

Monoubiquitination in Endocytosis

Raghunath Peesari and Simona Polo

IFOM, Fondazione Istituto FIRC di Oncologia Molecolare,
Via Adamello 16, 20139, Milan, Italy.

Introduction:

Ubiquitin (Ub) is a 76 amino acid residue protein that covalently binds to lysine residues in target proteins and regulates their stability, protein-protein interactions, sub-cellular localization and enzymatic activity. Protein ubiquitination occurs through a sequential three-step process involving ubiquitin-activating (E1), ubiquitin-conjugating (E2) and ubiquitin ligase (E3) enzymes (1-3). The addition of a single Ub moiety to a protein is known as monoubiquitination, whereas the modification of several lysine residues within a protein by single Ub moieties is known as multiple monoubiquitination. The addition of ubiquitin moieties that are themselves modified on lysine residues in an iterative fashion is known as polyubiquitination. Different types of polyubiquitination exist, depending on the particular modified lysine residue (i.e. Lys48- and Lys63-linked polyubiquitin chains) and are thought to regulate different cellular processes. Monoubiquitination is involved in regulating endocytosis of membrane proteins, DNA repair, histone activity and retroviral budding (1,3,4).

Monoubiquitination in Receptor Internalization and Endocytosis:

Ligand-induced ubiquitination of membrane receptors, such as receptor tyrosine kinases (RTKs), is implicated in receptor internalization and endocytosis (5-8). In yeast, it has been shown that monoubiquitination of the G-protein coupled receptor Ste2p leads to its internalization and subsequently degradation in the yeast vacuole, an equivalent of the mammalian lysosome (9). Many other transmembrane receptors, such as permeases, transporters and α -factor receptors, also undergo internalization upon monoubiquitination, although the additional modification with Lys63-linked polyubiquitin accelerates this process (10). In mammalian cells the situation is even more complex as both the transmembrane receptor and the endocytic adaptor proteins undergo ubiquitination upon stimulation with ligand. EGF-induced internalization and sorting to lysosomes of the epidermal growth factor receptor (EGFR) is the best characterized example of how Ub regulates receptor degradation. Early studies demonstrated that a protein chimera, consisting of a single Ub moiety fused to the cytoplasmic tail of EGFR, was constitutively internalized from the cell surface and targeted to late endosomal/lysosomal compartments for degradation. This finding indicated that monoubiquitin (monoUb) can serve as both an internalization and a sorting signal (11). More recently, analysis by quantitative mass spectrometry has shown that EGFR is multi-monoubiquitinated, as well as polyubiquitinated through Lys63-linked chains (12). These observations demonstrate that although monoUb is sufficient for internalization, as observed in yeast, polyubiquitination through Lys63 generates a more efficient internalization signal, possibly by increasing the binding avidity to Ub-receptor proteins (10).

Trafficking of membrane receptors:

Ub-dependent internalization/sorting of membrane receptors requires accurate molecular recognition of the ubiquitinated cargo by Ub-receptors. Ub-receptors (also known as ubiquitin binding domain (UBD)-containing proteins) are proteins that contain motifs or domains that

bind to monoUb or Ub moieties in polyUb chains (13-15). Different kinds of UBDs have been characterized in Ub-receptors, such as Ub-interacting motifs (UIMs) (16-18), Ub-associated (UBA), Ub E2 Variant (UEV) (18) and CUE1-homologous (CUE) (19) domains (Table 1). These UBDs bind ubiquitinated RTKs and are involved in trafficking receptor from the cell surface to lysosomes (20).

Table 1. Ubiquitin binding domains (UBDs) involved in endocytic sorting.

Ubiquitin Binding Domain (UBD)	UBD-containing Proteins in Yeast	UBD-containing Proteins in Mammals	Reference(s)
UIM	Ent1, Ent2, Vps27, Hse1	Epsin 1-3, Eps15/R, Hrs, STAM	(16,17,21-24)
UBA	Ede1	-	(25)
UEV	Vps23	Tsg101	(26-28)
CUE	Vps9	-	(19,29)
MIU	-	Rabex-5	(30)

The affinity of the interaction between a single UBD and a Ub moiety is very low, usually in the micromolar range (14,15). However, several molecular mechanisms exist to increase the affinity between ubiquitinated cargo and Ub-receptors making their interaction physiologically relevant. For example, in the case of the endocytic adaptor proteins Epsin or Eps15, multiple UBDs (UIMs) in the adaptor can bind to several Ub moieties in a multi-monoubiquitinated cargo (15) or to a single Ub moiety through different binding surfaces (30,31), thus increasing the overall affinity of the interaction.

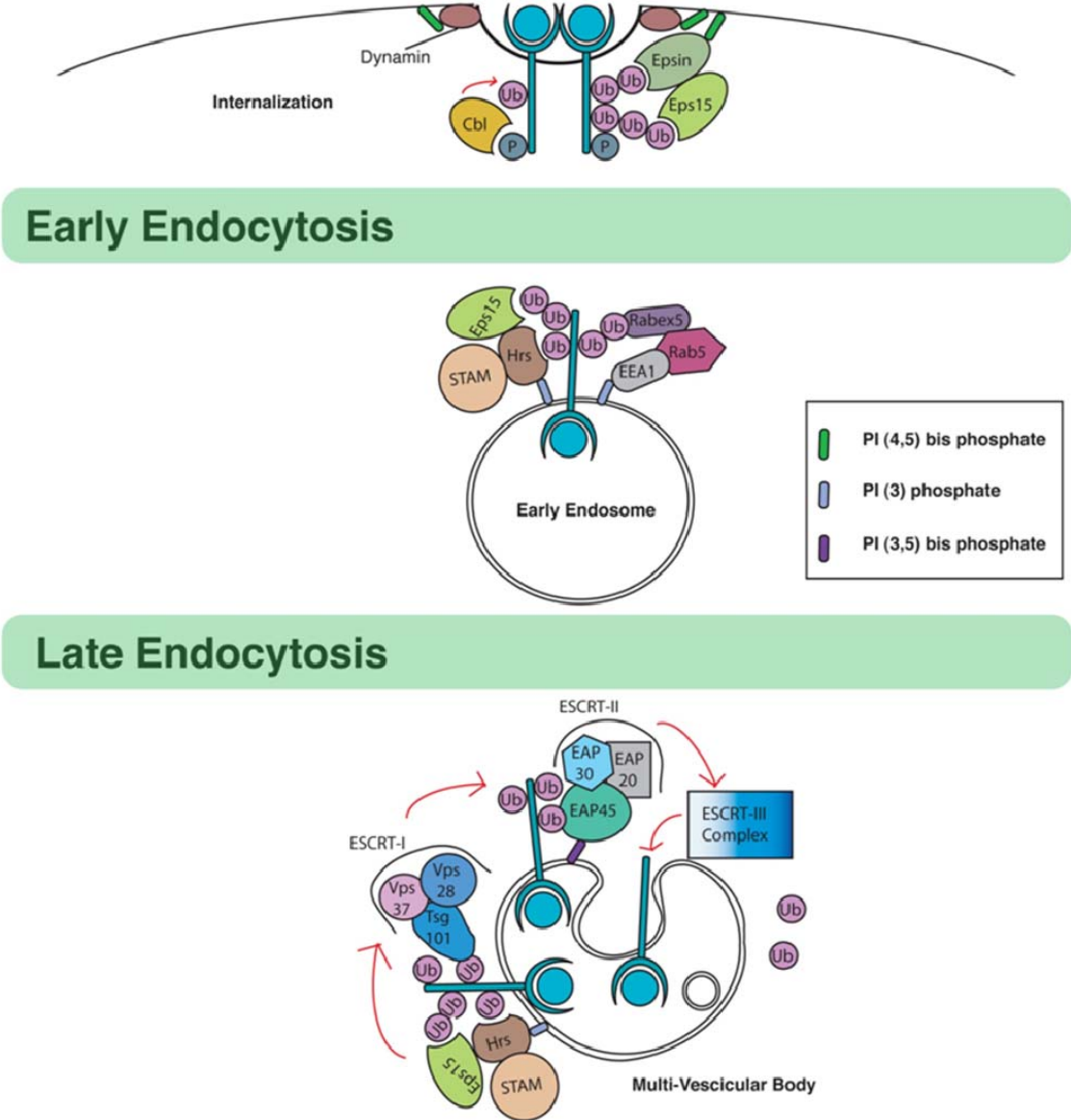
At later stages of endocytosis, Hrs, a UIM-containing endocytic adaptor protein, sorts the ubiquitinated cargo from early/late endosomes to the outer membrane of the multi-vesicular body (MVB), where the ubiquitinated cargo is first delivered to ESCRT-I (endosomal sorting complex required for transport), and then to ESCRT-II, and finally to the endosomal lumen through the ESCRT-III protein complex (32,33). It has also been shown that the Ub-receptors Eps15, Hrs and STAM, form an Ub-binding complex on early endosomes that sorts ubiquitinated cargo to the MVB pathway, which then delivers cargo to lysosomes for degradation (22). The transfer of the Ub-cargo from one Ub-receptor to another, is dependent on the ability of Ub-receptor UBDs to recognize alternative surfaces on Ub, besides the canonical Ile44 hydrophobic patch. The discovery of three novel UBDs that recognize non-canonical surfaces of Ub supports this mechanism: i) the ZnF_A20 domain present in Rabex-5 recognizes a polar patch centered on Asp58, which does not overlap with the canonical Ile44 surface on Ub (30,31); ii) the UBM (Ubiquitin binding motif) present in Y-family translesion synthesis (TLS) polymerases recognizes a surface of Ub centered on Leu8, which is near to, but not overlapping with, Ile44 (34); the ZnF_UBP domain present in the deubiquitinase, Isopeptidase T (IsoT), recognizes the C-terminal di-glycine motif, as well as Ile36 region, of Ub (35).

Monoubiquitination of Ub-receptors:

Several Ub-receptors have the ability to bind Ub and at the same time undergo monoubiquitination upon ligand stimulation (20). The UBDs of Ub-receptors, such as UIMs of Eps15, Hrs and Epsins, are important for their monoubiquitination (17,36). Such Ub-receptors undergo monoubiquitination through a molecular process called “coupled monoubiquitination”, which requires the presence of an intact UBD (37). The underlying molecular mechanism of coupled monoubiquitination has been elucidated using the Eps15 adaptor protein, which undergoes EGF-induced monoubiquitination, as a model system. This

process involves the direct interaction between the UIM of the Ub-receptor (Eps15) and a HECT-type E3 ligase (Nedd4) that has been already modified by ubiquitination. The modified E3 ligase now transfers the thiolester-conjugated Ub from its catalytic cysteine residue to the Ub-receptor, which then becomes monoubiquitinated (37). Similarly to the above mechanism, an Ubiquitin-like (Ubl) domain of an E3 ligase (Parkin) binds to the UIM of the Ub-receptor (Eps15) and monoubiquitinates it by directly transferring Ub from the bound E2 conjugating enzyme to the Ub-receptor (38).

Figure 1. Ubiquitination and Phosphoinositide-phosphates Network in Membrane Receptor endocytosis



Monoubiquitinated Ub-receptors might form several tiers of ubiquitination-dependent interactions in the endosome, by binding ubiquitinated cargo through their UBDs and recruiting another layer of Ub-receptors through a monoUb signal, thus leading to signal amplification and progression of ubiquitinated cargos along the endocytic pathway (39). The recruitment of Ub-receptors is also accomplished through a controlled spatial distribution of phosphoinositol lipids (PIs) and PI-binding proteins, which in turn bind to and recruit Ub-cargo binding proteins. A number of structurally distinct PI-binding domains anchor endocytic-sorting machinery to ubiquitinated cargo on membranes (Figure 1). The PH domain of dynamin and the ENTH domain of epsin binds to PI(4,5)P₂ that serves as a marker of the plasma membrane (40,41). PI(4,5)P₂ controls both early and late clathrin coated vesicle (CCV) formation by binding with AP2 subunits, epsin, dynamin and DAB2. The FYVE domain of Hrs and EEA1 binds to PI3P, which is critical for endosomal maturation and MVB budding (42,43). The PI3P and PI(3,5)P₂ participate in the membrane recruitment of Hrs and ESCRTs, respectively (42,44,45).

Monoubiquitination of Ub-receptors also plays an important regulatory role in determining their binding capacity to ubiquitinated cargos (46). Recently, it has been shown that monoubiquitination of endocytic proteins Sts1 and Sts2, results in an intra-molecular interaction between their UBDs and monoUb, thereby preventing the binding of the Ub-receptor to the ubiquitinated cargo (46). This phenomenon could explain how the Ub-receptors that constitute the endocytic sorting machinery can dynamically exchange their ubiquitinated cargoes along the endosomal compartments.

References:

1. Mukhopadhyay, D., and Riezman, H. (2007) *Science* **315**, 201-205
2. Weissman, A. M. (2001) *Nat Rev Mol Cell Biol* **2**, 169-178
3. Salmena, L., and Pandolfi, P. P. (2007) *Nat Rev Cancer* **7**, 409-413
4. Huang, T. T., and D'Andrea, A. D. (2006) *Nat Rev Mol Cell Biol* **7**, 323-334

5. Thien, C. B., and Langdon, W. Y. (2001) *Nat Rev Mol Cell Biol* **2**, 294-307
6. Miyake, S., Lupher, M. L., Jr., Druker, B., and Band, H. (1998) *Proc Natl Acad Sci U S A* **95**, 7927-7932
7. Levkowitz, G., Waterman, H., Zamir, E., Kam, Z., Oved, S., Langdon, W. Y., Beguinot, L., Geiger, B., and Yarden, Y. (1998) *Genes Dev* **12**, 3663-3674
8. Taher, T. E., Tjin, E. P., Beuling, E. A., Borst, J., Spaargaren, M., and Pals, S. T. (2002) *J Immunol* **169**, 3793-3800
9. Terrell, J., Shih, S., Dunn, R., and Hicke, L. (1998) *Mol Cell* **1**, 193-202
10. Galan, J. M., and Haguenaer-Tsapis, R. (1997) *EMBO J* **16**, 5847-5854
11. Haglund, K., Sigismund, S., Polo, S., Szymkiewicz, I., Di Fiore, P. P., and Dikic, I. (2003) *Nat Cell Biol* **5**, 461-466
12. Huang, F., Kirkpatrick, D., Jiang, X., Gygi, S., and Sorkin, A. (2006) *Mol Cell* **21**, 737-748
13. Dikic, I., Wakatsuki, S., and Walters, K. J. (2009) *Nat Rev Mol Cell Biol* **10**, 659-671
14. Hicke, L., Schubert, H. L., and Hill, C. P. (2005) *Nat Rev Mol Cell Biol* **6**, 610-621
15. Hurley, J. H., Lee, S., and Prag, G. (2006) *Biochem J* **399**, 361-372
16. Hofmann, K., and Falquet, L. (2001) *Trends Biochem Sci* **26**, 347-350
17. Polo, S., Sigismund, S., Faretta, M., Guidi, M., Capua, M. R., Bossi, G., Chen, H., De Camilli, P., and Di Fiore, P. P. (2002) *Nature* **416**, 451-455
18. Ponting, C. P., Cai, Y. D., and Bork, P. (1997) *J Mol Med* **75**, 467-469
19. Shih, S. C., Prag, G., Francis, S. A., Sutanto, M. A., Hurley, J. H., and Hicke, L. (2003) *EMBO J* **22**, 1273-1281
20. Di Fiore, P. P., Polo, S., and Hofmann, K. (2003) *Nat Rev Mol Cell Biol* **4**, 491-497
21. Bache, K. G., Brech, A., Mehlum, A., and Stenmark, H. (2003) *J Cell Biol* **162**, 435-442
22. Bache, K. G., Raiborg, C., Mehlum, A., and Stenmark, H. (2003) *J Biol Chem* **278**, 12513-12521
23. Bilodeau, P. S., Urbanowski, J. L., Winistorfer, S. C., and Piper, R. C. (2002) *Nat Cell Biol* **4**, 534-539
24. Bilodeau, P. S., Winistorfer, S. C., Kearney, W. R., Robertson, A. D., and Piper, R. C. (2003) *J Cell Biol* **163**, 237-243
25. Shih, S. C., Katzmann, D. J., Schnell, J. D., Sutanto, M., Emr, S. D., and Hicke, L. (2002) *Nat Cell Biol* **4**, 389-393
26. Katzmann, D. J., Babst, M., and Emr, S. D. (2001) *Cell* **106**, 145-155
27. Sundquist, W. I., Schubert, H. L., Kelly, B. N., Hill, G. C., Holton, J. M., and Hill, C. P. (2004) *Mol Cell* **13**, 783-789
28. Teo, H., Veprintsev, D. B., and Williams, R. L. (2004) *J Biol Chem* **279**, 28689-28696
29. Prag, G., Misra, S., Jones, E. A., Ghirlando, R., Davies, B. A., Horazdovsky, B. F., and Hurley, J. H. (2003) *Cell* **113**, 609-620

30. Penengo, L., Mapelli, M., Murachelli, A. G., Confalonieri, S., Magri, L., Musacchio, A., Di Fiore, P. P., Polo, S., and Schneider, T. R. (2006) *Cell* **124**, 1183-1195
31. Lee, S., Tsai, Y. C., Mattera, R., Smith, W. J., Kostelansky, M. S., Weissman, A. M., Bonifacino, J. S., and Hurley, J. H. (2006) *Nat Struct Mol Biol* **13**, 264-271
32. Raiborg, C., Bache, K. G., Gillyooly, D. J., Madshus, I. H., Stang, E., and Stenmark, H. (2002) *Nat Cell Biol* **4**, 394-398
33. Katzmann, D. J., Odorizzi, G., and Emr, S. D. (2002) *Nat Rev Mol Cell Biol* **3**, 893-905
34. Bienko, M., Green, C. M., Crosetto, N., Rudolf, F., Zapart, G., Coull, B., Kannouche, P., Wider, G., Peter, M., Lehmann, A. R., Hofmann, K., and Dikic, I. (2005) *Science* **310**, 1821-1824
35. Reyes-Turcu, F. E., Horton, J. R., Mullally, J. E., Heroux, A., Cheng, X., and Wilkinson, K. D. (2006) *Cell* **124**, 1197-1208
36. Katz, M., Shtiegman, K., Tal-Or, P., Yakir, L., Mosesson, Y., Harari, D., Machluf, Y., Asao, H., Jovin, T., Sugamura, K., and Yarden, Y. (2002) *Traffic* **3**, 740-751
37. Woelk, T., Oldrini, B., Maspero, E., Confalonieri, S., Cavallaro, E., Di Fiore, P. P., and Polo, S. (2006) *Nat Cell Biol* **8**, 1246-1254
38. Fallon, L., Belanger, C. M., Corera, A. T., Kontogiannea, M., Regan-Klapisz, E., Moreau, F., Voortman, J., Haber, M., Rouleau, G., Thorarinsdottir, T., Brice, A., van Bergen En Henegouwen, P. M., and Fon, E. A. (2006) *Nat Cell Biol* **8**, 834-842
39. Woelk, T., Sigismund, S., Penengo, L., and Polo, S. (2007) *Cell Div* **2**, 11
40. Itoh, T., Koshiba, S., Kigawa, T., Kikuchi, A., Yokoyama, S., and Takenawa, T. (2001) *Science* **291**, 1047-1051
41. Achiriloaie, M., Barylko, B., and Albanesi, J. P. (1999) *Mol Cell Biol* **19**, 1410-1415
42. Raiborg, C., Bremnes, B., Mehlum, A., Gillyooly, D. J., D'Arrigo, A., Stang, E., and Stenmark, H. (2001) *J Cell Sci* **114**, 2255-2263
43. Simonsen, A., Lippe, R., Christoforidis, S., Gaullier, J. M., Brech, A., Callaghan, J., Toh, B. H., Murphy, C., Zerial, M., and Stenmark, H. (1998) *Nature* **394**, 494-498
44. Slagsvold, T., Aasland, R., Hirano, S., Bache, K. G., Raiborg, C., Trambaiolo, D., Wakatsuki, S., and Stenmark, H. (2005) *J Biol Chem* **280**, 19600-19606
45. Whitley, P., Reaves, B. J., Hashimoto, M., Riley, A. M., Potter, B. V., and Holman, G. D. (2003) *J Biol Chem* **278**, 38786-38795
46. Hoeller, D., Crosetto, N., Blagoev, B., Raiborg, C., Tikkanen, R., Wagner, S., Kowanetz, K., Breitling, R., Mann, M., Stenmark, H., and Dikic, I. (2006) *Nat Cell Biol* **8**, 163-169