A field guide to ubiquitylation

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Abstract. The capacity for exquisite regulation of ubiquitylation provides eukaryotic cells with a means to fine-tune both protein function and levels. This complex set of processes affects myriad proteins and potentially impacts all cellular processes. Ubiquitylation is brought about through multienzyme processes, with specificity conferred primarily by interactions of substrates with specific ubiquitin protein ligases (E3s) in association with ubiquitin conjugating enzymes (E2s). Regulation of ubiquitylation occurs at multiple levels, including E2-E3 interactions, substrate recognition, chain elongation, binding of ubiquitin to conserved motifs and deubiquitylation. This review presents the fundamentals of the ubiquitin conjugating system.

Key words. Ubiquitin; ubiquitin protein ligase; ubiquitylation; RING finger; HECT; proteasome.

Introduction

The recognition that alteration in protein function by covalent modification with heterologous polypeptides is a common event represents a major conceptual advance in cell biology. While a number of polypeptide modifiers have now been identified [1], by far the most prevalent of these is the one aptly named ubiquitin [2–5]. Ubiquitin is a highly conserved 76-amino acid polypeptide encoded on multiple genes and expressed in all eukaryotes. These genes encode oligomers of ubiquitin or fusions of ubiquitin with other proteins, particularly small ribosomal subunits. When processed to its active monomeric form, ubiquitin can be covalently attached to other proteins through a complex, specific and highly regulated set of processes collectively known as ubiquitylation or ubiquitination. This modification has myriad cellular effects as a consequence of its ability to dramatically alter the fate and function of proteins to which it is attached. In one way or another ubiquitylation regulates almost all cellular processes. Accordingly, alterations of ubiquitylation pathways contribute to the pathogenesis of diseases from cancer to neurodegenerative disorders to viral infections.

The most well characterized role of ubiquitylation is to render proteins susceptible to degradation by the 26S proteasome. This occurs as a consequence of modification of proteins with chains of four or more ubiquitins linked through lysine 48 (K48) of ubiquitin and the specific recognition of these tagged substrates by the 19S cap of the 26S proteasome [6]. It is now apparent that the proteasome plays important roles beyond simply degrading proteins bearing K48 polyubiquitin chains. Among these functions are specific association with proteins that include ubiquitin domains (UBDs), and certain ubiquitin protein ligases (E3s) [7–9] as well as the targeting of select proteins, such as ornithine decarboxylase, for ubiquitin-independent degradation (see review by Phil Coffino, this issue). The proteasome also includes multiple intrinsic deubiquitylating activities [10–13] (see reviews by Bajorek and Glickman, this issue). Monoubiquitin or polyubiquitin chains linked through either K48 or other lysines, most notably K63, can also have marked effects through proteasome-independent mechanisms including protein kinase activation, DNA repair, modulation of transcription factor activity, and protein trafficking, including endocytosis and lysosomal targeting [14–17].

This review provides an overview of our rapidly evolving understanding of the ubiquitylation system, using specific examples to illustrate fundamental and newly emerging...
principles. Other recent reviews that cover specific issues in greater detail are referenced.

Ubiquitylation machinery

In general, ubiquitylation occurs as a result of the sequential action of three classes of enzymes, E1 or ubiquitin activating enzyme, E2 or ubiquitin conjugating enzyme, and E3 or ubiquitin protein ligase (fig. 1). E1, the first enzyme in the ubiquitylation pathway, forms a thiol-ester bond between its active site cysteine and the carboxyl-terminal glycine of ubiquitin. The activated ubiquitin on E1 is subsequently transferred to the active site cysteine of an E2 by transesterification. E3 binds ubiquitin-charged E2 and substrate and facilitates formation of an isopeptide linkage between the carboxyl-terminal glycine of ubiquitin and the \( \varepsilon \)-amino group of an internal lysine residue on the substrate, or an ubiquitin already attached to the protein [2, 3]. In some cases ubiquitin is attached to the free \( \alpha \)-amino group of the substrate rather than to a lysine [18–21].

Substrate specificity is largely determined by the E3. Considering the number of ubiquitylation substrates now known, it is not surprising that database analysis reveals hundreds of predicted E3s. The ability to predict E3s is attributable to the identification of E3 signature motifs, including the HECT (Homologous to E6-AP Carboxyl Terminus) [22], RING (Really Interesting New Gene) finger [23, 24], U-box [25, 26], and PHD (Plant Homeo-Domain) or LAP (Leukemia-Associated Protein) finger domains [27–31].

E1

It is generally believed that a single essential E1 governs ubiquitylation. However, E1- and E2-like domains exist within *Drosophila* dTAF1, and some evidence suggests a role for dTAF1 in histone monoubiquitylation [32]. The significance of this has yet to be established. In mammals utilization of two translation initiation sites results in two E1 isoforms referred to as E1a and E1b [33]. Cells expressing a temperature-sensitive E1 first led to the discovery that ubiquitylation is essential for cell cycle progression and provided in vivo evidence of its role in the proteolysis of short-lived proteins [34, 35]. To activate ubiquitin, E1 binds to MgATP and subsequently to ubiquitin, forming a ubiquitin adenylate that serves as the donor of ubiquitin to the active cysteine in E1 [36, 37]. Each fully loaded E1 carries two molecules of ubiquitin, one as a thiol-ester and the other as an adenylate. The activated ubiquitin is then transferred to the active site cysteine in E2. The carboxyl-terminal glycine of ubiquitin is essential for its activation by E1. The evolutionary conservation in activation for ubiquitin and other ubiquitin-like (UBL) protein modifiers is exemplified both by the presence of a carboxyl-terminal glycine in the active forms of most UBLs, such as SUMO/Pic-1/Sentrin, Nedd8/Rub1, ISG15/UCRP and FAT10 [1, 38], as well as by homology of E1-like molecules that activate UBLs to the ubiquitin E1 [39]. In the case of SUMO and Nedd8 these are heterodimers homologous to the amino and carboxyl portions of the ubiquitin E1 [40, 41].