Review

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Perilous journey: a tour of the ubiquitin–proteasome system

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Eukaryotic cells are equipped to degrade proteins via the ubiquitin–proteasome system (UPS). Proteins become degraded upon their conjugation to chains of ubiquitin where they are then directed to the 26S proteasome, a macromolecular protease. The transfer of ubiquitin to proteins and their subsequent degradation are highly complex processes, and new research is beginning to uncover the molecular details of how ubiquitination and degradation take place in the cell. We review some of the new data providing insights into how these processes occur. Although distinct mechanisms are often observed, some common themes are emerging for how the UPS guides protein substrates through their final journey.

The ubiquitin system: as easy as E1, E2, E3?

The ubiquitination of proteins involves the hierarchical action of three general families of ubiquitin enzymes (Figure 1A). An E1 enzyme must first activate ubiquitin, a highly conserved, 76 amino acid polypeptide, in an ATP-dependent manner. The E1 forms a covalent bond between the C-terminal end of ubiquitin and a cysteine residue in its active site. The thioesterified ubiquitin passes from the E1 active site to the next member of the cascade, the E2 or ubiquitin-conjugating enzyme. Finally, the E3 ubiquitin ligase binds to both the E2-bound ubiquitin and the protein substrate, promoting the transfer of ubiquitin onto the substrate. Note that in the mammalian ubiquitin E1–E2–E3 cascade, only two members of the E1 family are necessary to tag all E2s with ubiquitin, and the approximately 40 E2s that exist are sufficient to deliver ubiquitin to the more than 600 known E3s [1].

In general, proteins are ubiquitinated on lysine residues, where an isopeptide bond forms between the carboxyl end of ubiquitin and the lysine primary amine. Proteins can either be conjugated to one ubiquitin monomer (typically referred to as mono-ubiquitination) or to several ubiquitins to form a poly-ubiquitin chain. Ubiquitin contains seven lysine residues as well as a free amino end, and the primary amines associated with these moieties can all participate as acceptors of additional ubiquitins, thereby enabling the formation of poly-ubiquitin chains with different linkage types. Indeed, all eight of these linkages have been observed in cells [2,3]. To add to the complexity, ubiquitin does not always signal for protein degradation. For instance, ubiquitin can recruit other factors to mediate various cellular responses such as signaling, gene regulation, endocytosis, macro-autophagy, and DNA repair. The partitioning of a ubiquitinated substrate between these cellular responses or degradation is determined by both the protein ubiquitination state (mono- or poly-ubiquitinated) and by the chain linkage type (Box 1) [4].

As noted above, the size and scope of the repertoire of genes necessary to run the UPS are immense, and there are also 16 ubiquitin-like proteins whose conjugation to protein substrates is controlled by structurally related families of E1, E2, and E3 enzymes [5]. As an example, the ubiquitin-like protein Nedd8 (neural precursor cell expressed, developmentally downregulated gene 8) shares approximately 58% sequence identity with ubiquitin. The conjugation of Nedd8 to the cullin–RING (really interesting new gene) ligases, the largest family of E3s in the human proteome, is responsible for the regulation of their activity [1]. Although substantial structural similarity exists between ubiquitin and the ubiquitin-like proteins, the E1 and E2 enzymes display exquisite selectivity for their cognate substrates, thus avoiding unwanted cross-talk between the ubiquitin machinery and the related ubiquitin-like systems under physiological conditions.

Given the sheer scale of the UPS, it is not surprising that much of the early work on the pathway was centered on the discovery of the protein factors and their roles in ubiquitination and degradation. More recently, many groups in the ubiquitin field have been focusing their efforts on uncovering the molecular details for how proteins in the UPS function. The goal of this review is to summarize some of those findings.

In the beginning: ubiquitin-activating enzymes (E1)

E1s are multidomain enzymes that must activate ubiquitin and efficiently transfer it to the E2 active site. This function is crucial for cellular homeostasis because failure to activate ubiquitin, as seen by the chemical inhibition of E1 activity in the cell, results in the almost immediate

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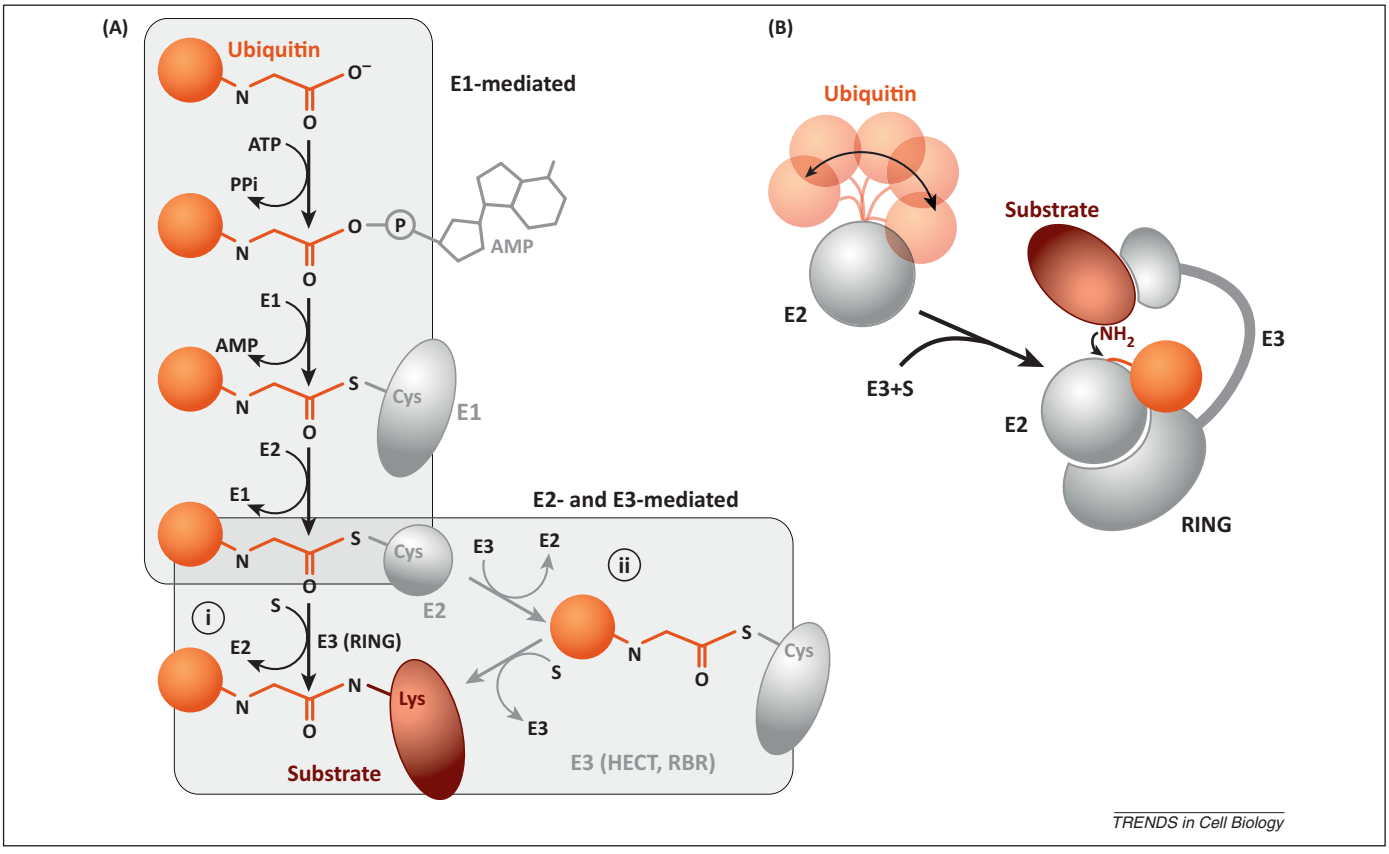

Figure 1. **(A)** Schematic representation of the different modifications occurring at the carboxyl end of ubiquitin during ubiquitination. (i) RING (really interesting new gene) and RING-like E3s mediate the direct transfer of ubiquitin from the E2 onto the substrate; (ii) an additional *trans*-thioesterification step is mediated by HECT (homologous to E6-AP carboxyl terminus) and RBR (ring between ring) E3s before substrate ubiquitination. **(B)** Model for substrate ubiquitination mediated by RING and RING-like E3s. The conformation of ubiquitin on the E2 can be labile due to its flexible tail. Binding of the E2∼ubiquitin (linked via a thioester bond) to the E3 serves to fasten the ubiquitin and its carboxyl tail against the E2, thereby accelerating the rate of ubiquitin transfer to substrate.

shutdown of the entire UPS [6]. Recent work has added to the understanding of the activation and transfer of ubiquitin (or ubiquitin-like proteins) to E2s by E1, thereby making it the best understood process in the UPS [7–13].

The E1-catalyzed reaction involves multiple molecular events, including the ATP-dependent adenylation of the C-terminal carboxyl group of ubiquitin, the formation of a thioester bond between the E1 catalytic cysteine residue and the C-terminus of ubiquitin, and the transfer of ubiquitin onto the E2 catalytic cysteine (Figure 1A). The structural analyses of either E1s for ubiquitin or for the ubiquitin-like proteins Nedd8 and SUMO (small ubiquitin-like modifier) revealed that multiple conformational changes occur during the reaction cycle.

For instance, structural studies of E1s in the presence of adenylated ubiquitin or ubiquitin-like proteins showed conformations where the distances between the E1 cysteine residues that form thioester bonds with either ubiquitin or ubiquitin-like proteins were far too great for this reaction to occur [11–13], suggesting that additional conformational changes would be required. Capturing the short-lived transition state intermediate necessitated the development of chemical methods to trap the enzyme in the proper conformation for thioester formation; however, this challenging work has led to a large increase in the understanding of how E1s function. Indeed, the recent crystallization of the SUMO E1 in complex with a reactive mimic of adenylated SUMO revealed a significant rearrangement of the E1 enzyme domain architecture. These large conformational changes bring the E1 cysteine to the adenylated SUMO moiety and serve to remodel the composition of the active site which reprograms the enzyme to catalyze thioester bond formation rather than SUMO adenylation.

Large conformational changes also occur during the transfer of ubiquitin from the E1 to the E2 active site. For example, it was shown that thioester formation between E1 and Nedd8 causes a large conformational change in the position of the E2-binding domain on the E1 (named the ubiquitin fold domain, UFD) that facilitates E2 binding [7]. Furthermore, a recent ubiquitin-specific E1–E2 co-crystal structure that was stabilized by disulfide crosslinking, as well as the molecular modeling of the transition between the E2 unbound and bound states, were consistent with a mechanism of ubiquitin transfer involving conformational changes [9]. Specifically, upon binding of the E2, the E1 UFD further rotates to juxtapose the E2 active site adjacent to the E1 active site that is conjugated to ubiquitin. The conformational flexibility of the UFD domain may assist in the ability of E1 to accommodate the binding and transfer of ubiquitin or ubiquitin-like proteins to multiple E2s [14]. Indeed, the E1 mechanism must be flexible enough to accommodate the approximately 40 different human E2s, and recent biochemical studies support this contention, demonstrating that the rate of ubiquitin transfer from E1 to E2 is relatively insensitive to the identity of E2 [15]. In conclusion, these