UFM1-Activating Enzyme 5 (Uba5) Requires an Extension to Get the Job Done Right

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Ubiquitin (Ub) and Ub-like proteins (Ubls) are posttranslational modifiers that regulate nearly every aspect of eukaryotic biology by altering the stability, intermolecular interactions, activity, and localization of modified target proteins [1]. The importance of Ub/Ubl signaling to human health is underscored by the fact that its dysregulation is implicated in a number of pathologies and that the pathways serve as targets for therapeutic intervention in cancer and other disorders. All Ub/Ubls are conjugated to target proteins through the sequential activities and interactions of parallel cascades of enzymes called E1, E2, and E3 that together serve to activate, shuttle, and ligate Ub/Ubls to target proteins, respectively [2].

Although Ub/Ubls and the enzymes in their conjugation cascades are structurally and mechanistically related, they regulate distinct repertoires of cellular processes, thus requiring mechanisms to ensure fidelity. This is primarily achieved by E1 enzymes, which serve as the gatekeepers of Ub/Ubl conjugation cascades by selectively binding and activating their cognate Ub/Ubls followed by transfer of the Ub/Ubl to cognate E2s [3–5]. All E1 enzymes activate Ub/Ubls through an ATP-dependent two-step catalytic mechanism comprising sequential adenylation and thioesterification reactions. The adenylation reaction comprises nucleophilic attack of a conserved C-terminal glycine carboxylate on the α-phosphate of ATP to form a Ub/Ubl-AMP acyl-adenylate intermediate and a pyrophosphate leaving group. The thioesterification reaction involves nucleophilic attack of the Ub/Ubl adenylate intermediate by the catalytic cysteine residue of E1 to form a high-energy thioester bond between the C-terminal glycine of the Ub/Ubl and the E1 catalytic cysteine along with an AMP leaving group. This is followed by recruitment of E2 enzymes and transfer of the Ub/Ubl from the E1 catalytic cysteine to the E2 catalytic cysteine in a process called E1–E2–Ubl thioester transfer [3–5].

Biochemical and structural studies have revealed remarkable diversity in the structural mechanisms that different E1s employ for catalysis of the adenylation, thioesterification, and transthiolation reactions [6–16]. This derives primarily from distinct structural features of the E1s, reflected by their organization into two groups, canonical and noncanonical [3]. Canonical E1s (Ub E1, Nedd8 E1, SUMO E1, Uba6, Uba7) have a common architecture comprising a pseudodimeric adenylation domain in which only one of the protomers is active, a Cys domain that harbors the catalytic cysteine, and a Ub-fold domain (UFD) that is involved in E2 recruitment. On the other hand, noncanonical E1s (Uba4, Uba5, Atg7) are obligate homodimers that harbor homodimeric adenylation domains in which both protomers are catalytically active, but lack the globular Cys domain and UFD present in canonical E1s. While canonical E1s recruit Ub/Ubls almost exclusively by interactions with the adenylation domains [6,7,9], unique structural elements in noncanonical E1s play important roles in Ubl recruitment in addition to the adenylation domains. These include the ECTD of Atg7, which plays a role in the multi-step recognition of the Ubl Atg8 [14,17,18], and the UIS of Uba5, which interacts with the Ubl UFM1 through a *trans* binding mechanism [15,19,20]. Notably, the homodimeric nature of noncanonical E1s results in two copies of the active functional elements (adenylation domains, catalytic cysteines, E2-interacting regions) in each protomer of the enzyme, which enables a *trans* E1-E2 thioester transfer mechanism for Atg7 [14,16,17,21,22] and Uba5 [15], in contrast to canonical E1s, which use a *cis* mechanism [10–12].

Despite an ever-increasing wealth of knowledge regarding the structure and function of E1 enzymes, little is known about potential mechanisms that might exist to regulate their activities. In this issue, Soudah and colleagues [23] present biochemical, biophysical, and structural data revealing that the activity of the noncanonical E1, Uba5, is modulated by an alternative splicing event that results in isoforms that differ only by the presence or absence of a stretch of 56 amino acids at the N-terminus of the enzymes (so-called long and short isoforms). All previous structural studies on Uba5 utilized the short isoform, and while the overall structures of the adenylation domain resembled those of previously characterized E1s, several surprising features of the Uba5 adenylation domain were observed (Fig. 1). First, despite harboring two copies of an active adenylation domain, only one copy interacted with nucleotide (ATP, AMP, or AMPPNP) [13,15]. Second, while ATP adopts a similar conformation in all other E1 structures, including bacterial ancestors of E1s (MoeB, ThiF, and MccB), the positions of the β- and γ-phosphates of ATP in the Uba5/ATP structure occupied strikingly different positions [13]. Third, the short isoform of Uba5 lacks a helical region present at the N-termini of adenylation domains in all other E1s (termed the H1/H2 region) [6–16], and a second region that is conserved and ordered in all other E1s (termed the g1/g2 region) [6–16] is disordered in structures of the short isoform of Uba5 [13,15]. Importantly, the H1/H2 and g1/g2 regions are located in adjacent protomers of the pseudodimeric adenylation domain and are positioned proximally where they engage in contacts that fortify the pseudodimer interface [6–16]. Both H1/H2 and g1/g2 regions mediate numerous contacts to ATP that are crucial for catalysis and altogether account for ~20%–25% of the surface area buried at E1/ATP interfaces.

In this study, Soudah and colleagues [23] determined that the long isoform of Uba5 has a significantly higher affinity for ATP and a faster rate of UFM1 activation compared to the short isoform. The authors narrowed down the region of the N-terminus responsible for these differences to residues 37–56 and further demonstrated that this region mediates a significant ATP-dependent increase in the thermal stability of Uba5 compared to the short isoform. To elucidate the molecular basis for these observations, the authors determined crystal structures of Uba5 harboring the 20-residue extension in complex with ATP•Mg, both in the presence and absence of UFM1 [23]. Analysis of the structures revealed that key regions of the Uba5 active site that are missing or disordered in structures of the short Uba5 isoform (i.e., the H1/H2 and g1/g2 regions mentioned above) are ordered in the longer fragment of Uba5 and that these regions make networks of interactions with ATP•Mg and with each other that are similar to those observed in previous structures of other E1s [23] (Fig. 1). This results in a repositioning of the β- and γ-phosphates of ATP, which make a number of contacts to residues in the H1/H2 and g1/g2 regions and a shift of the ATP:Uba5 molar ratio from 1:2 to 1:1 [23]. Altogether, the work of Soudah and colleagues [23] suggests that alternative splicing serves as a molecular switch that toggles Uba5 activity via remodeling of its active site. While the short isoform of Uba5 is capable of catalyzing Ufm1 activation and thioester transfer to Ufc1, its significantly lower level of activity compared to the longer

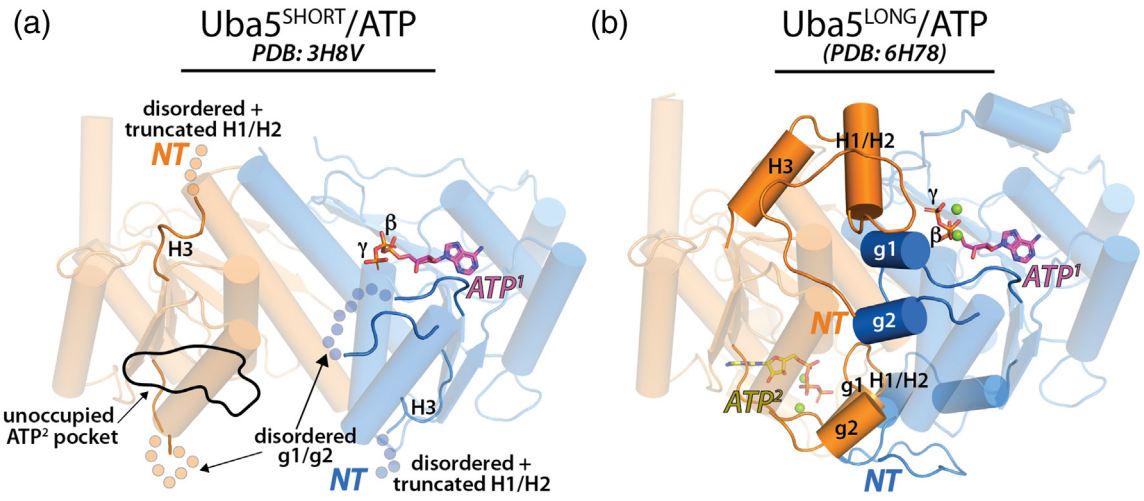

Fig. 1. Structural comparison of the short and long isoforms of Uba5 in complex with ATP. The Uba5^{SHORT} (a) and Uba5^{LONG} (b) structures are shown as cartoon representations with ATP presented as sticks. Key structural elements required for Uba5 activity as discussed in the text are labeled. Regions of disorder are shown as semitransparent spheres. NT is N-terminus.

isoform of Uba5 is likely due to suboptimal organization of its active site.

Ordering of the g1/g2 region in the longer fragment of Uba5 is likely due to contacts with the H1/H2 region that cannot occur in the short isoform. This structural interplay between the H1/H2 and g1/g2 regions is emerging as a key feature of E1 mechanism [24–26]. Disassembly of the E1 adenylation active site (largely at the H1/H2 and g1/g2 regions) has been demonstrated to accompany thioesterification, serving as means to drive the reaction forward in canonical E1s [24–26], whereas structures mimicking canonical E1–E2–Ubl thioester transfer reveal reassembly of the adenylation active site [10–12]. Furthermore, although the N-terminal extension of the long isoform of Uba5 is not involved in E2 (Ufc1) binding, Soudah and colleagues demonstrate through single-turnover biochemical analysis that the long isoform of Uba5 has a significantly faster rate of Uba5–Ufc1–Ufm1 thioester transfer compared to the short isoform in a manner dependent on ATP binding but not hydrolysis [23]. This suggests a structural role for ATP in thioester transfer, perhaps by fortifying the homodimer interface through its contacts with both H1/H2 and g1/g2, thereby promoting the *trans* thioester transfer mechanism of Uba5 [15]. This further suggests that the Uba5 active site is also likely to be assembled during thioester transfer, and the lack of a comparable "boost" in thioester transfer activity for the short isoform of Uba5 is likely due to its reduced ATP-binding affinity and consequent inability of the active site to adopt an active conformation, instead adopting a conformation with a less fortified homodimer interface. Finally, that the enhancement of thioester transfer by ATP does not require hydrolysis is consistent with previous studies demonstrating that Uba5 activates Ufm1 via a two-step reaction that involves binary complex formation, in contrast to canonical E1s that activate their Ubls through a three-step mechanism involving ternary complex formation [27].

A key question moving forward is to determine how alternative splicing of Uba5 might be important for biological activity. Further exciting mechanistic questions to be addressed include determining whether the Uba5 active site disassembles during thioesterification and reassembles during E1-E2 thioester transfer, as observed during canonical E1–E2–Ubl thioester transfer, and what conformational changes accompany these processes.

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