Cell biology

Transfer of ubiquitin protein is caught in the act

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A process termed ubiquitination mediates the regulated destruction of cellular proteins, thereby preventing disease or infection. Structural data now reveal how a crucial regulator of ubiquitination enzymes coordinates this process. **See p.461**

Many cellular functions that occur in eukaryotes (organisms whose cells contain a nucleus) are regulated by targeted protein destruction. This targeting is often achieved by a process called ubiquitination (or ubiquitylation), in which a protein selected for destruction is tagged with the protein ubiquitin. Ubiquitination is aided by enzymes known as E3 ligases, a subset of which are called cullin–RING ubiquitin ligases (CRLs)[1]. CRLs help to transfer ubiquitin from an E2 conjugating enzyme, to which it is bound, onto the target protein[1]. By default, CRLs are inactive, and they are activated when a protein called NEDD8 (which is similar in sequence to ubiquitin) becomes attached to the cullin subunit of the CRL[2–5]. But how this activation happens has been a mystery. On page 461, Baek *et al.*[6] report structural data obtained using a technique called cryo-electron microscopy (cryo-EM) that fills in some of the blanks.

CRLs contain a banana-shaped cullin subunit (one of five cullin proteins, CUL1 to CUL5). This binds (Fig. 1) at one end to a substrate-receptor subunit — which recruits the protein targeted for ubiquitination — and at the other end to what is termed a RING-finger protein, which is either RBX1 or RBX2 (refs 1,7). The RING-finger protein recruits a ubiquitin-attached E2 enzyme and stimulates the transfer of its ubiquitin to the target protein[1]. Previous structural analysis demonstrated that the attachment of NEDD8 to CUL5 enhances the potential of RBX2 and its ubiquitin-bound E2 enzyme to move towards the region adjacent to the substrate receptor and its bound target protein[3]. However, that work used a truncated version of CUL5 bound to RBX2, and lacked both a target protein bound to the substrate receptor and a ubiquitin-attached E2 enzyme, thus leaving to the imagination the mechanism by which NEDD8 stimulates ubiquitin transfer to the target protein.

Baek and colleagues therefore sought to capture a human NEDD8-attached CRL in the act of transferring ubiquitin to its target protein. To achieve this goal, the authors made a 'tribrid' molecule comprising three components. One component was a stretch of amino-acid residues derived from the protein IκB, which is a ubiquitination target that binds to a substrate receptor called β-TRCP. The second was an E2 enzyme termed UBE2D, and the third was ubiquitin. This tribrid provided a stable mimic of how the molecular components are arranged during the transition state, when ubiquitin is being transferred from the E2 enzyme to the target protein. Using cryo-EM, the authors obtained structural data for the complex that formed when the tribrid and β-TRCP assembled with the proteins CUL1, SKP1 and RBX1 (this complex is called $CRL1^{\beta\text{-}TRCP}$).

The type of structural information that can be obtained using X-ray crystallography is constrained by technical issues (packing forces in the crystals) that affect data collection. The cryo-EM approach taken by the authors avoids these constraints and enables multiple conformations of a structure to be obtained. The authors confirmed an earlier finding[3] that $CRL1^{\beta\text{-}TRCP}$ shows modest conformational flexibility in the absence of NEDD8, but that this flexibility increases when NEDD8 is attached. Furthermore, on addition of the tribrid, the ensemble of conformations converged to form one structure of a transition-state intermediate.

Baek and colleagues' structural data are nothing short of spectacular. Previous work[3] suggested that the active site of the E2 enzyme, where ubiquitin is transferred to the target protein, might not be fixed in location relative to the NEDD8-attached CRL because of the mobility of the RING-finger protein's RING domain. The transition state presented by Baek *et al.* shows the precise 3D relationship of three modules that form the whole complex: a catalytic module, an activation module and a substrate-scaffolding module. The catalytic module comprises ubiquitin-bound UBE2D and the RING domain of RBX1, and this module moves when NEDD8 becomes attached to CUL1. The activation module consists of a mobile domain in CUL1 called the WHB domain, to which NEDD8 attaches. The substrate-scaffolding module includes β-TRCP and portions of CUL1 and RBX1 that have a fixed spatial relationship to β-TRCP and IκB.

In Baek and colleagues' proposed activated structure, the catalytic module projects directly towards the substrate-scaffolding module, such that UBE2D touches β-TRCP (Fig. 1). The activation module coordinates the architecture of the transition state, with NEDD8 forming multiple contacts between UBE2D in the catalytic module and CUL1 in the substrate-scaffolding module. These

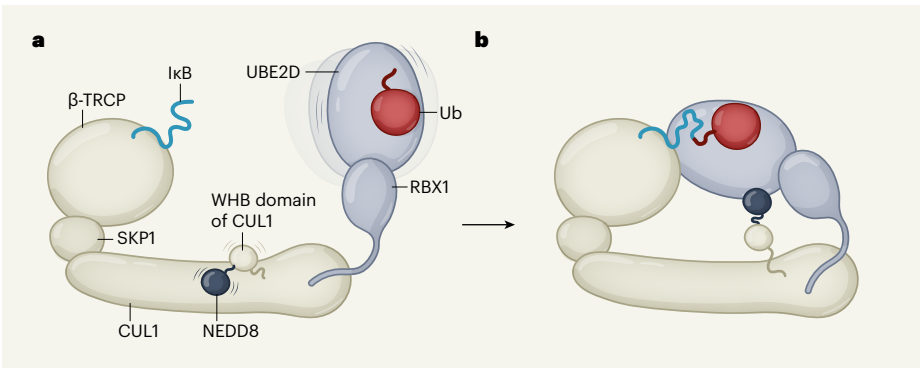

Figure 1 | Structural basis for how ubiquitination is stimulated by the NEDD8 protein. Baek *et al.*[6] used cryo-electron microscopy to analyse how the ubiquitin protein (Ub) becomes attached to a protein that is thereby marked for degradation. **a**, Ubiquitin binds to the enzyme UBE2D. The protein IκB is a ubiquitination target, and binds to a substrate receptor called β-TRCP. This receptor also binds to a protein complex consisting of SKP1–CUL1–RBX1, called CRL1, to form a complex termed $CRL1^{\beta\text{-}TRCP}$. The transfer of ubiquitin from UBE2D to IκB is aided by $CRL1^{\beta\text{-}TRCP}$. The NEDD8 protein tags the WHB domain of CUL1, thereby increasing the flexibility of the complex and enhancing ubiquitin transfer. **b**, The authors describe a transition-state complex consisting of three modules: an activation module (the NEDD8-bound WHB domain), a catalytic module (ubiquitin, UBE2D and the adjacent part of RBX1) and a substrate-scaffolding module (the remaining components). They report that extensive rearrangements of these modules occur after NEDD8 binds to CRL1, a finding that helps to explain how NEDD8 enhances ubiquitination. (Image based on Extended Data Fig. 2 of ref. 6.)

interactions stabilize the configurations of the WHB and RING domains and bring UBE2D's active site into close proximity with β-TRCP and its bound target protein.

To confirm these findings, the authors performed extensive and sophisticated kinetic analyses comparing wild-type complexes with those containing mutant proteins designed to disrupt interactions between the modules. All complexes containing a single mutant protein showed strongly reduced enzymatic activity compared with those of wild-type complexes, and complexes containing two mutant proteins had potent synergistic defects, which is consistent with the authors' model for how the complex functions.

This structure provides information that explains many previously confusing or contradictory observations. For example, it now makes sense why, during a bacterial infection, there is a catastrophic effect on CRL function when bacterial enzymes target the glutamine 40 amino-acid residue of NEDD8 (ref. 8). This is because modification of this residue would destabilize the activation module. In addition, the structure shows clearly how direct contacts between NEDD8 and UBE2D that occur away from UBE2D's catalytic site[9] work together with RBX1 to optimally position the catalytic module relative to the β-TRCP-bound target protein.

These structural insights pose new questions. Most notably, why does the transfer of the first ubiquitin to some CRL substrates require an extra RBX1-interacting complex of E3 and E2 enzymes (ARIH1 and UBE2L3, respectively[10]), given the extraordinary catalytic efficiency of the complex reported by Baek and colleagues? Moreover, how is the observed rapidity of ubiquitin transfer achieved, given the proposed requirement for the complex to undergo substantial structural rearrangements to reach the transition state? And what might the transition state look like for the NEDD8-stimulated process of chain elongation (the attachment of further ubiquitin molecules to the initial ubiquitin tag on a target protein), considering that ubiquitin-chain elongation is mediated by different E2 enzymes[11] from those that add the initial ubiquitin tag? With cryo-EM now firmly part of the toolkit for investigating ubiquitination, the answers might arrive sooner than we thought.

Importantly, these new structural data might help in the design of drugs known as proteolysis-targeting chimaeras (PROTACs), some of which can redirect specific CRL enzymes to ubiquitinate and thus destroy targets of clinical interest that are outside the enzymes' natural repertoire[12]. These drugs work by simultaneously binding substrate receptors of CRLs and a target protein. However, the formation of such complexes is not always sufficient to stimulate ubiquitin transfer[13]. The reason for this might become clear from the deeper understanding of CRL-mediated ubiquitin transfer gained through the work of Baek and colleagues.

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1. Lydeard, J. R., Schulman, B. A. & Harper, J. W. *EMBO Rep.* **14**, 1050–1061 (2013).
2. Saha, A. & Deshaies, R. J. *Mol. Cell* **32**, 21–31 (2008).
3. Duda, D. M. *et al. Cell* **134**, 995–1006 (2008).
4. Read, M. A. *et al. Mol. Cell. Biol.* **20**, 2326–2333 (2000).
5. Podust, V. N. *et al. Proc. Natl Acad. Sci. USA* **97**, 4579–4584 (2000).
6. Baek, K. *et al. Nature* **578**, 461–466 (2020).
7. Zheng, N. *et al. Nature* **416**, 703–709 (2002).
8. Cui, J. *et al. Science* **329**, 1215–1218 (2010).
9. Sakata, E. *et al. Nature Struct. Mol. Biol.* **14**, 167–168 (2007).
10. Scott, D. C. *et al. Cell* **166**, 1198–1214 (2016).
11. Sievers, Q. L., Gasser, J. A., Cowley, G. S., Fischer, E. S. & Ebert, B. L. *Blood* **132**, 1293–1303 (2018).
12. Verma, R., Mohl, D. & Deshaies, D. J. *Mol. Cell* https://doi.org/10.1016/j.molcel.2020.01.010 (2020).
13. Smith, B. E. *et al. Nature Commun.* **10**, 131 (2019).

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