All change: protein conformation and the ubiquitination reaction cascade

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Abstract

The structures of enzymes that collectively modify proteins by covalent addition of ubiquitin-like protein moieties have provided significant insights into the regulatory pathways they compose and have highlighted the importance of protein flexibility for the mechanism and regulation of the ubiquitination reaction.

Introduction and context

Protein modification by the addition of a small ubiquitin-like protein (Ubl) is a mechanism widely employed in eukaryotic cells to control protein activity after translation (reviewed in [1]). Ubis constitute a growing family of structurally similar molecules that include ubiquitin (Ub), NEDD8 (neural precursor cell expressed, developmentally downregulated 8), SUMO (small ubiquitin-like modifier), ISG15 (interferon-stimulated gene 15), and ATG8 (autophagy 8), and are reviewed in [2]. Ubis become conjugated to target proteins in a three-enzyme cascade. In the first reaction, an E1 (Ubl-activating) enzyme transfers an adenyl moiety from ATP to the C-terminal glycine residue of the Ubl, thus activating the Ubl for subsequent transfer to an E1 cysteine residue to generate a thioester linked E1-Ubl conjugate. In the next reaction, the Ubl is transferred to an E2 (Ubl-conjugating enzyme) active site cysteine residue, while in the final reaction, an E3 (Ubl ligase) catalyses transfer of the activated Ubl to its protein target (reviewed in [3–5]). In some cases, targets are modified by the addition of only a single Ubl, but in others, signal diversity is increased by the addition of chains of Ubis in which Ubl identity, chain length, and linkage type all contribute to specificity (reviewed in [6,7]).

Like many proteins involved in signalling pathways, E1, E2 and E3 enzymes are characterised by modular architectures in which regulator binding and enzymatic activities are segregated either to different domains within the same protein or, as is the case with many E3s, to different proteins. Recent structures determined by X-ray crystallography have provided detailed snapshots of E1-, E2-, and E3-containing complexes caught at different stages in their catalytic cycles and from these structures important insights into their mechanisms have been gained.

Major recent advances

The recent determination of the structure of a complex containing the NEDD8-E1 enzyme APPBP1-UBA3, a catalytically inactive variant of its cognate E2 (Ubc12), two NEDD8 molecules and Mg-ATP [8] (Figure 1a) elegantly demonstrated how a conformational change in an E1 can generate an affinity switch; only when the E1 is doubly loaded with its cognate Ubl does it acquire high affinity for its cognate E2. In this example, the APPBP1-UBA3 E1, once charged with two NEDD8 molecules at its adenylation (A)- and thioester (T)-active sites, transfers the (covalently bound) T-site NEDD8 to Ubc12 (an E2) and then adopts a conformation that promotes the release of the E2-Ubl conjugate. As the E2-binding sites for E1 and for E3 are mutually exclusive, the structure suggests that the E2 must dissociate from the E1 before it

Figure 1. Conformational change accompanies catalysis in E1s

(a) A conformational switch accompanies double-loading of an E1. A comparison of the structure of a singly loaded E1 complex (APPBP1-UBA3-NEDD8-ATP, PDB code 1R4N [11], left-hand side) and that of the doubly loaded complex (PDB code 2NVU [8], right-hand side) elegantly demonstrates the conformational switch within the E1 that accompanies double-loading of the E1 with its cognate Ubl. The direction of the rearrangement of the UFD domain of UBA3 (that binds to the E2) as well as that in which the NEDD8 is passed on from the A site to the T site are indicated with arrows. The molecular surfaces of APPBP1 and UBA3 are highlighted in light cyan and grey, respectively. In the right-hand panel, the E2 Ubc12 is represented in turquoise. The UBA3 UFD domain (black) is shown in ribbon representation. Bound NEDD8 molecules are coloured red (A site) and orange (T site). **(b)** Comparison of singly charged E1 structures. Left panel: Uba1-Ub complex (PDB code 3CMM [10]). Right panel: APPBP1-UBA3-NEDD8-ATP complex. The bound small ubiquitin-like protein (Ub or NEDD8, respectively) is coloured red and the UFD domains of both E1 enzymes are coloured black. Within the E1 structures, the two juxtaposed catalytic cysteine half domains that compose the catalytic cysteine domain [19] are coloured pale yellow and light blue (respectively), the crossover loop that connects the adenylation and catalytic cysteine domains is coloured dark blue, and the molecular surface of the rest of the E1 is coloured grey (Uba1 and UBA3) and light cyan (APPBP1). A, adenylation; APPBP1, amyloid beta precursor protein-binding protein 1; NEDD8, neural precursor cell expressed, developmentally downregulated 8; PDB, Protein Data Bank; T, thioester; Ub, ubiquitin; Ubl, small ubiquitin-like protein; UBA3, ubiquitin-activating enzyme 3; UFD, ubiquitin-fold domain.

can associate with a cognate E3. Indeed, enzymological studies, at least in some cases, have shown that this is in fact the case [9].

Subsequent determination of the structure of Uba1, a yeast Ub-E1 non-covalently bound to Ub, has demonstrated that the E1 mechanism, though broadly conserved, is subject to variation in detail [10] (Figure 1b). A comparison of the structures of the two copies of the Uba1-Ub complex in the crystal asymmetric unit further highlights the different positions that the ubiquitin-fold domain (UFD) of the E1 can adopt relative to the other E1 domains. Notably, in this singly Ub-loaded structure, the location of the UFD is similar to that of the UFD in the doubly NEDD8-loaded APPBP1-UBA3 structure and distinct from that observed in structures of NEDD8 [11] and SUMO [12] E1 enzymes singly Ubl-loaded at the A site. As discussed in Huang *et al.* [8], the reorientation of the UFD that accompanies double-loading of the NEDD8 E1 with NEDD8 is critical to subsequent transfer of the covalently bound T-site NEDD8 to the E2. In addition, the canyon formed between the UFD and the catalytic cysteine domain in the Uba1-Ub structure (Figure 1b) is much wider than that seen in APPBP1-UBA3. Taken together, these and other observations suggest that the conformational changes that accompany transfer of Ub from E1 to E2 will be much less dramatic than those associated with transfer of NEDD8 or SUMO.

As with E1 and E2 enzymes, an overriding theme to emerge from recent structural studies of E3s is the functional importance of conformational flexibility. E3s fall into three major subclasses according to the identity of the domain that binds the covalent E2-Ubl complex. These subclasses are referred to as 'homologous to the E6-AP carboxyl terminus' (HECT), 'really interesting new gene' (RING)/RING-like, and those E3s that have no resemblance to the HECT- or RING-containing enzymes. Recent structural and biochemical studies have begun to elucidate the mechanistic diversity of this broad enzyme class (reviewed in [4]), but in all examples, a recurrent theme is the importance of conformational flexibility to allow the E3 complexes to bind and modify different protein substrates.

A particular example, well documented by structural studies, is that of the SCF [Skp1 (S-phase kinase-associated protein 1)-cullin-F box] E3 Ub ligase family (reviewed in [13]). This E3 contains a RING domain that binds to the cullin subunit. The cullin functions as a scaffold within the complex and also binds to the adaptor protein Skp1 [14]. SCF activity is promoted by cullin neddylation [15] and inhibited by CAND1 (cullin-associated and neddylation-dissociated 1) binding [16].

Skp1 and the cullin together mediate the interaction between a diverse set of F-box-containing proteins that bring substrates to the E2, and E3 catalytic core. Models for SCF E3/E2 complexes have been constructed by superimposing structures of selected subcomplexes [14,17] (Figure 2a). However, it is difficult to see how catalysis will proceed as the E3 substrate bound to its cognate F-box protein is located approximately 50 Å away from the bound activated E2-Ubl [14].

The structure of a neddylated cullin-Rbx1 (ring-box 1) complex [17] (Figure 2b) and enzymological studies of selected SCFs [15,18] together provide valuable extra information to help solve this puzzle. Previous attempts to generate models for an SCF E3 complex had employed structures that contained a non-neddylated cullin subunit. A comparison of the neddylated and non-neddylated cullin structures bound to Rbx1 reveals that neddylation leads to the adoption by the cullin-Rbx1 complex of a more open structure in which the Rbx1 N-terminal linker is no longer constrained by interactions with the cullin, and as a result, the Rbx1 RING domain is also freed so that bound substrate is predicted to come significantly closer to charged E2 [17]. This prediction is supported by the fact that the neddylated SCF^{β-TrCP} complex, but not the non-neddylated form of this complex, promotes the BMPS [*N*-(β-maleimido-propyloxy)succinimide ester]-mediated crosslinking of UbCH5c (an SCF^{β-TrCP} E2) with a peptide from β-catenin (an SCF^{β-TrCP} substrate) [15].

As a further result of these structural changes, the cullin CAND1-binding site is also lost. Thus, the structure of the neddylated cullin-Rbx1 complex answers the question of how the distance between the bound substrate and E2 might be spanned and of how CAND1 inhibitor binding and cullin neddylation reciprocally regulate SCF activity: CAND1 binding stabilises the closed (inactive) cullin structure, whereas cullin neddylation promotes the more open (active) conformation and simultaneously prevents CAND1 association.

Future directions

Now that initial structures have given a glimpse of the structural diversity of the enzymes of the Ubl conjugation pathway, future work will focus on characterising complete complexes at different stages of their catalytic cycles. We expect further insights into this important signalling mechanism to emerge from visualising and characterising both the conserved and the unique mechanistic aspects of different Ubl-conjugating enzymes.

Figure 2. Conformational change accompanies catalysis in E3s

(a) Model for an E2/SCF^{Skp2} complex. This image was generated by merging the structures of Cul1-Rbx1-Skp1-Skp2 (1LDK) [14] and Skp1/Skp2 (1FQV) [20] as described in [14]. The E2 Ubch7 (2FBV) was then placed on to the Rbx1 RING domain guided by the c-Cbl-Ubch7 structure [21]. Skp1 and Skp2 are shown in blue and yellow (respectively), Cul1 in dark grey, Rbx1 in dark red, and the E2 Ubch7 in cyan. The catalytic cysteine is coloured pink. **(b)** Conformational change accompanies neddylation of Cul1. The panels highlight the change in the association of Rbx1 with Cul1 that accompanies Cul1 neddylation (dark red arrow). For comparison, only the Cul1 C-terminal domain (dark grey) is drawn. The left panel shows the non-neddylated structure in which Rbx1 (dark red ribbon) forms a close association with Cul1. Lys724 (non-neddylated) is drawn as blue spheres. The right panel depicts the neddylated Cul1-Rbx1 complex. Neddylation (NEDD8, red) promotes a more open Cul1-Rbx1 association. Lys724 covalently bound to NEDD8 is drawn as blue spheres. Cul1, cullin 1; NEDD8, neural precursor cell expressed, developmentally downregulated 8; Rbx1, RING-box protein 1; RING, really interesting new gene; SCF, Skp1 [S-phase kinase-associated protein 1]-cullin-F box; Skp, S-phase kinase-associated protein.

Abbreviations

A, adenylation; CAND1, cullin-associated and neddylation-dissociated 1; HECT, homologous to the E6-AP carboxyl terminus; NEDD8, neural precursor cell

expressed, developmentally downregulated 8; Rbx1, ring-box protein 1; RING, really interesting new gene; SCF, Skp1 [S-phase kinase-associated protein 1]-cullin-F box; Skp1, S-phase kinase-associated protein 1; SUMO, small ubiquitin-like modifier; T, thioester; Ub, ubiquitin; UBA3, ubiquitin-activating enzyme 3; Ubl, small ubiquitin-like protein; UFD, ubiquitin-fold domain.

Competing interests

The authors declare that they have no competing interests.

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