RESEARCH HIGHLIGHT

How "rock-and-roll" solved the cullin supply chain problem

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In a recent study published in *Cell*, Baek et al. used cryo-electron microscopy to capture transitional conformations of Cand1, a regulator of SCF ubiquitin ligases, depicting its utilization of a "rock-and-roll" mechanism to both displace substrate receptors from the catalytic core and allow new substrate receptors to bind in their place.

Cullin-RING ligases (CRLs) are the largest superfamily of ubiquitin ligases, catalyzing the ubiquitination of ~20% of all proteins bound for proteasomal degradation.[1] CRLs are multimeric molecular machines with interchangeable substrate receptors that endow CRLs the combinatorial versatility to adapt to a dynamic pool of substrates and affect cell physiology.

Mammalian CRLs comprise 7 subfamilies, including the CRL1 or "SCF" subfamily, whose name denotes its major subunits: Skp1, Cul1, and F-box proteins (Fbps).[2] These subunits form two pre-formed subcomplexes: one subcomplex consists of Cul1 and the RING-finger protein Rbx1, which catalyzes the transfer of ubiquitin onto substrates, and the other consists of the adaptor protein Skp1 bound constitutively to an Fbp that recruits substrates to the Cul1–Rbx1 catalytic core for ubiquitination.

Because ~70 Fbps share the same catalytic core, the cell requires ways to dynamically regulate SCF assembly. One regulatory mechanism involves neddylation, where the ubiquitin-like protein NEDD8 is conjugated onto a conserved lysine on Cul1's C-terminal WHB domain greatly increasing the efficiency of the ubiquitination reaction.[3] The COP9 signalosome (CSN) can then deneddylate idling SCFs, noting that bound substrates sterically hinder CSN–cullin association. Thus, SCFs are most efficiently deneddylated when there is no substrate available, and NEDD8-conjugated Cul1 is a marker for an active SCF.[3]

Another key regulator is Cand1, which associates with deneddylated Cul1–Rbx1 subcomplexes in a manner mutually exclusive from Skp1–Fbp binding.[4] Cand1 was initially believed to inhibit nascent SCF assembly by sequestering cullin-RING subcomplexes. Indeed, the first crystal structure of Cand1 depicts it wrapped sinuously around Cul1 with two critical contact points that enable Cand1's mode of action: (1) Cand1's first two HEAT repeats (comprising Cand1's "anti-neddylation" domain) bury Cul1's conserved neddylation site through their tight association with Cul1's WHB domain and (2) a crucial β-hairpin (in Cand1's "anti-Skp1" domain) occludes the Skp1-docking site on Cul1's N-terminus, competing with Skp1 for binding.[5]

However, other studies indicate that Cand1 plays a positive role in SCF activity. Cand1 loss-of-function mutations in *Arabidopsis* reduced turnover rates of SCF substrates[6] and adding Cand1 to a mixture of pre-formed $SCF^{\beta\text{-}TrCP}$ and free Skp1–Fbxw7 led to increased ubiquitination of Fbxw7 substrate cyclin E,[7] suggesting that Cand1 might catalyze the assembly of specific substrate receptors by recycling the cullin core from pre-existing SCFs. Furthermore, SCF assembly is dependent on the availability of their cognate substrates. Yet, while substrates can bind both free and Cul1-bound Fbps, without Cand1, substrates bound to free Fbps cannot assemble with the catalytic core, resulting in an accumulation of non-ubiquitinated substrates in the cell.[4] Cumulatively, these studies support a model by which Cand1, alongside ongoing neddylation and deneddylation, facilitates a constant "SCF assembly cycle" where both Cand1 and neddylation machinery mediate the continual assembly and disassembly of SCFs (Fig. 1). Then, SCFs are stabilized upon substrate binding, triggering Cul1 neddylation and Cand1 inhibition, allowing ubiquitination to proceed.

To obtain a structural rationale for Cand1-mediated remodeling, Baek et al. used cryo-electron microscopy to capture various conformations of Cand1–SCF.[8] By mixing Cand1, Cul1–Rbx1, and three different Skp1–Fbp complexes (representing the three different Fbp substrate recognition domains), they captured several different conformations of Cand1–SCF structures, immediately indicating that Cand1–SCF complexes do not form a single unstable intermediate, but several configurations as either Cand1 or Skp1–Fbp dissociates from Cul1–Rbx1.

During Skp1–Fbp dissociation, Cand1 adopts a "Cand1 engaged" or "rocked" conformation where Cand1's β-hairpin wedges into a crack between Cul1's N-terminal domain and Skp1. The crack is created by Cand1-mediated "rocking" of Skp1–Fbp, which increases Cul1–Cand1 contacts, decreases Cul1–Skp1 contacts, and maintains Cul1–Fbp contacts at the Fbp "pivot point". Skp1–Fbp is then forced to "pivot" on the remaining Cul1–Fbp contacts as the β-hairpin competes for access. Mutating the "pivot residues" on Fbxw7 resulted in an increased disassembly rate by Cand1 and decreased displacement of the idling SCF when the pivot mutant was added to mixture of Cand1 and pre-formed SCF^{Skp2}. Critically, these pivot mutants are specific to Cand1 since the Fbxw7 pivot mutant's ubiquitination activity was unaffected. Thus, Cand1 "rocks" the SCF using its β-hairpin to dissociate Skp1–Fbp from Cul1.

When Cand1's β-hairpin is deleted, the authors found that the β-hairpin mutant adopts a "rolling" conformation both in the presence and absence of Skp1–Fbp, suggesting that loss of binding between Cand1's β-hairpin and Cul1 triggers Cand1 "rolling" around Cul1–Rbx1. Unlike the "Cand1 engaged" conformation, the "Cand1 rolling" conformation depicts a disengaged anti-Skp1 domain and a loosened grip on Rbx1's RING domain by Cand1's anti-neddylation domain, although contacts with Cul1's C-terminus remain intact. Further rolling (likely facilitated by Rbx1's RING domain, which is known to rotate to

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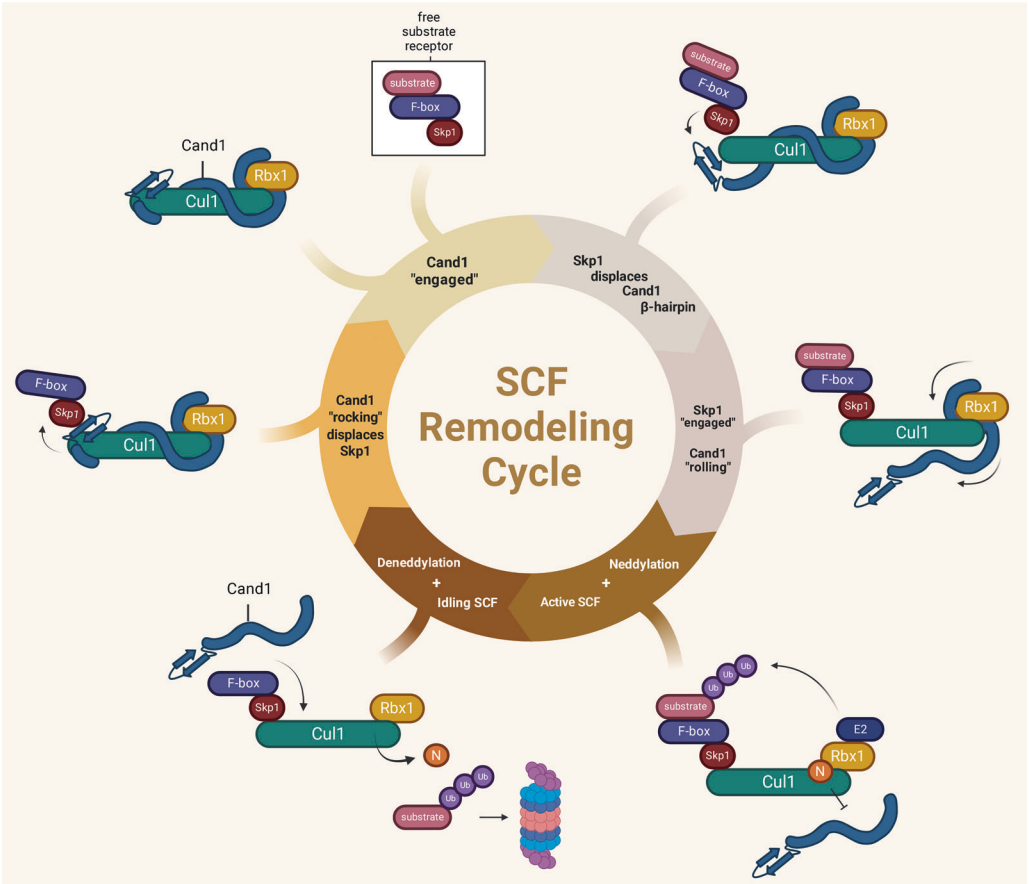

Fig. 1 Cand1-mediated recycling of the Cul1–Rbx1 catalytic core as part of an SCF remodeling cycle. As shown by Baek et al.,[8] Cand1 utilizes a "rock-and-roll" mechanism to displace Skp1–Fbp from idling SCFs and facilitate the assembly of nascent SCFs. Cand1, in conjunction with neddylation machinery, mediates the recycling of Cul1–Rbx1 catalytic subcomplexes, which are the limiting factor in SCF-mediated ubiquitination.

accommodate neddylation) would reduce the remaining contacts, causing Cand1 to dissociate. The authors further show that a Cand1 "β-hairpin++" mutant, which contains two mutations that preserved key Cul1 contacts but was defective in SCF rocking, also predominantly adopted the "rolling" conformation and impaired dissociation of pre-formed SCFs. Finally, expressing either of the Cand1 β-hairpin mutants in cells where Cand1 and its paralog Cand2 were knocked out did not rescue the Cand1/2 knockout phenotype, whereas expressing wild-type Cand1 restored the cellular repertoire of SCF ligases to levels comparable to parental cells. Therefore, during the reassociation of a new substrate receptor to a Cand1-bound catalytic core, Skp1 outcompetes Cand1's β-hairpin for binding to Cul1's N-terminus, triggering Cand1 "rolling" and dissociation.

In conclusion, the authors provide a structural basis for the mechanism by which Cand1 promotes both the assembly and disassembly of Skp1–Fbp subcomplexes from the Cul1–Rbx1 catalytic core (Fig. 1). Through the analysis of Cand1–SCF intermediate configurations, they determined the trajectory of Cand1-mediated SCF remodeling, clarifying Cand1's role in SCF-mediated ubiquitination. Combined with neddylation machinery, Cand1 is critical for maintaining the dynamic landscape of SCF ligases. Most studies on Cand1 are centered around SCFs; thus, future research could explore whether the same mechanisms apply to other subfamilies of CRLs as well as how Cand1 distribution is coordinated across the different CRL subfamilies.

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ADDITIONAL INFORMATION

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