Different mechanisms that promote protein monoubiquitination

Pilar Puig-Sàrries* and Bernat Crosas*

[1]Institut de Biologia Molecular de Barcelona, CSIC, Barcelona Science Park, Baldiri i Reixac 15- 21, 08028 Barcelona, Spain. [2]Institut Químic de Sarrià, Universitat Ramon Llull. Via Augusta 390, 08017, Barcelona, Spain

Diferentes mecanismos que promueven la monoubicuitinación de proteínas

Diferents mecanismes que promouen la monoubicuitinació de proteïnes

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RESUMEN

La monoubicuitinación es una modificación post-traduccional que consiste en la conjugación de una única molécula de ubicuitina a un sustrato. Se ha descrito que la monoubicuitinación controla la localización y la función de proteínas implicadas en procesos tales como la reparación del ADN, la regulación de histonas, la expresión génica y la endocitosis. A pesar de que todavía existen muchas incógnitas respecto a los mecanismos por los cuales la monoubicuitinación regula la función proteica, sí que se conocen algunos de los mecanismos que promueven la monoubicuitinación de sustratos. En este trabajo se discutirán algunos de los principios de los procesos que generan la monoubicuitinación *in vivo* a través de enzimas conjugadoras de ubicuitina (E2), de ubicuitina ligasas (E3), de las proteasas desubicuitinasas y de otros co-factores.

Palabras clave: monoubicuitinación, monoubicuitinación acoblada, modificación post-traduccional, ubicuitina

SUMMARY

Protein monoubiquitination is a post-translational modification that consists of the conjugation of a single ubiquitin molecule to a target protein residue. Monoubiquitination regulates protein activity and localization and is involved in DNA repair, histone regulation, and receptor endocytosis. Although the mechanisms by which monoubiquitination regulates protein function are still not well understood, there are some insights into the mechanisms that promote this modification. In this work, we discuss some of the principles of the processes that produce monoubiquitination *in vivo*; i.e., how ubiquitin conjugating enzymes, ubiquitin ligases or other co-factors can produce direct substrates monoubiquitination, and how ubiquitin specific proteases can indirectly convert polyubiquitinated to monoubiquitinated proteins.

Keywords: coupled monoubiquitination, monoubiquitination, post-translational modification, ubiquitin

RESUM

La monoubicuitinació és una modificació post-traduccional que consisteix en la conjugació d'una sola molècula d'ubicuitina a un substrat. S'ha descrit que la monoubicuitinació controla la localització i la funció de proteïnes implicades en processos tals com la reparació de l'ADN, la regulació d'histones, l'expressió gènica i l'endocitosi. Tot i que encara hi ha moltes incògnites respecte als mecanismes pels quals la monoubicuitinació regula la funció proteica, sí que es coneixen alguns dels mecanismes que promouen la monoubicuitinació de substrats. En aquesta revisió s'hi discutiran alguns dels principis dels processos que generen la monoubicuitinació *in vivo* a través d'enzims conjugadors d'ubicuitina (E2), d'enzims ubicuitina lligasa (E3), de les proteases desubicuitinases i d'altres co-factors.

Paraules clau: monoubicuitinació, monoubicuitinació acoblada, modificació post-traduccional, ubicuitina

*Corresponding authors: ppsbmc@ibmb.csic.es, bcnbmc@ibmb.csic.es

INTRODUCTION

Post-translational modifications are regulatory processes altering the composition of a protein, usually through the covalent addition of a small molecule to one of the amino acid residues. Phosphorylation, methylation, acetylation, glycosylation, lipidation, ubiquitination, SUMOylation and proteolysis are some of the modifications that proteins undergo (Hochstrasser, 2000; Pickart, 2001).

Ciechanover and collaborators first described post-translational modification by ubiquitin, in their discovery of small formed covalent conjugates with endogenous reticulocyte proteins (Ciechanover *et al*, 1980). Since then, the ubiquitin field seems to be ever expanding. Ubiquitin participates in multiple processes and its complex and diverse regulatory roles make it one of the most versatile signaling molecules in the cell. Ubiquitination patterns can be grouped into three main classes, each of which specifies a different fate for the substrate protein. Modification of a protein by a single ubiquitin moiety is called monoubiquitination. When multiple lysine residues within a protein are modified with one ubiquitin, the substrate is termed multi-monoubiquitinated. Finally, when the process of ubiquitin addition is repeated to create a chain of at least four ubiquitins, the protein is termed polyubiquitinated. Ubiquitin chains can contain one or more than one type of linkage. The first case refers to homotypic chains in which just one lysine participates in the conjugation of ubiquitin. The second situation refers to the use of distinct lysine residues to connect ubiquitin moieties- i.e., Lys 6/11, Lys 27/29, Lys 29/48 or Lys 29/33 (Kim *et al*, 2007). Ubiquitin can also be connected to other ubiquitin-like modifiers such as Sumo-2 and Sumo-3 giving rise to heterologous Ub chains (Tatham *et al*, 2001). Finally, Ub-Ub linkages can also be formed on Met1 producing linear chains. In these chains, the C-terminal glycine of ubiquitin is linked to the Met1 of the next ubiquitin (Kirisako *et al*, 2006).

In this review, we discuss the different mechanisms that have been described so far to promote monoubiquitination.

THE PROCESS OF UBIQUITINATION

Ubiquitin (Ub) is an essential protein of 76 amino acids (~8KDa) and one of the most conserved proteins in eukaryotes: only four of its amino acids differ among yeast, plants and animals (Glickman & Ciechanover, 2002; Catic & Ploegh, 2005; Zuin *et al*, 2014). Ubiquitin can be conjugated to substrate proteins or to itself by means of a covalent isopeptide bond between the C-terminal glycine and a lysine residue of the protein substrate, a process known as ubiquitination. Additionally, ubiquitin can bind to specific surfaces (ubiquitin-binding domains – UBD) forming non–covalent interactions either with ubiquitin moieties or with ubiquitin chains (Dikic et al, 2009).

Ubiquitination is involved in the regulation of multiple and divers cellular processes, such as proteasomal-dependent protein degradation, antigen processing, apoptosis, biogenesis of organelles, cell cycle and division, DNA transcription and repair, differentiation and development, neural and muscular degeneration, morphogenesis of neural networks, modulation of cell surface receptors, the secretory pathway, response to stress and extracellular modulators, ribosome biogenesis, immune system or viral infection (Finley *et al*, 1989; Deshaies & Joazeiro, 2009; Raiborg & Stenmark, 2009; Ulrich & Walden, 2010; Zinngrebe *et al*, 2013; Nakamura, 2011; Kloetzel, 2001; Glickman & Ciechanover, 2002; Hamilton & Zito, 2013).

Ubiquitin contains 7 lysines (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48, and Lys63) that can all be linked to the C-terminus of another ubiquitin or to the N-terminal methionine of ubiquitin, resulting in the formation of polyubiquitin polymers. Eukaryotes possess a multi-enzyme system comprising a cascade of three classes of enzymes required for ubiquitination of a substrate protein: ubiquitin-activating (E1), ubiquitin-conjugating (E2), and ubiquitin ligase (E3) enzymes (Figure 1). In the first step, a ubiquitin-activating enzyme (E1) catalyzes an ATP-dependent high-energy thioester linkage between a ubiquitin's carboxyl terminus and itself. Next, ubiquitin is transferred to the active-site cysteine of a ubiquitin-conjugating enzyme, E2. Finally, a member of the ubiquitin-protein ligase family, an E3 enzyme, catalyzes the formation of an isopeptide bond between the C-terminal glycine of ubiquitin and the substrate. (A Ciechanover, Elias, Heller, & Hershko, 1982; Glickman & Ciechanover, 2002; Hershko et al., 1983).

There are two major types of E3s in eukaryotes: the HECT and the RING types. HECT (Homologous to E6AP C-Terminus) enzymes interact simultaneously with the E2 enzyme and the substrate. A ubiquitin molecule is transferred from the E2 to the catalytic site of the ligase forming an intermediate thioester. Next, ubiquitin is ligated to the substrate, catalyzing substrate ubiquitination (Rotin & Kumar, 2009). The RING (Really Interesting New Gene) ligase family binds simultaneously the E2-ubiquitin intermediate and the targeted protein promoting substrate ubiquitination. The main difference between RING and HECT ligase members is that the former ones transfer directly ubiquitin from the E2 to the substrate, while HECT ligases transiently bind ubiquitin through an obligate thioester bond at its active-site cysteine.

In most cases, ubiquitin is conjugated to the epsilon-amino group of a lysine (Glickman & Ciechanover, 2002) but it can also be conjugated to the NH2-terminal group of its substrate (Ciechanover & Ben-Saadon, 2004), or to Cys, Ser, and Thr residues of target proteins (Ravid & Hochstrasser, 2007; Cadwell & Coscoy, 2005). In yeast, a ubiquitin chain elongation factor, E4, binds to the ubiquitin moieties of preformed short conjugates and catalyzes ubiquitin chain elongation. These polyubiquitinated substrates are often subsequently targeted, recognized and degraded by the Ubiquitin-Proteasome System (Crosas *et al*, 2006; Koegl *et al,* 1999).

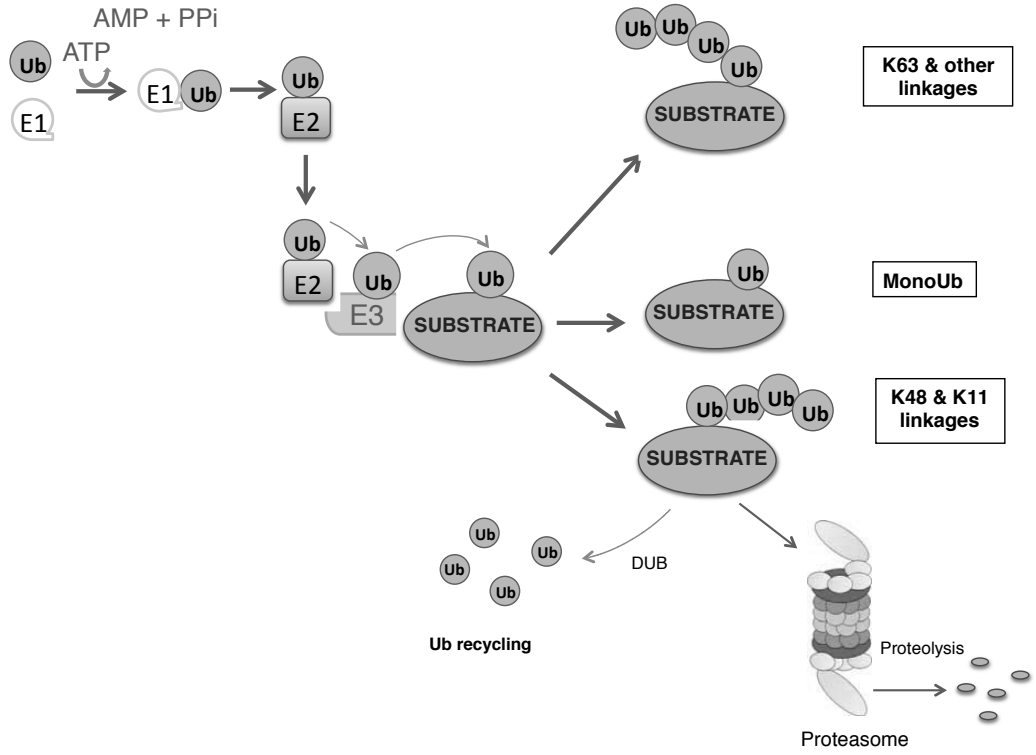

Figure 1. *Scheme of the Ubiquitin-Proteasome System and some of the fates of ubiquitinated substrates. DUB: deubiquitylases responsible for the recycling of ubiquitin and chain editing.*

MECHANISMS OF MONOUBIQUITINATION

Post-translational modification by ubiquitin playd a role on the subcellular localization, stability, and protein-protein interactions. Monoubiquitination regulates DNA repair, histone function, gene expression, and receptor endocytosis (Hicke, 2001; Di Fiore *et al*, 2003; Hoeller *et al,* 2006; Bergink & Jentsch, 2009).

E2s, E3s, or the substrate itself determine whether only one lysine on the substrate is modified (monoubiquitination), more than one lysine is modified (multi-monoubiquitination) or if a lysine on the substrate is modified with a chain of ubiquitin molecules (polyubiquitination). Next, we describe different mechanisms that can generate monoubiquitinated proteins.

a) E2-mediated coupled monoubiquitination

Proteins containing UBD are defined as ubiquitin receptors. They can be monoubiquitinated following a process known as "coupled monoubiquitination" where their UBD is required. Once a UBD-containing protein is monoubiquitinated, it undergoes a change in its conformation due to an intramolecular binding between the UBD and the ubiquitin moiety. The protein is then intrinsically switched off and is unable to bind ubiquitinated substrates due to the autoinhibitory intereference of its conjugate ubiquitin moiety (Di Fiore *et al,* 2003; Hoeller *et al*, 2006; Woelk *et al*, 2006).

UBD-containing proteins can be monoubiquitinated in an E3-independent step. The ubiquitin attached to the active site of an E2 interacts with the UBD and ubiquitin is transferred directly to the substrate, provided the substrate protein contains a UBD (Hoeller *et al,* 2007). Hoeller and colleagues performed *in vitro* ubiquitination reactions in the presence of a panel of E2 enzymes (UbcH2, UbcH3, UbcH5A, UbcH5B, UbcH5C, UbcH6, and UbcH10), but no E3 ligases. Several proteins (Stam2, Eps15, Pol ι and Pol κ, HDAC6 and Sts1) with different functional UBD types (UBA, UIM, UBM, ZnF, and UBZ) were used as substrates. Ub-loaded E2 enzymes were able to promote monoubiquitination on these substrates in an E3-independent manner (Hoeller *et al,* 2007).

b) E3-mediated coupled monoubiquitination

Eps15 is an endocytic protein that contains two ubiquitin-interacting motifs (UIM) that are a class of UBDs. Eps15 undergoes coupled monoubiquitination by two different ways involving two different E3 ubiquitin ligases, Nedd4 and Parkin that contain a HECT and a RING domains, respectively. When Eps15 is ubiquitinated by Nedd4, the ligase needs to be first modified by ubiquitination and contain a thiolester conjugated ubiquitin. Next, the UIM2 of Eps15 binds the ubiquitin moiety linked to the Nedd4 ligase and then the thiolester-bound ubiquitin is transferred to Eps15, generating monoubiquitinated Eps15 (Woelk *et a*l, 2006). On the other hand, Parkin simultaneously interacts with an E2 and the UIM of Eps15 through its ubiquitin-like domain. This interaction facilitates the transfer of a ubiquitin molecule from the E2 to Eps15 resulting in its monoubiquitination (Fallon *et al*, 2006). In both cases, once Eps15 is monoubiquitinated, the UIM interacts intramolecularly with the attached Ub, avoiding further ubiquitin chain extension in Eps15.

Analogously to Eps15, the UIM of the transcription factor Met4 was found to both restrict chain elongation on Met4 and prevent the recognition and proteolysis of polyubiquitinated Met4 by the proteasome (Flick *et al,* 2006).

It has been suggested that the UIM of the proteasomal ubiquitin receptor Rpn10 interacts with the ubiquitins linked to the lysines in Rpn10 (Isasa *et al*, 2010). The intramolecular interaction supports a mechanism to both regulate the interaction with polyubiquitinated substrates and prevent these UBD-containing proteins from being polyubiquitinated.

c) E2-mediated monoubiquitination

Histones, proteins that associate with DNA forming the nucleosomes, can also be mono- and polyubiquitinated in an E3-independent manner (Kim & Roeder, 2009). Rad6, an E2 conjugating enzyme 8 with an acidic carboxyl-terminal tail, ubiquitinates histones *in vitro* (Morrison et al, 1988). Genetic deletion of the acidic tail abolishes histone ubiquitination, suggesting that a direct interaction between a region in the histone and the acidic tail of Rad6 is necessary to monoubiquitinate the histone (Sung *et al,* 1988; Sullivan & Vierstra, 1991). The interaction that occurs between the E2 and the substrate may bring the ubiquitin to be transferred closer to the target lysine, which would enable a direct ubiquitination with no E3s involvement. Fanconi anemia is an illness produced by the inactivation of the Fanconi anemia tumor suppressor pathway, responsible for DNA repair. DNA repair is promoted by the monoubiquitination of one of the proteins of the pathway, FANCD2 (Siddique *et al*, 2001; Gregory *et al,* 2003). Alpi and collaborators found that the E2 conjugating enzyme Ube2t dictates site-specific FANCD2 monoubiquitination in conjunction with the E3 ubiquitin ligase FANCL (Alpi *et al,* 2008). Interestingly, they observed that FANCD2 was polyubiquitinated in a reaction with FANCL and the E2 Ubch5b, indicating that monoubiquitination of FANCD2 is specific to Ube2t. Although the activity of an E3 ligase is mandatory to ubiquitinate FANCD2, it is the E2 that determines whether FANCD2 is mono or polyubiquitinated.

d) E3-mediated monoubiquitination

Some E3 ligases adjust the ubiquitin conjugating activity of E2s, determining whether a substrate will be mono- or polyubiquitinated. The RING E3 Ubr1 ligase, together with Rad6, polyubiquitinates N-end rule substrates (Xie & Varshavsky, 1999). On the other hand, the RING Rad18 E3 ligase blocks the ubiquitin-chain synthesis activity of the Rad6 enzyme promoting monoubiquitination of the proliferating cell nuclear antigen, PCNA during DNA repair, which signals for recruitment of damage-tolerant polymerases and leads to error-free DNA repair (Hibbert *et al*, 2011). Rad6 contains a region opposite of its active site that interacts with ubiquitin, the *backside* (Hibbert *et al,* 2011). Additionally, Rad18 interacts with Rad6 via the N-terminal RING domain and a C-terminal binding domain that recognizes the *backside* of Rad6. Thus, Rad18 competes with the binding of free ubiquitin for the backside of Rad6 and inhibits the generation of polyubiquitin chains on the substrate.

A recent study shows that the RING E3 ligase Bre1, together with Rad6, monoubiquitinates yeast histone H2B at K123 (Turco *et al*, 2014). Bre1 interacts through a region outside of its RING domain, the Rad 6 binding domain (RBD), with the *backside* of Rad6. However, in contrast with the previous example, this interaction does not explain why H2B gets only monoubiquitinated: a Bre1 mutant lacking RBD can also monoubiquitinate the substrate. Turco et al. showed that the RBD promotes ubiquitin discharge from Rad6 to H2B, suggesting that the RBD could help tether Rad6 in proximity of the RING of Bre1 and the substrate. The conformation adopted by the Bre1-Rad6-H2B complex would allow the transfer of one ubiquitin molecule from the E2 to a specific lysine in H2B and would prevent addition of more ubiquitins.

Similarly, for histone H2A monoubiquitination by Bmi1/Ring1b ubiquitin ligases which are components of the Polycomb repressive complex 1, it has been proposed that the rigidity of the E2-E3 complex assembled to nucleosomal DNA promoted K119 specific monoubiquitination (Bentley *et al*, 2011).

e) Deubiquitination catalyzes monoubiquitination

Ubiquitination is reversed through the action of a large family of deubiquitylases (DUBs), which remove ubiquitin moieties from polypeptides and polyubiquitin chains. Monoubiquitination can also be promoted by the catalytic activity of DUBs that trim polyubiquitin chains on substrates leaving just one ubiquitin molecule (Kee et al., 2005). The yeast E3 ubiquitin ligase Rsp5 preferentially assembles K63-linked ubiquitin chains, whereas the DUB Ubp2 disassembles them, promoting monoubiquitination (Kee *et al*, 2005). Rsp5 was shown to polyubiquitinate the ER membrane protein Spt23 *in vitro*, however, the addition of Ubp2 reversed Rsp5-catalyzed polyubiquitination (Kee *et al,* 2005). The activity of Ubp2 would explain that Spt3 is monoubiquitinated by Rsp5 *in vivo* (Rape *et al,* 2001). The RNA polymerase II subunit Rpb1 is poly- and monoubiquitinated *in vivo by* Rsp5. Rsp5 binds the C-terminal of Rpb1 and promotes K63 ubiquitin chain elongation. Nonetheless, the activity of Ubp2 modifies the substrate resulting in a monoubiquitinated form (Harreman *et al,* 2009). The Rsp5-Ubp2 association has also been shown to control the levels of monoubiquitination of theproteasomal subunit, Rpn10, in vivo (Isasa et al, 2010).

f) External co-factor regulates monoubiquitination.

The process of monoubiquitination can be induced by an external protein cofactor that modulates enzyme processivity, such as Vps23. When the arrestin family protein Rim8/Art9 is monoubiquitinated by Rsp5, the UBD of Vps23 interacts with the ubiquitin linked to Rim8/Art9, which then prevents its further polyubiquitination (Herrador *et al*, 2010, 2013).

In summary, monoubiquitination is a post-translational modification that can be produced through a diversity of mechanisms. It is apparent that the nature of the substrate, the type of E2 or E3, the activity of other co-factors or DUBs play a key role in the mono/poly-ubiquitination fate of the substrate. The variety of the different strategies that generate monoubiquitination is a sign that monoubiquitination cannot be explained by a general rule and needs to be studied in a detailed and specific way.

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