Grabbing E2 by the tail

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Ubiquitin-like proteins, including NEDD8, regulate a wide range of cellular processes and are mobilized by parallel biochemical pathways. A recent crystal structure explains how the NEDD8-specific E1 enzyme specifically recruits its cognate E2 enzyme by binding to a flexible N-terminal extension.

Ubiquitylation is a form of post-translational modification in which the small protein ubiquitin is ligated by its C terminus to a lysine side chain of the target protein[1]. Many cellular proteins receive this modification[2] and consequently experience a range of possible fates including, but not limited to, degradation by the proteasome[3], endocytosis[4] and targeting to the lysosome[5]. The consequences of this process are profound; ubiquitylation seemingly affects many, if not most, facets of cellular homeostasis and regulation. Not surprisingly (in retrospect) evolution has diversified this powerful system to form a range of ubiquitin-like proteins (Ubls) that also serve as post-translational modifiers through conjugation to target proteins[6]. One of the best-known Ubls is NEDD8, which is essential in fission yeast[7] and mice[8], and functions to modify p53 (ref. 9) and the cullin subunits of ubiquitin ligases[10].

Ubiquitin, NEDD8 and other Ubls are activated and ligated to their substrate proteins by parallel pathways[11]. An E1 enzyme activates the Ubl C terminus in a two-step process of adenylation and subsequent transfer to a thioester bond with a cysteine side chain of the E1. This 'activated' Ubl is then transferred to the active site cysteine of an E2 enzyme, and subsequently to lysine side chains of the target substrate proteins in a process that usually involves an E3 enzyme. The ubiquitin pathway expands from a single E1 enzyme through 11 E2 enzymes and even more E3s to modify >1,000 of the 6,000 protein gene products in yeast[2]. Other Ubl pathways have a more conservative profile; for example, analysis of available eukaryotic sequences suggests that NEDD8 has just one E2 in all species.

As with all mechanisms of cellular regulation, questions of specificity are of critical importance for the Ubl systems. This includes the first step in the pathways, in which the Ubl-specific E1 enzymes must recognize the appropriate Ubl and corresponding E2(s) from an array of homologs. Previously published crystal structures from Schulman and colleagues have explained how the E1 enzyme for NEDD8 (the APPBP1–UBA3 heterodimer) binds ATP[12] and specifically recognizes NEDD8 (ref. 13). As the latest step in this unfolding story, Huang *et al.*[14] on page 927 of this issue report new structural and biochemical data on the human proteins that explain how APPBP1–UBA3 specifically recruits the NEDD8 E2 enzyme (Ubc12) via a peptide that extends N-terminally from Ubc12's core E2 domain.

Huang *et al.*[14] start their analysis with the observation that Ubc12 differs from most other E2s in having an N-terminal extension of 26 residues. They showed that this extension is important for NEDDylation *in vitro* and for proliferation in a cell culture assay. Furthermore, kinetic analysis and competition binding studies demonstrate that the N-terminal extension is required for efficient binding to APPBP1–UBA3. Ultimately, these biochemical data are given solid form by the crystal structure of APPBP1–UBA3 determined in complex with a peptide corresponding to the 26 N-terminal residues of Ubc12 (**Fig. 1**). Residues 1–13 are visible in this structure, where they lie in an extended conformation in a groove at the edge of the UBA3 β-sheet. Residues 14–26 lack defined electron density and are presumably mobile in the crystal. Binding is stabilized by numerous hydrogen bonding interactions to main chain groups of the peptide and by burial of the peptide Phe5 and Leu7 side chains in hydrophobic environments.

The structure suggests that the N-terminal extension is flexible in the unbound Ubc12 protein and binding of residues 1–13 leaves residues 14–26 as a flexible tether connecting the N-terminal binding epitope to the ordered E2 domain in the APPBP1–UBA3 complex. Huang *et al.*[14] support their model by demonstrating that the kinetics of Ubc12–NEDD8 thioester formation is unchanged upon insertion of seven additional residues or deletion of five residues from the flexible connecting region. The simplest interpretation of the current data is that binding of Ubc12 to APPBP1–UBA3 is bipartite; one contact is with the N-terminal extension, as seen in the crystal structure, whereas the other contact is with the body of the folded E2 core domain. Specific contacts with the E2 core domain are presumably required for efficient thioester transfer and are probably highly similar to contacts other Ubls make with their cognate E1 enzymes. Thus, for Ubc12, binding of the N terminus contributes to binding affinity, whereas the E2 enzymes of other Ubls, most of which lack N-terminal extensions, derive all of their E1 binding affinity from interactions of their core domains. This model therefore explains how APPBP1–UBA3 specifically recognizes Ubc12, although it does not explain how other E1 enzymes select their cognate E2 enzymes.

Combination of the information provided by the new structure with previously determined APPBP1–UBA3 structures[12,13] allows construction of a composite model for activation of ubiquitin, NEDD8 and other Ubls (**Fig. 1**). In this view, which is also supported

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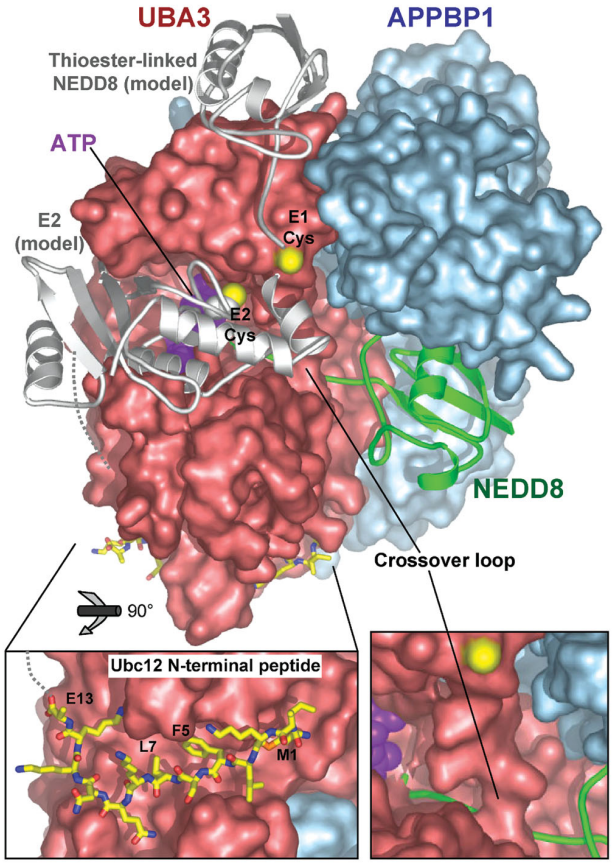

Figure 1 Composite model of the APPBP1–UBA3 E1 and its interactions. This figure was constructed by superposition of three APPBP1–UBA3 structures: the APPBP1–UBA3 complex with the Ubc12 N-terminal peptide (standard atom colors) reported in this issue by Huang *et al.*[14], and the ATP (purple)[12] and NEDD8 (green; adenylation binding site)[13] complexes. A possible position for the second NEDD8 bound as a thioester to UBA3 Cys216 is shown in white. The core E2 domain of Ubc12 (white) was positioned by manually docking the equivalent domain of yeast Ubc13 under the constraints that E1 and E2 cysteines (yellow) should be close together, the core domain is tethered to the last ordered residue of the N-terminal peptide by an eight-residue connecting peptide (dotted line), and major steric clashes are avoided. The Ubc13 structure was used because the Ubc12 core domain structure has not been determined and all known E2 core domain structures are highly conserved. Shown in the lower left panel is the Ubc12 N-terminal peptide (yellow) binding to UBA3 as viewed from the underside. The lower right panel shows the crossover loop through which the NEDD8 C terminus passes to reach the ATP.

by kinetic studies of the ubiquitin E1 (refs. 11,15) and APPBP1–UBA3 (ref. 16), the first step in Ubl activation involves its specific binding through noncovalent interactions in a cleft on the right side of **Figure 1** (NEDD8, green), whereas ATP is bound in another cleft on the left side. These two clefts are separated by a loop (crossover loop), through which the Ubl C terminus extends to reach the bound ATP and undergo adenylation[12,13,17]. Subsequently, the second step in activation is transfer of the Ubl C terminus from the adenylate to form a thioester with UBA3 Cys216, which is adjacent to the crossover loop and is conserved in E1 sequences. Although this second step may seem unnecessary from a chemical perspective because the adenylated Ubl C terminus is already activated, it may have evolved to avoid a topological trap in which the Ubl and E2 enzyme are stuck on opposite sides of the UBA3 E1 crossover loop[17]. Once covalently bound to UBA3 Cys216, the Ubl can be displaced from its initial binding site to allow binding of a second Ubl and formation of adenylate, while the first Ubl is still bound as a thioester[15,16]. The orientation of Cys216-bound Ubl is currently unknown; it might even be highly mobile and lack any significant noncovalent interactions with the E1 enzyme, although this suggestion is purely speculative.

The binding site characterized by Huang *et al.*[14] places the peptide's last ordered residue, Glu13, ~55 Å from Cys216 of UBA3 (**Fig. 1**). Assuming a fully extended conformation for eight connecting residues, as indicated by their biochemical data, Huang *et al.*[14] note that an E2 core domain model can be positioned with the Ubc12 catalytic cysteine adjacent to Cys216 of UBA3. We have built a simple model to illustrate this point and found that without allowing any conformational changes and only minimal steric overlap, the E1 and E2 cysteines are separated by ~9 Å (**Fig. 1**). Minor adjustments would allow them to approach each other more closely and possibly accommodate the E2 core domain more deeply into the E1 cleft. Thus, although the N-terminal peptide-binding site is distant from the E1 cysteine, transfer to E2 can, at least in principle, be accommodated without invoking major structural rearrangements.

In summary, Huang *et al.*[14] have explained how the NEDD8-specific E1 enzyme recognizes Ubc12, and also provided data upon which to build models of other E1–E2 complexes. Several important questions remain, however, to spur further effort. For example, the structure of an E1–E2 core domain complex will be needed to explain how other E1s select their cognate E2 enzymes and determine the geometry of the E1-to-E2 thiolester transfer. Another remaining mechanistic question is how do E1s transfer their Ubl from the adenylate site to the thioester site—a process that apparently requires considerable conformational change[12,17]. It seems that the more we learn about the structural biology of the essential E1 enzymes, the more intriguing they become.

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