Nature Reviews Molecular Cell Biology | AOP, published online 9 December 2009; doi:10.1038/nrm2824

PROTEIN DEGRADATION

Step by step

Ubiquitylation involves the activation of ubiquitin by an E1 enzyme and its subsequent transfer to a ubiquitin-conjugating enzyme (E2), which interacts with an E3 ligase to ubiquitylate the substrate. Although it is known that at least four ubiquitin molecules in a chain are needed to target proteins for degradation, the mechanism of chain assembly has been elusive owing to the rapid speed of the reaction. Now, two studies by Deshaies and colleagues provide important insights by measuring the ubiquitin transfer reaction with millisecond time resolution.

Chain extension could occur by stepwise addition of ubiquitin monomers, by transfer of a preassembled chain from the enzymatic machinery to the substrate (*en bloc* transfer) or by a combination of the two mechanisms. Pierce *et al.* visualized ubiquitylation of a model substrate by the Cdc34–SCF^{Cdc4} (E2–E3) complex in a single-encounter reaction and found that ubiquitylation was complete in 30 seconds — too quick to discern the mechanism of chain extension. So, they measured the distribution of ubiquitin chain lengths on Cdc34 and the number of ubiquitin transfer events from Cdc34 to the substrate, and tested the results in a theoretical model of the mechanisms of chain formation. The results predicted that Cdc34–SCF^{Cdc4} assembles ubiquitin chains on substrates primarily by sequential transfers of single ubiquitin molecules. This 'step by step' polyubiquitylation mechanism was confirmed by data from single-encounter reactions on a quench flow apparatus, which allowed the authors to take measurements of the product distribution on a millisecond timescale.

Kleiger *et al.* studied how the speed of ubiquitylation is achieved. They measured the dynamics of the Cdc34–SCF^{Cdc4} interaction and found that the two enzymes bind and dissociate rapidly. Based on normal rates of protein association, the rates of E2 recruitment and dissociation for an E2–E3 complex with submicromolar affinity would be too slow to sustain the maximal rate of synthesis of a ubiquitin chain reported by Pierce *et al.* (~5 molecules per second). Substrates would dissociate before chain synthesis could be completed. However, rapid association kinetics have been previously observed for interacting proteins that align through electrostatic interactions, and Cdc34 has an acidic tail that might behave in the same way. Indeed, deletion of the acidic tail of Cdc34 caused defects in

SCF^{Cdc4} binding, suggesting that electrostatic interactions might be important for complex formation.

The authors identified a positively charged surface (known as the 'basic canyon') that could accommodate the acidic tail of Cdc34 in the cullin subunit of the E3 complex, and binding was confirmed by cross-linking experiments. Notably, mutations in the basic canyon or the acidic tail block Cdc34–SCF^{Cdc4} processivity, suggesting that this interaction is important for the function of the E2–E3 complex.

These studies provide an unprecedented glimpse into the mechanisms of ubiquitylation and show for the first time that ubiquitin chains are built on SCF^{Cdc4} substrates by sequential transfer of single ubiquitin molecules. In the future, studying how proteins are tagged for degradation will be much easier.

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ORIGINAL RESEARCH PAPERS Kleiger, G. *et al.* Rapid E2-E3 assembly and disassembly enable processive ubiquitylation of cullin-RING ubiquitin ligase substrates. *Cell* **139**, 957–968 (2009) | Pierce, N. W. *et al.* Detection of sequential polyubiquitylation on a millisecond timescale. *Nature* 3 Dec 2009 (doi:10.1038/nature08595)

FURTHER READING Ye, Y. & Rape, M. Building ubiquitin chains: E2 enzymes at work. *Nature Rev. Mol. Cell Biol.* **10**, 755–764 (2009)