only upon increased substrate demand.

In order to gauge the model's predictive strength, Liu et al. (2018) simulated effects of varying the concentrations of SCF components and predicted that overexpression of CUL1, but not the FBP that targets phosphorylated IκBα, would rescue defects in the rate of its degradation in DKO cells. Experimental validation of these predictions presented a new paradox: if CUL1 upregulation can obviate the need for CAND exchange, why does such a complex system exist in the first place? A potential answer came from calculating a matrix of response coefficients, which suggested that increasing the total FBP concentration would delay substrate degradation specifically in DKO cells. Indeed, gross overexpression of an FBP in DKO cells that lack dynamic CAND-mediated exchange clogs the system: this restricts CUL1 from accessing other FBPs, thereby stabilizing their ubiquitylation substrates (Liu et al., 2018). The authors conclude that CAND-driven exchange permits the SCF system to tolerate changes in FBP expression associated with development, without requiring CUL1 levels to change in diverse regulatory settings. Nonetheless, some SCF substrates (p27, CyclinE) are stabilized in DKO cells only when total FBP levels are increased by overexpression, implying that these substrates can be efficiently degraded independently of CAND exchange. Why some SCF substrates do not require CAND-dependent FBP exchange is unclear, but could reflect variations in the levels of their cognate FBPs, or differences in dissociation rates of these substrates versus phosphorylated IκBα. Whatever the case, it will be interesting to see whether this model can predict threshold conditions for substrates that require CAND-dependent exchange.

Moving forward, the new mathematical model opens doors for understanding the SCF network, where activity of a component is blunted through mutation or altered in expression in diseased states, and during therapeutic intervention. Computational modeling could reveal underappreciated secondary or tertiary effects of network perturbation and how these might contribute to disease. It will also be interesting to see how CAND-driven exchange functions, and to what extent it is required, in organisms like *C. elegans* and *D. melanogaster* that express multiple Skp1-related genes with divergent sequences in CUL1-binding loops that prevent simultaneous binding to CAND1. With the availability of an accurate mathematical model, these and other mysteries of SCF and CRL networks CAN(D) now be solved.

DECLARATION OF INTERESTS

The authors declare application for a patent (application number PCT/US2016/052493) entitled Methods and compositions of inhibiting DCN1-UBC12 interaction.

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Uneven Braking Spins RNA Polymerase into a Pause

Irina Artsimovitch[1,*] and Georgiy A. Belogurov[2]
[1]Department of Microbiology and The Center for RNA Biology, The Ohio State University, Columbus, OH, USA
[2]Department of Biochemistry, University of Turku, Turku, Finland
*Correspondence: artsimovitch.1@osu.edu
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In this issue of *Molecular Cell*, Guo et al. (2018) and Kang et al. (2018) report structures of paused transcription complexes in which asynchronous translocation inhibits nucleotide addition, allowing for global rearrangements in RNA polymerase stabilized by RNA hairpin and NusA.

RNA polymerase (RNAP) pausing is a key regulatory mechanism in all domains of life. Pausing is frequent, ubiquitous, and vital for proper control of every step of RNA synthesis. Pausing aids folding of structural and catalytic RNAs, facilitates recruitment of proteins and small ligands, melts chromatin structure near promoters, and is a prelude to termination. In addition, pausing couples RNA synthesis to DNA repair, RNA splicing, polyadenylation, and translation. Based on similar responses to pause-inducing sequences (Larson et al., 2014) among phylogenetically diverse multisubunit RNAPs, the core mechanism of pausing is thought to be conserved. At a pause site, RNAP interactions with nucleic acids trigger isomerization into a short-lived elemental pause state in which nucleotide addition is inhibited; a hairpin structure formed in the nascent RNA or backtracking of RNAP can further stabilize the paused state (Zhang and Landick, 2016). A widely accepted model based on comprehensive studies of *Escherichia coli* RNAP pausing at a hairpin-dependent site in the *his* operon attenuator by Bob Landick and collaborators, with contributions from other groups, posits that RNAP translocation is inhibited, the catalytic bridge helix (BH) and trigger loop (TL) are remodeled, and the clamp opens. The ubiquity of the clamp-opening mechanism is bolstered by reports that NusG/Spt5 proteins, the only universally conserved transcription factors, inhibit pausing by favoring the closed clamp (Werner, 2012). Detailed structural information would be needed to decipher contributions of numerous RNAP-nucleic acid contacts implicated in controlling pausing, to visualize fine changes in the active site and larger changes hypothesized to occur in several distant RNAP domains, and to understand mechanisms of pause-promoting (e.g., NusA) and pause-inhibiting (e.g., NusG) factors. However, a high-resolution structure of a paused elongation complex (PEC) remained elusive. In this issue of *Molecular Cell*, the Darst/Landick and Weixlbaumer groups report cryo-EM structures of *E. coli* hisPEC with (Guo et al., 2018) and without (Kang et al., 2018) NusA that answer some questions and pose new ones.

These structures reveal that, contrary to the model predictions, the hisPEC is in a hybrid state in which the RNA has translocated completely, but the template DNA (tDNA) has not, and the clamp does not open (Figure 1). Instead of a much-anticipated widening of the main channel upon clamp opening, more subtle movements of many RNAP elements, including the rotation of a large swivel module that includes the clamp, explain how nucleotide addition is inhibited in the hisPEC and rationalize the known effects of changes in RNAP on pausing. Kang et al. (2018) present convincing crosslinking evidence arguing that swiveling, albeit more subtle, is sufficient to support the pause-stabilizing effect of the hairpin, without a need for clamp opening. Although the new structures do not support contacts of the TL to the RNA 3′ end previously captured by crosslinking, they explain how the hairpin controls TL state (Toulokhonov et al., 2007). Swiveling traps SI3, a domain inserted into the TL, in a position incompatible with TL folding, which is critical for catalysis.