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Mini review

Regulation of catalytic activities of HECT ubiquitin ligases

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Abstract

Studies in yeast and mammalian cells over the past decade have shown that HECT domain ubiquitin ligases (HECT E3 enzymes) are involved in diverse physiological pathways. Many substrates of specific HECT E3s have been identified, as well as many adaptor proteins that aid in defining substrate specificity or intra-cellular localization of HECT E3s. Here we review some recently discovered mechanisms for regulation of the catalytic activities of HECT E3s, including regulation at the level of E2 recruitment, phosphorylation-dependent relief of inhibitory intra-molecular interactions, and regulation by association with a deubiquitinating enzyme.

Keywords: Ubiquitin; HECT ubiquitin ligases; Rsp5; Smurf2; Itch

Conjugation of ubiquitin to target proteins involves three groups of enzymes, the E1, E2, and E3 enzymes, which function cooperatively in a cascade of ubiquitin transfer reactions [1]. The E3 enzymes, or ubiquitin ligases, interact with both an upstream E2 enzyme and specific target proteins facilitating protein ubiquitination. RING type E3s, which include both monomeric proteins (e.g., Mdm2, Cbl) and multimeric protein complexes (e.g., cullin-based E3s, the APC), function primarily as scaffolds, orienting the E2–ubiquitin thioester complex and target protein for ubiquitin transfer. HECT domain E3s are unique among the several classes of E3s in that ubiquitin is transferred from the E2 to an active-site cysteine within the HECT domain, forming an E3–ubiquitin thioester complex [2]. Ubiquitin is then transferred to target proteins that are bound to the substrate recognition determinants of the E3.

The HECT domain, itself, is an approximately 350 amino acid domain that is always found at the C-terminus of the ligase, and structural information is available for three HECT domains and one HECT domain–E2 complex [3–5]. Briefly, the HECT domain consists of a larger N-lobe that contains the E2 binding site, and a smaller C-lobe that contains the active-site cysteine. The lobes are connected by a short flexible linker, and conformational flexibility about this linker appears to be critical for juxtaposing the active-site cysteines of the E2 and E3 in order to facilitate the transthiolation reaction. Most aspects of isopeptide bond catalysis are uncharacterized, particularly with respect to how polyubiquitin chains are formed, although one key requirement for ubiquitin transfer from the active-site cysteine to the target protein is a phenylalanine residue located, in most HECT E3s, four amino acids from the end of the protein [6]. This may function to properly orient the ubiquitin molecule that is tethered to the active-site cysteine.

The smallest HECT E3s are approximately 90 kDa and the largest are over 500 kDa. The region upstream of the HECT domain contains determinants of substrate specificity, intra-cellular localization, and in some cases, as discussed below, regulation. E6AP was the first HECT E3 identified, and this protein mediates the ubiquitination of p53 in cells that express the human papillomavirus (HPV) E6 oncoprotein. This appears to be an unregulated reaction in that cells that express E6 constitutively ubiquitinate p53, causing it to be constantly degraded. There are approximately 50 HECT E3s in humans and five in *Saccharomyces cerevisiae*. Only a few of these have been characterized in

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detail, although the best characterized subgroup of HECT E3s, with respect to both substrate identification and regulation, are the C2-WW-HECT E3s [7]. These proteins contain an N-terminal C2 phospholipid binding domain and multiple (from two to four) WW domains in the central portion of the protein that mediate enzyme–substrate interactions. Yeast Rsp5 belongs to this group, as well as nine human proteins, including Nedd4, Smurf1, Smurf2, and Itch. The C2-WW-HECT E3s have been more amenable to analysis than other HECT E3s, in part because of genetic tools in yeast and the fact that it has been relatively simple to isolate proteins (substrates) that bind specifically to WW domains. The regulation of substrate recognition by C2-WW-HECT E3s, as well as the physiologic functions of these enzymes, has been reviewed recently [7]. We will focus here on mechanisms of regulation of the catalytic activity of HECT E3s.

Regulation at the level of E2 recruitment

The TGF-β signaling pathway has multiple effects on growth and differentiation processes. The pathway is regulated at many different levels to achieve balanced and temporal responses, including downregulation of the TGF-β receptor, itself [8]. Smurf1 and Smurf2 are C2-WW-HECT E3s that mediate ubiquitination and degradation of the TGF-β receptor, and these proteins are regulated at several points by Smad7, an inhibitory Smad (I-Smad). Smad7 consists of an N-terminal domain (NTD), a central region with a PY motif that binds to the WW domains of Smurf1 and Smurf2, and an MH2 domain, which engages the TGF-β receptor. Smad7 therefore recruits Smurf1 and Smurf2 to the receptor, where they then mediate receptor downregulation. In addition to mediating enzyme–substrate interactions, Smad7 also activates Smurf2 by enhancing its ability to interact with the activating E2 enzyme, UbcH7 [4]. This study found that while the PY motif of Smad7 binds to the WW domains of Smurf2, the NTD of Smad7 binds to both the HECT domain and UbcH7 and facilitates the interaction between the E2 and E3 enzymes (Fig. 1). At the same time, the crystal structure of the Smurf2 HECT domain revealed that, while being overall similar to other HECT domain structures (E6AP and WWP1), the predicted E2 binding surface of Smurf2 lacked certain key hydrophobic residues that were critical for E2 interaction in the E6AP–UbcH7 structure (corresponding to E6AP residues I656 and F691) [4]. The corresponding residues in Smurf2 are hydrophilic amino acids (His547 and Tyr581). This suggested that Smurf2 might have an inherently low affinity for its E2 enzyme, and that Smad7 might activate Smurf2 by aiding in the recruitment of UbcH7. Indeed, when the key residues in the E2 binding pocket of the Smurf2 were replaced with the hydrophobic residues found in E6AP, Smurf2 became constitutively active.

Interestingly, a recent study determined the affinity of the E2–E3 interaction for E6AP and UbcH7 and found it to be quite low, approximately 6 μM [9]. In addition, alteration of residues at the E2–E3 interface that increased the binding affinity decreased the ability of the E2 to be charged with ubiquitin by E1. This is consistent with structural studies that have indicated that the surface of the E2 that interacts with the E3 is likely to significantly overlap with the surface that interacts with the E1 enzyme [3,10,11]. Together, these results suggest that polyubiquitination catalyzed by HECT E3s (as well as RING E3s) requires multiple rounds of E2–E3 binding and release,

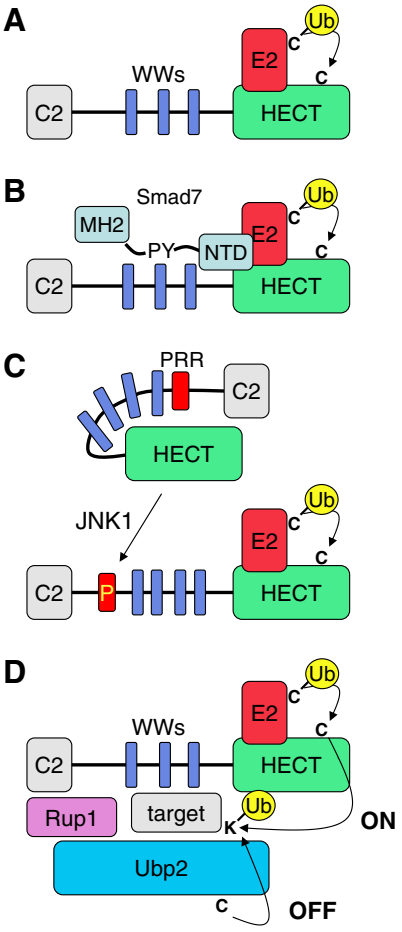

Fig. 1. Modes of regulating or modulating the catalytic activities of C2-WW-HECT E3s. (A) Some C2-WW-HECT E3s, such as Rsp5, appear to be constitutively active and can be charged with ubiquitin in the presence of only ATP, E1 enzyme (not shown), and an appropriate E2 enzyme. The arrow indicates ubiquitin transfer (a transthiolation reaction) from the E2 active-site cysteine to the E3 active-site cysteine. (B) Smad7 activates Smurf2 by recruitment of the E2 enzyme, UbcH7. The PY motif of Smad7 interacts with the WW domain region of Smurf2, while the NTD interacts with both the HECT domain and the E2. These interactions increase the affinity of Smurf2 for UbcH7. (C) Itch is a C2-WW-HECT E3 that adopts a "closed" inactive conformation due to intra-molecular interactions between the WW-PRR region and the HECT domain. In response to signaling events, JNK1 is recruited by a D domain within the HECT domain and then phosphorylates multiple sites within the PRR region. This relieves the inhibitory interactions, leading to activation of the E3. (D) While Rsp5 appears to be constitutively active, its activity is modulated following ubiquitination by Rup1- and Ubp2-dependent deubiquitination. Rup1 and Ubp2 (a ubiquitin-specific cysteine protease) are stably associated with Rsp5, but do not inhibit substrate association.