SUMOYLATION

No half measures

IMAGE SOURCE

The covalent conjugation of ubiquitin (Ub) and Ub-like (Ubl) proteins, such as small ubiquitin-related modifier (SUMO), to other proteins is vital for differentiation, apoptosis and the cell cycle. Attachment of Ub and Ubl proteins is tightly regulated and requires three enzymes. An E1 enzyme activates the Ub or Ubl protein and transfers it to an E2 conjugating enzyme, which, together with E3 ligases, promotes Ub or Ubl protein conjugation. Activation of Ub and Ubl proteins by E1s occurs through two half reactions. The first uses ATP and magnesium to couple an adenylate group to the Ub or Ubl carboxyl terminus and the second results in the formation of a thioester bond between an E1 catalytic Cys and the Ub or Ubl C terminus, with release of AMP. Although several previously determined structures showed that E1 was apparently poised to undergo adenylation, no adenylate was ever seen in the E1 active site. In addition, the E1 catalytic Cys was too far away to catalyse the thioester transfer reaction. Two crystal structures of E1 in complex with SUMO now help to solve the mystery.

To capture the intermediate E1-catalysed states, Lu *et al.* synthesized SUMO mimics that resemble either the adenylate intermediate or an intermediate during thioester formation, and Olsen *et al.* determined the structures of human E1 with both SUMO constructs. As in previous structures, E1 was seen to have multiple domains, including a catalytic Cys domain and two adenylation domains. When Olsen *et al.* compared the new structures with the previous ones bound to ATP, magnesium and SUMO, they found that after adenylation, the E1 active site undergoes substantial changes to promote thioester bond formation.

Many side chains that were initially in contact with the ATP and magnesium move out of the way to facilitate the release of pyrophosphate and magnesium, and the Cys domain rotates 130° to place the helix containing the E1 catalytic Cys into the active site. At the same time, the crossover loop and the re-entry loop, which link the Cys domain to the adenylation domains, alter their conformation, as do two helices needed for adenylation.

Nearly half of the active site residues required for adenylation are replaced, mostly by residues from the Cys domain that are required for thioester bond formation. These observations were confirmed through biochemical and mutational analysis, which indicates that these residues are essential in other E1 enzymes.

Previous examples of active-site alterations have involved whole domain movements rather than the refolding of secondary structural elements and the movement of so many specific residues. The next questions are: why is it catalytically favourable to make such dramatic changes to the active site and are similar changes also required during thioester transfer between the E1 and E2?

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ORIGINAL RESEARCH PAPERS Olsen, S. K. *et al.* Active site remodelling accompanies thioester bond formation in the SUMO E1. *Nature* **463**, 906–912 (2010) | Lu, X. *et al.* Designed semisynthetic protein inhibitors of Ub/Ubl E1 activating enzymes. *J. Am. Chem. Soc.* **132,** 1748–1749 (2010)
FURTHER READING Geiss-Friedlander, R. & Melchior, F. Concepts in sumoylation: a decade on. *Nature Rev. Mol. Cell Biol.* **8**, 947–956 (2007)