Nature Reviews Molecular Cell Biology | AOP, published online 12 September 2007; doi:10.1038/nrm2265

POST-TRANSLATIONAL MODIFICATION

The importance of being inactive

DOI:
10.1038/nrm2265

Protein stability is controlled by the formation of polyubiquitin chains on proteins, which target them for degradation by the proteasome. Ubiquitylation can be catalysed by HECT-E3 ligases that transfer ubiquitin (Ub) from a conserved Cys residue in the catalytic HECT domain onto target proteins, as well as onto other sites on the ligase itself. However, how the activity of HECT-E3 ligases is controlled is poorly understood. In *Cell*, Wiesner *et al.* report that activity of the HECT-E3 ligase SMURF2 is regulated by an autoinhibitory mechanism that prevents futile cycles of protein production and degradation.

SMURF2 is a modular protein, composed of an N-terminal C2 domain that mediates membrane localization, three WW domains involved in binding substrates and adaptor proteins, and the HECT domain. Using nuclear magnetic resonance (NMR) analysis, Wiesner *et al.* found that the C2 domain interacted with the HECT domain at a position that was close to its catalytic Cys residue.

So, does this interaction have functional implications? The authors showed that deleting the C2 domain led to the increased formation of Ub linkages to the catalytic Cys of the HECT domain, and that mutating residues F29 and F30, which are required for the C2–HECT interaction, increased the autoubiquitylation of SMURF2. Therefore, the C2 domain has an inhibitory effect on activity of the HECT domain.

Some ubiquitylating enzymes are thought to operate as dimers, suggesting that the HECT domain could be inhibited by the C2 domain within the same protein or from a separate protein. To distinguish between these possibilities, the authors measured levels of ubiquitylation of the constitutively active (F29A/F30A) mutant in the face of increasing levels of isolated C2 domain or catalytically dead (C716A), full-length SMURF2. In contrast to the isolated domain, the C2 domain from full-length C716A SMURF2 had no effect on SMURF2 (F29A/F30A) ubiquitylation, indicating that C2-mediated inhibition arises from an intramolecular interaction.

The authors next examined the ubiquitylation of RhoA, which is a known substrate for the closely related HECT-E3 ligase SMURF1, but not SMURF2. However, SMURF2 (F29A/F30A) could ubiquitylate RhoA, which suggests that the C2–HECT interaction may play a part in determining substrate specificity. So, how might the autoinhibitory mechanism be relieved upon substrate recognition? The adaptor protein SMAD7 couples the HECT-domain activity of SMURF2 to substrate recruitment *in vivo,* and the authors showed that SMAD7 interfered with the inhibitory C2–HECT domain interaction. This elegant mechanism couples substrate recognition with the release of the autoinhibitory C2 interaction.

The autoinhibitory mechanism was found to be conserved in several E3 ligases that have a C2–WW–HECT domain architecture. Therefore, this general mechanism maintains a set of E3 enzymes in an inactive state, which prevents these enzymes and their substrates from being ubiquitylated and degraded in an unregulated manner *in vivo*.

James Pickett

> “...an autoinhibitory mechanism that prevents futile cycles of protein production and degradation.”

ORIGINAL RESEARCH PAPER Wiesner, S. *et al.* Autoinhibition of the HECT-type ubiquitin ligase Smurf2 through its C2 domain. *Cell* **130**, 651–662 (2007)