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PROTEIN DEGRADATION

Fits like a glove

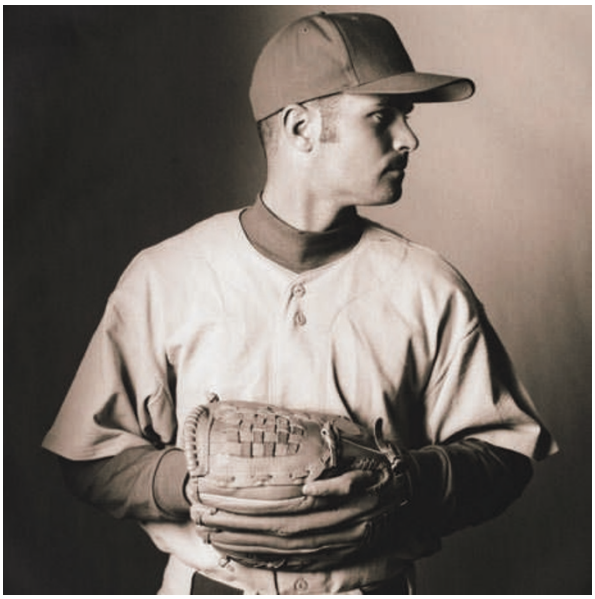
> …the ClpS residues that form the hydrophobic pocket are important for substrate recognition…

Protein degradation is an irreversible process that requires specific recognition of targets. Reporting in *Molecular Cell*, Baker and colleagues now reveal the molecular mechanisms that underlie this recognition.

The highly conserved 'N-end rule pathway' selects proteins for degradation on the basis of the identity of their amino terminal residue. In bacteria, the adaptor protein ClpS recognizes N-end rule substrates and transfers them to the ATP-dependent Clp protease complex (ClpAP) for degradation.

Baker and colleagues determined the 1.15 Å crystal structure of ClpS in complex with a mimic peptide that contained an N-terminal Tyr, which is one of the universally recognized N-end rule residues. They found that the Tyr side chain 'fits like a glove' into a deep hydrophobic pocket on the surface of ClpS, and also found that the target peptide α-amino group forms hydrogen bonds with ClpS side chains at the entrance to the pocket.

Sequence alignments of ClpS orthologues revealed a high evolutionary conservation of the ClpS side chains that make the specific contacts with the N-end rule peptide. These data suggest that the ClpS residues that form the hydrophobic pocket are important for substrate recognition and subsequent degradation. Indeed, by mutating the ClpS residues that interact with the N-terminal α-amino group of the substrate peptide, Baker and colleagues showed that substrate degradation by ClpAP was impaired *in vitro*.



The crystal structure also suggested that the side chain of a Met residue in the ClpS hydrophobic pocket worked as a gatekeeper by preventing β-branched amino-acid residues (such as Ile or Val) from entering the pocket. This could explain why these residues are not recognized by ClpS and are not degraded by the N-end rule pathway. To test this hypothesis, the authors mutated the Met residue at position 40 of *Escherichia coli* ClpS to an Ala residue and found that the adaptor could recognize substrates with β-branched amino-acid residues at the N terminus *in vitro*.

Together, these results provide the first mechanistic insights into how N-end rule substrates are recognized by the ClpS adaptor protein and degraded. In eukaryotes, N-end rule substrates are ubiquitylated by E3 ubiquitin ligases and degraded by the proteasome. The similarity between ClpS and a subset of E3 ligases suggests that a common mechanism for N-end rule recognition has evolved in different organisms.

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ORIGINAL RESEARCH PAPER Wang, K. H. *et al.* The molecular basis of N-end rule recognition. *Mol. Cell* **32**, 406–414 (2008)
FURTHER READING Ravid, T. & Hochstrasser, M. Diversity of degradation signals in the ubiquitin–proteasome system. *Nature Rev. Mol. Cell Biol.* **9**, 679–689 (2008)

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