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POST-TRANSLATIONAL MODIFICATION

UBE1, you're not alone

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Ubiquitin and other ubiquitin-like molecules (UBLs) are post-translationally attached to proteins through a sequential enzymatic cascade that uses an E1 activating enzyme, an E2 conjugating enzyme and an E3 ligase. It was presumed that a single E1 — UBE1 (ubiquitin-activating enzyme E1) — activates ubiquitin and that the substrate specificity of the cascade (that is, which proteins are modified with ubiquitin) was exclusively due to the diversity of E2s and E3s. Jin *et al.* show both of these assumptions to be incorrect by identifying a second E1 for ubiquitin that defines a separate ubiquitin-conjugating pathway.

E1 enzymes are characterized by an adenylation domain that is composed of two ThiF-homology motifs that bind and adenylate (activate) the UBL, a catalytic cysteine domain (CCD) to which the adenylated UBL is transiently attached during the cascade and a C-terminal ubiquitin-fold domain (UFD) that recruits E2. Jin *et al.* searched the human genome for genes that encode a ThiF-homology domain. They identified all known E1s and a previously uncharacterized protein, UBA6 (also known as UBE1L2 (ubiquitin-activating enzyme E1-like 2)). UBA6 also contains a CCD and a UFD so, by domain composition alone, UBA6 closely resembles an E1 enzyme.

> "…these findings show an unanticipated level of regulation in the ubiquitin-conjugation cascade."

The ability of UBA6 to activate different UBLs, including several for which the E1 is unknown, was tested *in vitro*. Surprisingly, UBA6 activated ubiquitin but not the other UBLs tested. This was confirmed *in vivo*: UBA6 that was conjugated to ubiquitin could be isolated from cells under conditions that stabilized the E1–ubiquitin intermediate.

Next, the authors compared the specificity of UBA6 to transfer ubiquitin to different E2s with that of UBE1. Although some E2s were charged by UBA6 and UBE1 with equal efficiencies, the two E1s showed different specificities. Almost half of the E2s tested were charged by UBE1 only, whereas a previously uncharacterized E2 was identified as the first UBA6-specific E2 (USE1). The differential charging of E2s was conserved *in vivo*: small-interfering-RNA-mediated depletion of UBA6 selectively reduced the levels of charged USE1 but not those of a UBE1-specific E2. UBA6 and USE1 form part of a second ubiquitin-conjugating cascade; however, the E3s and target substrates for USE1 remain unknown. The specificity of the different E1s is predominantly generated by the UFD because switching the UFDs between UBA6 and UBE1 reversed the specificity towards some (but not all) E2s.

Together, these findings show an unanticipated level of regulation in the ubiquitin-conjugation cascade. Previously, the ubiquitin dependence of a process has often been investigated by inhibiting UBE1 and, therefore, a re-evaluation of true ubiquitin independence is now required.

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ORIGINAL RESEARCH PAPER Jin, J. *et al.* Dual E1 activation systems for ubiquitin differentially regulate E2 enzyme charging. *Nature* **447**, 1135–1138 (2007)
FURTHER READING Welchman, R. L. *et al.* Ubiquitin and ubiquitin-like proteins as multifunctional signals. *Nature Rev. Mol. Cell Biol.* **6**, 599–609 (2005)