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Set them free: F-box protein exchange by Cand1

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Cand1 (Cullin-associated and neddylation-dissociated protein 1) has long been known as a regulator of SCF ubiquitin ligases, but details remained puzzling due to conflicting results from *in vitro* and *in vivo* experiments. Three recent reports, one in *Cell* and two in *Nature Communications*, propose Cand1 as a protein exchange factor with interesting mechanism that reconciles Cand1 genetics and biochemistry.

Most eukaryotic proteins are modified by the small protein ubiquitin at some point during their life. Ubiquitin tags can mark them for degradation in the proteasome, or control other protein properties such as localization, activity, and interactions. Ubiquitin ligases (E3 enzymes) play a particularly important role in the E1-E2-E3 ubiquitylation cascade as they directly select substrates for ubiquitin attachment. E3s define a large protein family with over 600 members in human cells that control ubiquitin transfer onto thousands of substrate proteins [1]. The complexity of this system comes with conceptual challenges that are particularly apparent for the largest group of E3s, the multisubunit Cullin-RING ubiquitin ligases (CRLs). The archetypal CRLs, the Skp1/Cul1/F-box protein (SCF) complexes, assemble on the Cul1 scaffold, with the small RING protein Rbx1 and E2 bound to the C-terminus, and the adapter protein Skp1 associated with the N-terminal region. Skp1 binds to one of many F-box proteins (FboxP), which confer specificity by selectively recruiting substrate proteins for ubiquitin transfer [2] (Figure 1, right). Up to 69 FboxPs in humans, and possibly 700 in plants, compete for the Cul1 core. How cells adjust abundance of the different SCF ligases in response to cell cycle and environmental cues to dynamically match substrate demand is one of the major questions in the field. Since identification of Cand1 over 10 years ago, its involvement in SCF complex formation has been evident [3, 4]. However, its true function was somewhat of a mystery. Cand1 acted as a potent SCF inhibitor *in vitro* by displacing the FboxP-Skp1 pair from Cul1, but genetic experiments classified Cand1 as a positive regulator of SCF and other CRLs *in vivo* [5]. An additional layer of complexity is added by covalent modification of cullins with the ubiquitin-like protein Nedd8. Neddylation (modification with Nedd8) induces a conformational rearrangement of Cul1 that stimulates ubiquitin transfer by the SCF-bound E2 and also obscures the Cand1 binding site on Cul1 [6]. Nedd8 deconjugation is catalyzed by the COP9 signalosome (CSN). Strikingly, the paradox observed for Cand1 is also evident for CSN, because CSN clearly functions as a negative regulator of SCF *in vitro*, yet genetic data suggest a positive role for SCF activity *in vivo* [5]. A prevailing model has been that SCF and other CRLs must undergo neddylation cycles whereby deneddylated cullins are sequestered by Cand1, allowing substrate receptor exchange followed by reactivation of the assembled CRL by neddylation. However, mechanistic insight was scarce.

In a recent study published in *Cell* [7], Deshaies and colleagues provide a biochemical framework that not only explains the CSN and Cand1 paradoxes, but also suggests a model for how SCF composition adjusts to varying substrate demand. They used *in vitro* real-time fluorescence resonance energy transfer (FRET) assays to monitor binding dynamics between FboxP-Skp1 and Cul1-Rbx1 complexes. Fbxw7-Skp1 formed an astonishingly tight complex with Cul1-Rbx1 (K_D = 200 fM) that could not be replaced by other FboxP-Skp1 complexes. However, addition of Cand1 accelerated spontaneous dissociation of SCF^{Fbxw7} over one-million-fold. Kinetic measurements demonstrated that Cand1 acts neither as a competitive nor allosteric inhibitor of Fbxw7-Skp1 binding to Cul1-Rbx1. Instead, Cand1 specifically increases the dissociation rate of the FboxP-Skp1 complex while having little effect on association rates. The authors point out that such a kinetic effect is reminiscent of guanine nucleotide exchange factors (GEFs). Accordingly, they suggest the term substrate receptor exchange factor (SREF) for Cand1 and functionally similar factors.

Cand1's SREF activity was beautifully illustrated *in vitro* using the two different F-box proteins Fbxw7 and β-TrCP. When $SCF^{\beta\text{-TrCP}}$ was combined with purified Fbxw7-Skp1 in an *in vitro* ubiquitylation reaction, no ubiquitylation of cyclin E (Fbxw7 substrate) was observed. This was not surprising because the tight binding of β-TrCP-Skp1 to Cul1 was expected to prevent assembly of SCF^{Fbxw7}. Remarkably, addition of Cand1 dramatically stimulated cyclin E ubiquitylation, likely through dissociation of β-TrCP-Skp1, thus establishing a new equilibrium

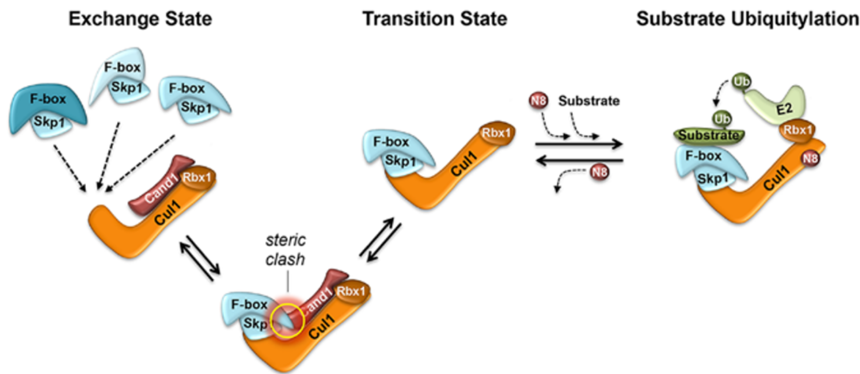

Figure 1 Cand1-driven substrate receptor exchange model (based on Pierce *et al.* [7]). Substrate availability protects the stable *substrate ubiquitylation state* (right). Depletion of substrates enhances CSN-mediated deneddylation shifting the SCF complex into a *transition state* that either finds new substrates and becomes reactivated by Nedd8 (N8) conjugation, or forms a transient complex with Cand1. The transient complex is highly unstable because of steric interference between F-box protein and Cand1 causing cycles of Cand1 and FboxP-Skp1 eviction. The *exchange state* allows the repertoire of formed SCF complexes to sample for substrates and, upon engagement, transit into the stable *substrate ubiquitylation state*.

of $SCF^{\beta\text{-TrCP}}$ and SCF^{Fbxw7} complexes. This assay design exposed Cand1 as an activator of SCF *in vitro*, which is consistent with its positive regulator role revealed by genetic experiments. The important findings that the FboxP-Skp1 complex can remove tightly bound Cand1 from Cul1, and indication of a transient complex of Cand1 with fully assembled SCF led to proposal of a model for SCF dynamics driven by substrate demand (Figure 1). A key feature of the model is based on recent evidence that substrate binding to CRLs can significantly reduce CSN access and CRL deneddylation [8, 9]. When substrates are exhausted, accelerated deneddylation shifts the active SCF complex into a deneddylated *transition state*, which can either bind new substrate and become reactivated by Nedd8 conjugation, or enter the *exchange state*. The latter is characterized by a Cand1-bound transition complex that controls dissociation and association of FboxP-Skp1 complexes. This concept extends the previous neddylation cycle model based on a strong biochemical foundation and provides a hypothesis for dynamic remodeling of the SCF landscape by substrate demand. Pierce *et al*. [7] support this biochemical concept with findings *in vivo* demonstrating significant shifts in the SCF landscape when Cand1 is absent.

The importance of Cand1 as a F-box protein exchange factor is reinforced by two recent studies in yeast. Zelma *et al*. [10] demonstrate the role of Cand1 in remodeling the SCF repertoire in response to changing growth conditions, and Wu *et al*. [11] provide additional evidence for Cand1 as an F-box protein exchange factor *in vivo*. Clearly there are more challenges ahead to understanding CRL dynamics, but the significance of these findings may reach beyond ubiquitin biology as it introduces the concept of *protein exchange factors* that govern association of protein binding platforms with large numbers of interactors.

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