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Review

Non-canonical ubiquitylation: Mechanisms and consequences☆

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ABSTRACT

Post-translational protein modifications initiate, regulate, propagate and terminate a wide variety of processes in cells, and in particular, ubiquitylation targets substrate proteins for degradation, subcellular translocation, cell signaling and multiple other cellular events. Modification of substrate proteins is widely observed to occur via covalent linkages of ubiquitin to the amine groups of lysine side-chains. However, in recent years several new modes of ubiquitin chain attachment have emerged. For instance, covalent modification of non-lysine sites in substrate proteins is theoretically possible according to basic chemical principles underlying the ubiquitylation process, and evidence is building that sites such as the N-terminal amine group of a protein, the hydroxyl group of serine and threonine residues and even the thiol groups of cysteine residues are all employed as sites of ubiquitylation. However, the potential importance of this "non-canonical ubiquitylation" of substrate proteins on sites other than lysine residues has been largely overlooked. This review aims to highlight the unusual features of the process of non-canonical ubiquitylation and the consequences of these events on the activity and fate of a protein.

Contents

1. Mechanisms of ubiquitylation

Ubiquitylation, the specific addition of ubiquitin groups to targeted proteins, regulates processes as diverse as proteasomal and lysosomal degradation, subcellular localization and DNA damage repair (Kerscher et al., 2006). Of these, our fullest understanding comes from many years of biochemical investigation of the degradation of ubiquitylated proteins by the proteasome (reviewed in (Hershko and Ciechanover, 1998; Varshavsky, 1997b), see Fig. 1).

The process of ubiquitylation begins with the adenylation of the C-terminus of ubiquitin (Ub), an 8.5 kDa, 76-residue protein, by the Ub-activating (E1) enzyme using energy from the hydrolysis of ATP (Hershko et al., 1981). This modification activates the

Abbreviations: Ub, ubiquitin; SCF, Skp-Cullin-F-box; HECT, homologous to E6 carboxyl terminus; RING, really Interesting New Gene; SUMO, small ubiquitin-like modifier; UPS, ubiquitin proteasome system; UFD, ubiquitin fusion degradation; ERAD, endoplasmic reticulum-associated degradation; HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital.

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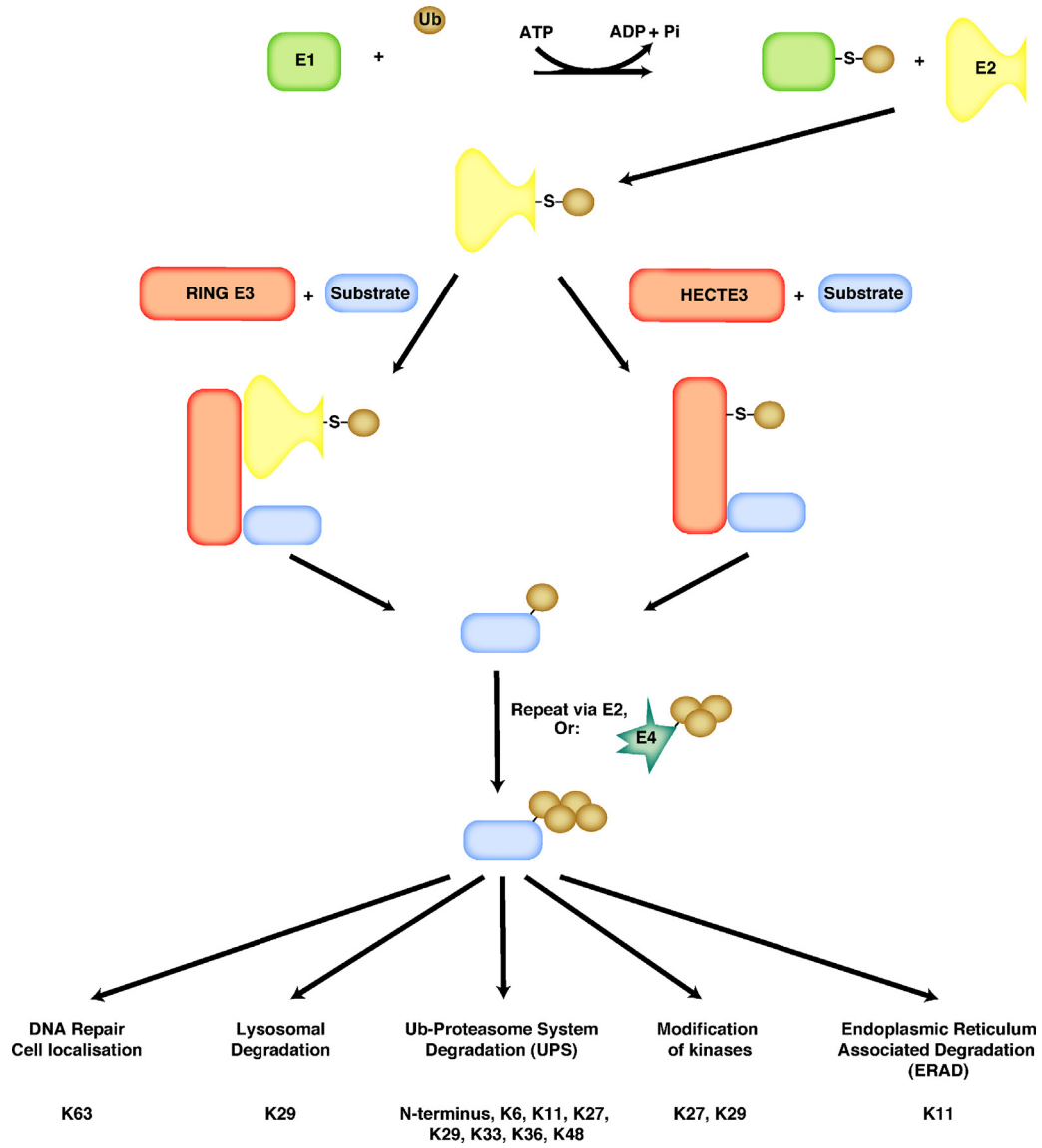

Fig. 1. The ubiquitylation cascade. Ub is activated by an Ub-activating (E1) enzyme using energy from ATP hydrolysis and passed to an Ub-conjugating (E2) enzyme. Ub can then be passed to a substrate protein, specified by the distinct E3 ligase that binds both the substrate and the E2. Many E3 ligases, such as the Really Interesting New Gene (RING) E3 ligases, act as a scaffold to pass Ub from the E2 directly to the substrate protein. In contrast, HECT (homologous to E6-AP carboxy-terminus) E3 ligases form a covalent bond with Ub themselves. The Ub moiety, covalently bound to the substrate protein, is then itself ubiquitylated by either successive rounds of ubiquitylation or by addition of a pre-assembled polyUb chain by the action of an E4 ligase. Once ubiquitylated to the appropriate extent, the substrate is targeted for a variety of cellular processes. Ubs can be cleaved from the protein at all stages by the action of de-ubiquitylating enzymes (DUBs).

Ub moiety energetically throughout the ubiquitylation cascade for eventual transfer to the substrate protein. Ub is then covalently fused via a thioester linkage to a cysteine residue in the E1 by attack of the cysteine at the C-terminus of ubiquitin, releasing AMP (Ciechanover et al., 1981). The E1 cannot just take Ub straight to the substrate (though such a concept is possible, such as in non-ribosomal polypeptide synthesis (Cane and Walsh, 1999)). Instead, Ub is shuttled from the E1 onto a cysteine residue of an Ub-conjugating (E2) enzyme (Hershko et al., 1983), of which there are at least 50 in the mammalian genome (Wu et al., 2003). This E2 can then pass on this Ub moiety to protein targets in concert with an Ub (E3) ligase.

Ubiquitylation may involve the E2 passing the Ub directly onto the substrate protein using the E3 ligase as a scaffold, such as with the Skp-Cullin-F-box (SCF) class of E3 ligase (Jackson et al., 2000). Alternatively, the E2 passes the ubiquitin to a cysteine residue of a homologous to the E6-AP carboxyl terminus (HECT) E3 ligase, which then passes the ubiquitin to its protein target (Huibregtse et al., 1995). In either case, the Ub is "canonically" passed onto a lysine residue of the substrate protein via an isopeptide bond. The cycle can then be repeated to add further Ub moieties to the Ub already attached to the substrate. In some cases, E4 ligases can add a polyUb chain to a monoubiquitylated site: for instance p300 can carry out this function on a site monoubiquitylated by Mdm2 on p53 (Grossman et al., 2003). It is, however, the E3 ligase that confers specificity for the final ubiquitylation of the substrate protein (Hershko, 1988) and this class of enzymes show the greatest diversity within the ubiquitylation machinery, numbering several hundred in mammals (Huibregtse et al., 1995; Jackson et al., 2000; King et al., 1995; Sudakin et al., 1995).

The particular site of ubiquitin chain linkage between Ub molecules can be determined either by the E2 alone (David et al., 2010) or in some cases more specifically by the E3 ligase (David et al., 2011). Various linkages of polyUb chains may confer distinct properties. For example, K48-linked chains target proteins for proteasomal degradation (Thrower et al., 2000) as can K11-linked