The role of ubiquitylation in receptor endocytosis and endosomal sorting

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Summary

Ligand-induced activation of transmembrane receptors activates intracellular signaling cascades that control vital cellular processes, such as cell proliferation, differentiation, migration and survival. Receptor signaling is modulated by several mechanisms to ensure that the correct biological outcome is achieved. One such mechanism, which negatively regulates receptor signaling, involves the modification of receptors with ubiquitin. This post-translational modification can promote receptor endocytosis and targets receptors for lysosomal degradation, thereby ensuring termination of receptor signaling. In this Commentary, we review the roles of ubiquitylation in receptor endocytosis and degradative endosomal sorting by drawing on the epidermal growth factor receptor (EGFR) as a well-studied example. Furthermore, we elaborate on the molecular basis of ubiquitin recognition along the endocytic pathway through compartment-specific ubiquitin-binding proteins and highlight how endocytic sorting machineries control these processes. In addition, we discuss the importance of ubiquitin-dependent receptor endocytosis for the maintenance of cellular homeostasis and in the prevention of diseases such as cancer.

This article is part of a Minifocus on Ubiquitin. For further reading, please see related articles: 'Ubiquitin and SUMO in DNA repair at a glance' by Helle D. Ulrich (*J. Cell Sci.* **125**, 249-254). 'Emerging regulatory mechanisms in ubiquitin-dependent cell cycle control' by Annamaria Mocciaro and Michael Rape (*J. Cell Sci.* **125**, 255-263). 'Cellular functions of the DUBs' by Michael J. Clague et al. (*J. Cell Sci.* **125**, 277-286). 'HECT and RING finger families of E3 ubiquitin ligases at a glance' by Meredith B. Metzger et al. (*J. Cell Sci.* **125**, 531-537). 'Non-canonical ubiquitin-based signals for proteasomal degradation' by Yelena Kravtsova-Ivantsiv and Aaron Ciechanover (*J. Cell Sci.* **125**, 539-548). 'No one can whistle a symphony alone – how different ubiquitin linkages cooperate to orchestrate NF-κB activity' by Anna C. Schmukle and Henning Walczak (*J. Cell Sci.* **125**, 549-559).

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Introduction

Ubiquitylation is a highly controlled process that labels proteins by covalent attachment of ubiquitin to lysine residues. Conjugation of ubiquitin typically occurs in a three-step process by the sequential action of ubiquitin-activating (E1), ubiquitin-conjugating (E2) and ubiquitin-ligating (E3) enzymes. This reaction leads to the formation of an isopeptide bond between the carboxyl group of the C-terminal glycine residue (Gly76) of ubiquitin and the ε-amino group of a Lys residue in or the N-terminus of the substrate (Hershko and Ciechanover, 1998; Pickart and Eddins, 2004; Varshavsky, 1997). Because ubiquitin itself contains seven lysine residues (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48 and Lys63) that can serve as acceptor sites for chain elongation, chains that contain multiple ubiquitin moieties can be formed by either homotypic or heterotypic linkages (Ikeda and Dikic, 2008). Cellular proteins can thus be subject to diverse types of ubiquitin modifications: (1) monoubiquitylation, the attachment of a single ubiquitin, (2) multiple monoubiquitylation, the attachment of multiple single ubiquitin molecules to several lysine residues in the target protein, and (3) polyubiquitylation, a modification with ubiquitin chains of diverse lengths and linkages (Ikeda and Dikic, 2008). The ubiquitin modification can be reversed by the action of deubiquitylating enzymes (DUBs) that recycle ubiquitin to the cytoplasmic pool (Komander et al., 2009) [see the Commentary by Michael Clague, Judy Coulson and Sylvie Urbé in this issue (*J. Cell Sci.* **125**, 277-286)]. Ubiquitylation is thus a highly versatile and reversible process.

Ubiquitin modifications on target proteins are recognized by an array of ubiquitin-binding domains (UBDs) that bind non-covalently to ubiquitin and which are found in a plethora of effector proteins. The formation of complex ubiquitin–UBD interaction networks controls numerous cellular processes (Dikic et al., 2009); whereas Lys48-linked polyubiquitylation labels proteins for proteasomal targeting and degradation, other forms of protein ubiquitylation control diverse cellular functions, including receptor endocytosis, endosomal sorting, NF-κB signaling and DNA repair (Bergink and Jentsch, 2009; Haglund and Dikic, 2005; Hershko and Ciechanover, 1998; Raiborg and Stenmark, 2009).

Ligand binding to transmembrane receptors induces signaling cascades that control essential cellular processes, such as cell proliferation, differentiation, migration and survival (Schlessinger, 2000). Following binding to their cognate ligands and signal initiation, many cell surface transmembrane proteins and receptors undergo endocytosis, followed by their intracellular trafficking along an endocytic path. Activated signaling receptors (such as receptor tyrosine kinases; RTKs) undergo clathrin-mediated endocytosis. In this pathway, receptors accumulate in clathrin-coated pits that pinch off from the plasma membrane into the cytoplasm through the action of the GTPase dynamin (Fig. 1) (Doherty and McMahon, 2009). Receptors can also undergo endocytosis by several clathrin-independent mechanisms (Hansen and Nichols, 2009; Mayor and Pagano, 2007). Following internalization either by clathrin-dependent or clathrin-independent endocytosis, receptors are routed to early endosomes (Sorkin and

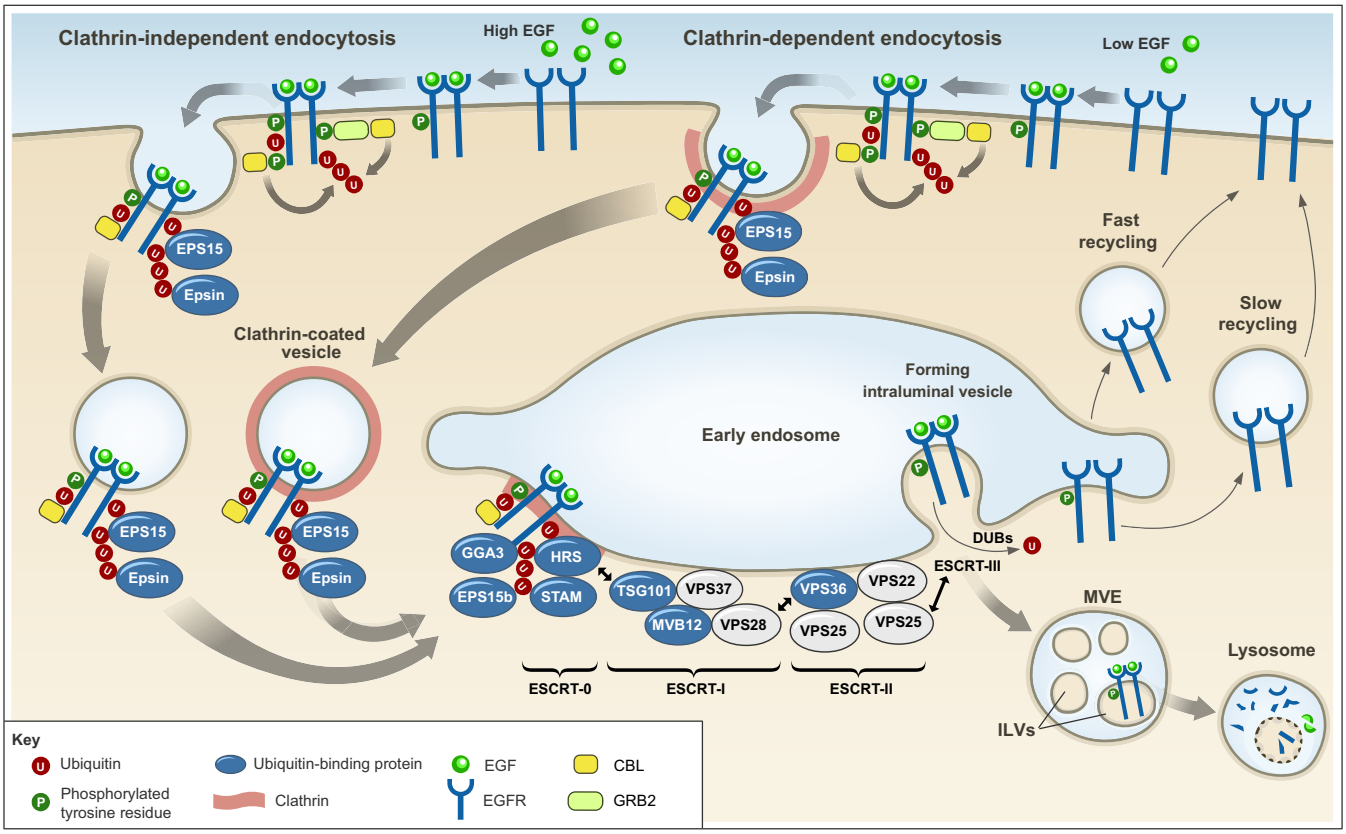

Fig. 1. Ubiquitylation in receptor endocytosis and endosomal sorting. Following ligand binding, signaling receptors (in this example the EGFR), can undergo clathrin-mediated endocytosis (right) or clathrin-independent endocytosis (left) (Goh et al., 2010; Scita and Di Fiore, 2010; Sigismund et al., 2005). In both cases, receptors are routed to early endosomes, from where they can be sorted into ILVs of the MVE and subsequently targeted for lysosomal degradation (ubiquitylated receptors) or recycled to the plasma membrane (non-ubiquitylated receptors). As described in the main text, the contribution of ubiquitylation to EGFR endocytosis seems to depend on the experimental system used (Goh et al., 2010; Madshus and Stang, 2009; Sigismund et al., 2005). The CBL family of ubiquitin ligases has a key role in mediating RTK ubiquitylation. CBL associates with activated receptors either directly or indirectly, for example through GRB2. Ubiquitin is an essential signal for endosomal sorting of EGFRs into the ILVs of MVEs. Components of the endocytic machinery, including EPS15, epsins, the ESCRT-0 components HRS and STAM, the ESCRT-I components TSG101 and Mvb12p (in yeast) (and the novel ESCRT-I component UBAP1), the ESCRT-II component VPS36 (EAP45), as well as EPS15b and GGA3 contain ubiquitin-binding domains and have been implicated in recognizing and sorting ubiquitylated receptors either at the plasma membrane or at endosomes, as indicated in the figure. The ESCRT-I and -II components assemble in supercomplexes and have been proposed to organize buds at the endosomal membrane. The ESCRT-III complex associates with ESCRT-II and forms polymers that drive membrane scission and ILV biogenesis. DUBs catalyze the removal of ubiquitin from receptors before their translocation into the ILV, without allowing cargo to escape.

von Zastrow, 2009). Importantly, receptor signaling can occur both from the plasma membrane and endosomes (Miaczynska and Bar-Sagi, 2010; Sorkin and von Zastrow, 2009). Once located in early endosomes, receptors can either be recycled back to the plasma membrane, to allow for repeated receptor activation, or become sorted into intraluminal vesicles (ILVs) of multivesicular endosomes (MVEs), which targets them for lysosomal degradation and thereby results in termination of receptor signaling (Katzmann et al., 2001; Raiborg and Stenmark, 2009) (Fig. 1). Fidelity in endosomal receptor sorting is thus important for restricting the duration of receptor signaling and the maintenance of cellular homeostasis by preventing prolonged and excessive activation of signals downstream of the receptor that could contribute to pathologies such as cancer (Abella and Park, 2009; Bache et al., 2004; Haglund et al., 2007; Mosesson et al., 2008).

Extensive studies in the past 15 years have established the importance of receptor ubiquitylation in controlling receptor endocytosis and degradative endosomal sorting, and have identified molecular mechanisms and machineries governing these processes. In this Commentary, we will review the roles of ubiquitylation in receptor endocytosis and endosomal sorting, and provide an overview of the endocytic sorting machineries with ubiquitin-binding abilities. We will particularly draw on knowledge gained from studies of the epidermal growth factor receptor (EGFR) and other receptor tyrosine kinases. Furthermore, we will discuss how components of the endocytic sorting machinery are regulated by monoubiquitylation and highlight the importance of receptor ubiquitylation in the homeostatic control of biological processes and in malignant conditions such as cancer.

Historical overview of ubiquitylation and endocytosis

The first evidence for ubiquitin having a role in receptor endocytosis and degradative receptor sorting to the vacuole and/or lysosome came from studies of the G-protein-coupled receptor (GPCR) Ste2p and transporters (uracil permease and the ABC-transporter Ste6p) in *Saccharomyces cerevisiae*. In particular, monoubiquitylation of uracil permease was sufficient for its endocytosis, and Lys63-

Table 1. Ubiquitylation of RTKs

RTK	E3 ubiquitin ligase(s)	E3 ubiquitin ligase interaction site	Type of ubiquitylation (Ub)	Known function of RTK ubiquitylation	References
EGFR	CBL, CBLB	Tyr1045-*P*[a]	Multiple monoUb, Lys63-linked polyUb	Endosomal receptor sorting and lysosomal degradation	(Haglund et al., 2003; Huang and Sorkin, 2005; Levkowitz et al., 1998; Mosesson et al., 2003; Sigismund et al., 2005; Szymkiewicz et al., 2002)
IGF1R	CBL	N.D.	Lys48-linked polyUb	Endocytosis through caveolae	(Sehat et al., 2008)
	MDM2	N.D.	Lys63-linked polyUb	Clathrin-mediated endocytosis	
	NEDD4	N.D.	?	Receptor degradation	(Vecchione et al., 2003)
PDGFR	CBL, CBLB	Tyr1021-*P*[b]	Multiple monoUb, PolyUb	Endosomal receptor sorting and lysosomal degradation	(Haglund et al., 2003; Joazeiro et al., 1999; Lennartsson et al., 2006; Miyake et al., 1998; Mori et al., 1992; Szymkiewicz et al., 2002; Reddi et al., 2007)
CSF1R	CBL	Tyr973-*P*	?	Receptor degradation	(Lee et al., 1999; Wang et al., 1999; Weilhemsen et al., 2002)
KIT	CBL, CBLB	Tyr568-*P*, Tyr936-*P*[a]	MonoUb	Internalization and lysosomal degradation	(Masson et al., 2006; Miyazawa et al., 1994; Mori et al., 1995)
VEGFR-1	CBL	Tyr1333-*P*	?	Receptor degradation	(Duval et al., 2003; Kobayashi et al., 2004)
VEGFR-2	NEDD4	N.D.	?		(Murdaca et al., 2004)
FGFR	CBL	N.D.[a]	Ub on multiple sites	Lysosomal degradation, but not internalization	(Haugsten et al., 2008; Wong et al., 2002a)
TRKA	CBL	N.D.	?	Lysosomal degradation	(Takahashi et al., 2011)
	TRAF6	N.D.	Lys63-linked polyUb	Receptor internalization	(Geetha et al., 2005; Geetha and Wooten, 2008)
	NEDD4-2	N.D.	Multiple monoUb	Lysosomal degradation	(Arevalo et al., 2006)
MET (also known as HGFR)	CBL, CBLB	Tyr1003-*P*	?	Lysosomal degradation	(Peschard et al., 2001)
EPHB1	CBL	N.D.	?	Lysosomal degradation	(Fasen et al., 2008)
RET	CBL	N.D.[a]	?	Receptor degradation	(Scott et al., 2005)

[a]Indirect recruitment of CBL to activated RTKs through adaptor proteins (e.g. GRB2 for EGFR, KIT and RET, and fibroblast growth factor receptor substrate 2 and GRB2 for FGFR) (Jiang et al., 2003; Scott et al., 2005; Sun et al., 2007; Wong et al., 2002a).
[b]For the PDGF β-receptor (PDGFRB).
The table has been adapted and updated from Acconcia et al. (Acconcia et al., 2009). N.D., not determined.

linked ubiquitin chains further stimulated the process (Galan and Haguenauer-Tsapis, 1997; Hicke and Riezman, 1996; Kolling and Hollenberg, 1994). Subsequent studies in mammalian cells have shown accordingly that fusion of ubiquitin to different types of receptors can drive their endocytosis and endosomal sorting for degradation (Haglund et al., 2003; Nakatsu et al., 2000; Raiborg et al., 2002; Strous et al., 1996).

Whereas the role of ubiquitylation in receptor endocytosis remains elusive for certain receptor types in mammalian cells, ubiquitylation has been established as an important signal that directs sorting of receptors into the ILVs of the MVE to target them for lysosomal degradation. In the following sections, we will focus on the role of ubiquitylation in RTK endocytosis and MVE sorting, with particular emphasis being placed on the EGFR, which is one of the most well-characterized RTKs. For a discussion of the roles of ubiquitylation in endocytosis and endosomal sorting of other types of receptors [including transforming growth factor beta (TGF-β) receptors, GPCRs and Notch receptors] and of other transmembrane proteins (channels, transporters, junctional proteins) the reader is referred to several excellent reviews that have been published recently (Hislop and von Zastrow, 2011; Kjenseth et al., 2010; Le Bras et al., 2011; Miranda and Sorkin, 2007; Moustakas et al., 2001).

Ubiquitylation in endocytosis and endosomal sorting of RTKs

RTKs constitute a large family of receptors that includes more than 50 members in humans and which have essential roles in controlling a variety of cellular and biological processes, including cell proliferation, differentiation, migration and survival (Lemmon and Schlessinger, 2010). Following their ligand-induced activation at the plasma membrane, a large number of RTKs undergo rapid ubiquitylation (Table 1). A major family of E3 ubiquitin ligases that is involved in ubiquitylation of RTKs is the family of CBL (for Cas-Br-M ecotropic retroviral transforming sequence) RING-type E3 ubiquitin ligases (Joazeiro et al., 1999; Levkowitz et al., 1999; Levkowitz et al., 1998; Thien and Langdon, 2001). In the case of the EGFR, CBL E3 ligases are either recruited directly to a specific phosphorylated tyrosine residue (Tyr1045-*P*) in the cytoplasmic region of the activated receptor through their tyrosine-kinase-binding (TKB) domain, or indirectly through interaction with the adaptor protein growth factor receptor-bound protein 2 (GRB2) to promote EGFR ubiquitylation at the plasma membrane (Fig. 1) (de Melker et al., 2004; Jiang et al., 2003; Schmidt and Dikic, 2005; Stang et al., 2000; Thien and Langdon, 2001). Studies aimed at elucidating the nature of ligand-induced EGFR ubiquitylation have revealed that EGFRs become modified with multiple monoubiquitin units, as well as a mixture of ubiquitin chains, predominantly Lys63-linked chains (Haglund et al., 2003; Huang et al., 2006; Mosesson et al., 2003). In the following sections, we will discuss the mechanisms by which ubiquitylation regulates endocytosis and endosomal sorting of the EGFR as a representative member of the RTK family.

Ubiquitylation and internalization of the EGFR

Clathrin-mediated endocytosis represents a major pathway for EGFR internalization, but EGFR endocytosis can also occur through clathrin-independent pathways (Fig. 1) (Goh et al., 2010; Scita and Di Fiore, 2010; Sigismund et al., 2005). The roles of ubiquitylation in EGFR endocytosis have been and are still under thorough investigation, and recent studies have indicated that the ligand concentration and extent of ubiquitylation can affect the

internalization route (Sigismund et al., 2005). These findings suggest that at low doses of epidermal growth factor (EGF), ubiquitylation of the EGFR cannot be detected and that, under these conditions, receptors mainly undergo clathrin-mediated endocytosis (Sigismund et al., 2005). By contrast, higher ligand concentrations result in a substantial proportion of the receptors becoming ubiquitylated and undergoing clathrin-independent, but lipid-raft-dependent, endocytosis (Sigismund et al., 2005). The specific internalization route was found to affect EGFR fate and thus the duration of receptor signaling (Sigismund et al., 2008). EGFRs entering cells through clathrin-mediated endocytosis were not targeted for degradation but were rather recycled to the cell surface (Sigismund et al., 2008). In this way, clathrin-dependent EGFR endocytosis prolonged the duration of EGFR-activated signaling pathways and ultimately EGFR-induced biological responses (Sigismund et al., 2008). By contrast, in these studies clathrin-independent EGFR endocytosis preferentially targeted receptors for degradation (Sigismund et al., 2008; Sigismund et al., 2005). In addition to the above-mentioned reports, other recent studies have indicated that multiple mechanisms collectively control clathrin-mediated endocytosis of the EGFR (Goh et al., 2010). These mechanisms include ubiquitylation of Lys residues in the kinase domain of EGFR, interaction of the receptor with the clathrin adaptor protein (AP)-2 complex, interaction with the adaptor protein GRB2 and acetylation of C-terminal Lys residues (Goh et al., 2010). It is possible that the relative contribution of these mechanisms varies depending on the cell type and experimental conditions, that not all mechanisms are simultaneously employed by a single cell and that certain mechanisms are preferentially employed under physiological conditions. In this context, it also has to be considered that, in some studies, ubiquitylation of EGFRs is detected even at low EGF concentrations, and that ubiquitylation can promote the translocation of the receptors to clathrin-coated pits (Madshus and Stang, 2009). On the basis of these available data, the contribution of ubiquitylation to clathrin-mediated EGFR endocytosis still remains somewhat elusive and might be influenced by the exact experimental conditions used (Goh et al., 2010; Huang et al., 2007; Madshus and Stang, 2009; Sigismund et al., 2005).

Ubiquitylation in endosomal sorting of the EGFR

Following their endocytosis and trafficking to early endosomes, EGFRs can either recycle back to the plasma membrane or become sorted into the ILVs of MVEs and thereby become targeted for lysosomal degradation (Fig. 1) (Futter et al., 1996; Levkowitz et al., 1998; Longva et al., 2002). Several lines of evidence show that ubiquitylation is a signal that is required for degradative receptor sorting into MVEs and consequent lysosomal degradation (Katzmann et al., 2002; Raiborg et al., 2002; Raiborg and Stenmark, 2009). This is supported by the observation that fusion of ubiquitin to the transferrin receptor, which is normally recycled, prevents its recycling and targets it for degradative endosomal sorting (Raiborg et al., 2002). Moreover, mutant EGFRs that lack the ability to recruit CBL, and thus do not become ubiquitylated, are not targeted for lysosomal degradation and are instead rapidly recycled to the plasma membrane (Peschard and Park, 2003). Similarly, oncogenic CBL mutants, whose ubiquitin ligase activity is impaired, block EGFR degradation, and receptors are instead recycled from endosomes to the plasma membrane (Peschard and Park, 2003).

In addition, two alternative EGFR ligands, TGF-α and EGF, which have different effects on EGFR ubiquitylation also differentially affect degradation. TGF-α dissociates from the EGFR at the low pH (5.5–6.0) of early endosomes, which leads to a rapid decrease of EGFR ubiquitylation and consequent receptor recycling (Longva et al., 2002). EGF, by contrast, has a higher affinity for the EGFR and remains persistently bound to the receptor along the endocytic route, thereby favoring continued EGFR ubiquitylation and degradation (Longva et al., 2002). Indeed, following EGF stimulation, CBL remains associated with EGFRs and promotes receptor ubiquitylation along the endocytic route, thereby ensuring that the receptors are sorted to MVEs and targeted for lysosomal degradation (de Melker et al., 2001; Grovdal et al., 2004; Haglund et al., 2003; Levkowitz et al., 1998; Longva et al., 2002; Umebayashi et al., 2008). Taken together, these data support the notion that ubiquitylation of receptors, such as the EGFR, is a signal for MVE sorting and lysosomal degradation that shunts receptors away from the recycling pathway.

Ubiquitylation and endocytosis of other RTKs

Other RTKs that undergo ligand-induced ubiquitylation include the platelet-derived growth factor receptor (PDGFR) (Miyake et al., 1998; Miyake et al., 1999; Mori et al., 1992; Mori et al., 1993), the vascular endothelial growth factor receptor (VEGFR) (Duval et al., 2003; Kobayashi et al., 2004), the hepatocyte growth factor receptor (HGFR, also known as MET) (Peschard et al., 2001), the fibroblast growth factor receptor (FGFR) (Haugsten et al., 2008; Wong et al., 2002a), the colony-stimulating factor-1 receptor (CSF1R) (Lee et al., 1999; Wang et al., 1999), KIT (Masson et al., 2006; Miyazawa et al., 1994; Mori et al., 1995), RET (Scott et al., 2005), the insulin-like growth factor-type 1 receptor (IGF1R) (Vecchione et al., 2003) and the nerve growth factor (NGF) receptor NTRK1 (also known as TRK and TRKA) (Arevalo et al., 2006; Geetha et al., 2005; Geetha and Wooten, 2008; Takahashi et al., 2011) (Table 1). Ubiquitylation of all of these receptors promotes their degradation and is, at least in part, also mediated by the CBL family of ubiquitin ligases (Table 1). Ubiquitylation is not required for the endocytosis of, for example, FGFR1, but is required for its degradative endosomal receptor sorting and termination of receptor signaling (Haugsten et al., 2008). By contrast, Lys63-linked polyubiquitylation by the E3 ubiquitin-protein ligase TRAF6 is important for NTRK1 internalization, which is in turn required for receptor-induced signaling and neurite outgrowth (Geetha et al., 2005). Ubiquitylation of this receptor by both E3 ubiquitin-protein ligase NEDD4-like (NEDD4L, also known as NEDD4-2) and CBL, however, promotes receptor degradation in lysosomes and thereby termination of receptor signaling (Arevalo et al., 2006; Takahashi et al., 2011).

Importantly, RTK ubiquitylation itself is also controlled in order to fine-tune receptor signaling and downregulation. The activity of CBL ubiquitin ligases is negatively regulated by several mechanisms, such as self-ubiquitylation of CBL, which promotes its proteasomal degradation, and specific modulators that can inhibit CBL-mediated ubiquitylation, including Sprouty 2 (SPRY2), suppressor of T-cell receptor signaling 2 (STS2, also known as UBS3A and UBASH3A) and low-density-lipoprotein-related protein 1 (LRP1) (Kowanetz et al., 2004; Takayama et al., 2005; Wong et al., 2002b). Thus, robust mechanisms are in place to regulate RTK ubiquitylation, endocytosis and degradation, and ultimately control the duration of RTK activation, thereby modulating receptor signaling output.

Ubiquitin-binding proteins in endosomal receptor trafficking

The presence of UBDs within numerous compartmentalized endocytic adaptor proteins has provided insight into the molecular basis for ubiquitin-dependent trafficking of receptors along the endocytic pathway (Fig. 1, Table 2). Here, we give an overview of some of these proteins and their roles in receptor endocytosis and endosomal sorting.

Ubiquitin-binding proteins in early endocytosis

Several endocytic adaptor proteins involved in regulating the early steps of endocytosis contain UBDs. EPS15 (for EGFR substrate 15), epsin 1 (EPN1, EPS15-interacting protein 1) and epsin 2 (EPN2) interact with the clathrin adaptor AP-2 complex at the plasma membrane and participate in the assembly of clathrin-coated vesicles (Benmerah et al., 1998; Chen et al., 1998). Each of these proteins contains ubiquitin-interacting motifs (UIMs), which are able to mediate the interaction with monoubiquitin (Table 2) (Di Fiore et al., 2003; Polo et al., 2002). Knockdown of EPS15 and epsin 1 and 2 causes impairment of EGFR endocytosis (Kazazic et al., 2009; Sigismund et al., 2005; Stang et al., 2004), and an intact UIM in Ent1p, one of the yeast epsin homologues, has been shown to be required for receptor endocytosis in yeast strains that lack wild-type epsins (Ent1p and Ent2p) and EPS15 (Ede1p) (Shih et al., 2002).

Ubiquitin-binding proteins in endosomal sorting

Ubiquitin-binding proteins that reside at the limiting membrane of early endosomes participate in binding and directing ubiquitylated receptors into MVEs (Dikic et al., 2009; Raiborg and Stenmark, 2009). The endosomal sorting complex required for transport (ESCRT) machinery has major roles in the sorting of ubiquitylated cargo into MVEs and in MVE biogenesis (Hurley and Hanson, 2010; Raiborg and Stenmark, 2009; Shields and Piper, 2011). The ESCRT complex consists of four subcomplexes (0, I, II and III), which in turn comprise a number of different proteins (Fig. 1) (Hurley, 2010; Katzmann et al., 2002; Raiborg and Stenmark, 2009; Shields and Piper, 2011; Wollert and Hurley, 2010). ESCRT-0, which consists of HRS [hepatocyte growth factor-regulated tyrosine kinase substrate, also known as HGS and, in yeast, vacuolar protein sorting (Vps) 27p] and STAM (signal-transducing adaptor molecule; Hse1p in yeast), is recruited to endosomal membranes through the interaction of the HRS FYVE domain with phosphatidylinositol 3-phosphate [PtdIns3*P*] (Burd and Emr, 1998; Gaullier et al., 1998; Raiborg et al., 2001b). ESCRT-0 has the ability to recruit the ESCRT-I complex [which is made up of TSG101 (tumor susceptibility gene 101; Vps23p in yeast), VPS28, VPS37 and MVB12 (multivesicular body subunit 12, also known as FAM125A) (Chu et al., 2006; Katzmann et al., 2001; Kostelansky et al., 2007; Morita et al., 2007; Oestreich et al., 2007; Stuchell et al., 2004)] through an interaction between HRS and TSG101 (Bache et al., 2003; Katzmann et al., 2003; Lu et al., 2003). ESCRT-I and ESCRT-II, which comprise a single VPS36 (also known as EAP45), a single VPS22 and two VPS25 subunits, can form a supercomplex through interactions between VPS28 and VPS36 (also see below) (Gill et al., 2007; Im and Hurley, 2008). ESCRT-II can interact with ESCRT-III [comprising CHMP6 (VPS20), CHMP4A (VPS32), CHMP3 (VPS24) and the CHMP2 (VPS2) subunits]. In a model for ILV formation at the MVE, ESCRT-I and ESCRT-II form buds that subsequently are cut by membrane scission catalyzed by the polymer formed by the ESCRT-III complex (Hanson et al., 2008; Hurley, 2010; Im et al., 2009; Raiborg and Stenmark, 2009; Wollert and Hurley, 2010; Wollert et al., 2009).

Several of the subunits of the ESCRT machinery contain UBDs and accumulating evidence suggests that these allow the ESCRT machinery to capture and sort ubiquitylated receptors into MVEs (Fig. 1, Table 2) (Dikic et al., 2009; Hurley and Stenmark, 2011; Raiborg and Stenmark, 2009; Shields and Piper, 2011). The ESCRT-0 complex contains several UBDs: HRS contains one double-sided UIM and one VHS (VPS27, HRS, STAM) domain (yeast Vps27p contains two UIMs), and STAM (yeast Hse1p) contains one UIM and one VHS domain (Table 2). Convincing evidence in both yeast and mammalian cells suggests that ESCRT-0 can recognize and sort ubiquitylated cargo at endosomes, because loss of the UBDs in the yeast ESCRT-0 or of the UIM of mammalian HRS causes defects in capturing and sorting of ubiquitylated cargo (Bilodeau et al., 2002; Hirano et al., 2006; Raiborg et al., 2002; Ren and Hurley, 2010; Urbe et al., 2003). Ubiquitylated cargo was originally thought to be sorted sequentially from ESCRT-0 to ESCRT-I and subsequently to ESCRT-II. However, recent studies suggest a model in which ESCRT-0 can recognize ubiquitylated cargo and recruit it to an ESCRT-I–ESCRT-II supercomplex that contains multiple UBDs with the ability to cooperatively recognize and coordinate sorting of ubiquitylated cargo into ILVs (Gill et al., 2007; Hurley and Hanson, 2010; Im and Hurley, 2008; Shields et al., 2009; Shields and Piper, 2011). The ESCRT-I subunit TSG101 contains a UEV (ubiquitin-conjugating enzyme E2 variant) domain and yeast Mvb12p of ESCRT-I also contains a UBD with the ability to interact with ubiquitin and sort ubiquitylated cargo (Table 2) (Shields et al., 2009; Sundquist et al., 2004; Teo et al., 2004). Moroever, a newly discovered ESCRT-I component, ubiquitin-associated protein 1 (UBAP1), contains two ubiquitin-associated domains (UBAs) that interact with ubiquitin and are required for ubiquitin-dependent endosomal sorting of EGFRs (Stefani et al., 2011). UBAP1 contains a UMA (for UBAP1-MVB12-associated) domain that is conserved in MVB12 and has been suggested to be part of an endosome-specific ESCRT-I complex (Stefani et al., 2011). The VPS36 subunit of ESCRT-II contains a ubiquitin-binding GLUE (for Gram-like ubiquitin binding in EAP45) domain and the yeast homologue Vps36p, contains two Npl4 zinc finger (NZF) domains, one of which binds ubiquitin (Alam et al., 2004; Slagsvold et al., 2005). Thus, taken together, ESCRT-0, -I and -II contain an array of UBDs that are involved in capturing and sorting ubiquitylated cargo into the ILVs of MVEs (Table 2). As described above, the ESCRT-III complex subsequently completes ILV biogenesis by membrane scission. Before receptor translocation into the ILV of the MVE, ubiquitin is removed from receptors by DUBs. This ensures that ubiquitin molecules are recycled to maintain cellular ubiquitin levels, without allowing cargo to escape (Clague and Urbe, 2006).

A number of additional ubiquitin-binding proteins present at endosomes are able to participate in degradative sorting of ubiquitylated cargo. The endosome-localized isoform of EPS15, EPS15b, associates with ESCRT-0, contains two UIMs and participates in sorting of activated EGFRs for degradation (Roxrud et al., 2008). Moreover, GGA (for Golgi-localized, gamma ear-containing, Arf-binding protein) proteins and TOM1 (target of myb1), which associate with ESCRT-I, are candidate proteins for alternative ESCRT-0 complexes, because they sort ubiquitylated cargo and, similar to ESCRT-0, contain UBDs, namely, GAT (GGA and TOM1) and VHS domains, as well as clathrin-binding domains

Table 2. Endocytic adaptor proteins with ubiquitin-binding domains

Endocytic UBD-containing adaptor protein		UBD	Affinity for ubiquitin in vitro (K_d, μM)	UBD-dependent mono-ubiquitylation	Proposed functions of UBD in endocytosis and endosomal sorting	References
Yeast	Mammals					
Ent1p, Ent2p	EPN1, EPN2	UIM	N.D.	Yes	Recognition of ubiquitylated cargo at the plasma membrane and interactions with the endocytic machinery	(Dores et al., 2010; Miller et al., 2004; Oldham et al., 2002; Polo et al., 2002; Shih et al., 2002)
Ede1p	EPS15	UBA (yeast), UIM (mammals)	~80 (monoubiquitin), ~900 (mono- or poly-ubiquitin)	Yes	Recognition of ubiquitylated cargo at the plasma membrane and interactions with the endocytic machinery	(Fisher et al., 2003; Klapisz et al., 2002; Miller et al., 2004; Oldham et al., 2002; Polo et al., 2002; Shih et al., 2002; Swanson et al., 2006)
–	EPS15b	UIM	N.D.	N.D.	Sorting of ubiquitylated cargo at endosomes (interacts with HRS of ESCRT-0)	(Roxrud et al., 2008)
Vps27p	HRS	UIM	~100–300 (monoubiquitin), ~30 (lysine-63-linked polyubiquitin)	Yes	Recognition and sorting of ubiquitylated cargo at endosomes. ESCRT-0 component, recruits ESCRT-I (TSG101)	(Bilodeau et al., 2002; Bishop et al., 2002; Fisher et al., 2003; Hirano et al., 2006; Katz et al., 2002; Miller et al., 2004; Polo et al., 2002; Raiborg et al., 2002; Ren and Hurley, 2010; Shekhtman and Cowburn, 2002; Shih et al., 2002; Swanson et al., 2003)
		VHS	~1500 (monoubiquitin)			
Hse1p	STAM1, STAM2	UIM	~200 (monoubiquitin)	N.D.	Sorting of ubiquitylated cargo at endosomes. ESCRT-0 component	(Bilodeau et al., 2002; Fisher et al., 2003; Hong et al., 2009; Kanazawa et al., 2003; Mizuno et al., 2003; Ren and Hurley, 2010)
		VHS	~50–220 (monoubiquitin)			
Vps23p	TSG101	UEV	~500 (monoubiquitin)	N.D.	Sorting of ubiquitylated cargo to MVEs. ESCRT-I component	(Babst et al., 2000; Bishop et al., 2002; Katzmann et al., 2001; Pornillos et al., 2002)
Mvb12p	MVB12	UBD (yeast)[a]	N.D.	N.D.	Contribution to sorting of ubiquitylated cargo to MVEs. ESCRT-I component	(Shields et al., 2009)
–	UBAP1	UBA	N.D.	N.D.	Sorting of ubiquitylated EGFRs to MVEs. Novel ESCRT-I component	(Stefani et al., 2011)
Vps36p	VPS36 (also known as EAP45)	NZF (yeast), GLUE (mammals)	~200 (monoubiquitin) or ~460 (monoubiquitin)	N.D.	Sorting of ubiquitylated cargo to MVEs. ESCRT-II component	(Alam et al., 2004; Slagsvold et al., 2005)
Vps9p	RABGEF1	CUE (yeast),	~1 (monoubiquitin)	Yes	Sorting of ubiquitylated cargo to vacuole or MVE	(Davies et al., 2003; Lee et al., 2006; Mattera and Bonifacino, 2008; Penengo et al., 2006; Prag et al., 2003; Shih et al., 2003)
		A20 ZnF	~20 (monoubiquitin)			
		and MIU (mammals)	~30 (monoubiquitin)			
–	STS1, STS2 (also known as UBASH3B and UBASH3A)	UBA	N.D.	Yes	Inhibition of RTK endocytosis. UBA interacts with RTK–Ub chimera	(Hoeller et al., 2006; Kowanetz et al., 2004; Raguz et al., 2007)
Ggas	GGAs	GAT	~200 (monoubiquitin)	Yes	Sorting of ubiquitylated cargo to MVE	(Kawasaki et al., 2005; Prag et al., 2005; Puertollano and Bonifacino, 2004; Shiba et al., 2004)
		VHS	N.D.			
Tom1p	TOM1	GAT	~400 (monoubiquitin)	N.D.	Recruitment of ubiquitylated cargo to endosomes	(Akutsu et al., 2005; Katoh et al., 2004)
		VHS	N.D.			
Sla1p	SH3KBP1 (also known as CIN85)	SH3	~40 (monoubiquitin) or ~200–1000 (monoubiquitin)	N.D.[b]	CBL-interacting adaptor protein involved in endocytosis of RTKs, SH3 domains mediate interaction with CBL and ubiquitin	(Bezsonova et al., 2008; Petrelli et al., 2002; Soubeyran et al., 2002; Stamenova et al., 2007)

Several proteins, including Vps27p and HRS, Hse1p and STAM1 and STAM2, RABGEF1, Gga3 and GGA3, and TOM1 contain two types of UBDs that have been implicated in ubiquitin binding, both of which are shown. A20 ZnF, A20 zinc finger; CUE, coupling of ubiquitin conjugation enzyme-like; CIN85, CBL-interacting protein of 85 kDa; MIU, motif interacting with ubiquitin; SH3KBP1, SH3-domain kinase binding protein 1; N.D., not determined; Ub, ubiquitin.

[a]A UBD in the C-terminal region of yeast Mvb12p has been identified so far (Shields et al., 2009).

[b]CIN85 undergoes CBL-mediated monoubiquitylation following EGF stimulation (Haglund et al., 2002).

(Table 2) (Katoh et al., 2004; Puertollano and Bonifacino, 2004; Yamakami et al., 2003). The molecular modes of action and structural details of the ubiquitin-binding proteins at endosomes are still under thorough investigation.

Mechanisms of ubiquitin recognition during endocytic trafficking

The UBDs in the endocytic proteins discussed above all have low affinity for ubiquitin in vitro (i.e. their dissociation constants lie in the micromolar range; Table 2) (Dikic et al., 2009). Appropriate sorting of ubiquitylated cargo in vivo might be accomplished by increasing the avidity for ubiquitin through multivalent interactions, by increasing the local concentration of ubiquitylated cargoes and through the presence of multiple ubiquitin moieties attached to the cargo (Dikic et al., 2009). For example, the UIM of HRS forms a double-sided α-helix with two ubiquitin-binding surfaces, which bind two ubiquitin moieties with equal affinity, and the ESCRT complexes have the ability to form supercomplexes containing several UBDs, as described above (Hirano et al., 2006; Shields and Piper, 2011). In addition to promoting efficient ubiquitin interaction, HRS has the ability to increase the local concentration of cargo in microdomains on sorting endosomes by capturing cargo in flat clathrin coats that are assembled as a result of HRS interacting with PtdIns3*P* and clathrin (Raiborg et al., 2002; Raiborg et al., 2001a; Raiborg et al., 2001b; Sachse et al., 2002). A prominent example of cargo that is ubiquitylated on multiple sites is EGFR, which becomes modified with Lys63-linked polyubiquitin chains and multiple monoubiquitin modification following ligand stimulation (Haglund et al., 2003; Huang et al., 2006; Mosesson et al., 2003). Interestingly, certain UBDs have been shown to have a considerably increased affinity for Lys63-linked polyubiquitin chains compared with that for monoubiquitin, and this selectivity might be important during EGFR sorting (Dikic et al., 2009; Ren and Hurley, 2010). The UBDs in the endocytic proteins described above all bind the hydrophobic patch of ubiquitin around Ile44, and could thus compete for the binding to ubiquitylated cargo (Dikic et al., 2009). The fact that the interactions are of low affinity might allow transfer from one UBD-containing protein to another. Thus, overall, sorting of ubiquitylated receptors along the endocytic pathway to target them for lysosomal degradation is coordinated by the action of an array of compartmentalized UBD-containing endocytic adaptor proteins.

Monoubiquitylation of endocytic proteins

Several UBD-containing proteins involved in endocytosis are themselves monoubiquitylated through a process that is dependent on their UBDs and is referred to as coupled monoubiquitylation (Fig. 2). Endocytic proteins known to undergo coupled monoubiquitylation include EPS15, EPS15R, epsin 1, epsin 2, HRS, STAM, RABGEF1 (also known as RABEX5; Vps9p in yeast) as well as STS1 and STS2 (also known as UBASH3B and UBASH3A) (Hoeller et al., 2006; Katz et al., 2002; Klapisz et al., 2002; Oldham et al., 2002; Polo et al., 2002; Urbe et al., 2003) and for many of them this process takes place following the stimulation of cells with growth factors (Di Fiore et al., 2003; van Delft et al., 1997). Curiously, UBD-containing proteins lose their ability to interact with ubiquitin once they become monoubiquitylated, and this has been suggested to result from intramolecular interactions between the UBD and the covalently attached monoubiquitin moiety on the same molecule (Fig. 2) (Hoeller et al., 2006). Interestingly, coupled monoubiquitylation of UBD-containing proteins can be mediated independently of an E3 ubiquitin ligase but in a manner that requires

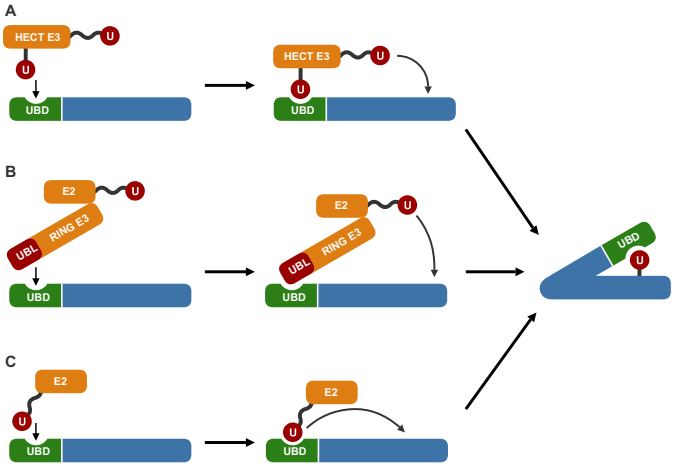

Fig. 2. Mechanisms of coupled monoubiquitylation of UBD-containing proteins. (**A**) A monoubiquitylated HECT-type E3 ubiquitin ligase (e.g. NEDD4) can interact with the UBD of a UBD-containing endocytic adaptor (e.g. EPS15). The HECT E3 ubiquitin ligase subsequently transfers the thiolester-conjugated ubiquitin molecule to a lysine residue in the UBD-containing protein, which thereby becomes monoubiquitylated. (**B**) Similarly, the ubiquitin-like (UBL) domain of a RING-type ubiquitin ligase (e.g. parkin) can interact with the UBD of a UBD-containing endocytic adaptor (e.g. EPS15). The RING E3 ubiquitin ligase then mediates monoubiquitylation of a lysine residue of the UBD-containing protein by transferring ubiquitin from an E2-conjugating enzyme to the substrate. (**C**) A ubiquitin-charged E2-conjugating enzyme can interact directly with the UBD of the endocytic adaptor, which leads to the direct transfer of ubiquitin from the E2 to a lysine residue in the substrate. Following coupled monoubiquitylation by one of these three mechanisms, the UBD of the endocytic adaptor can interact with the monoubiquitin attached to itself, leading to an intramolecular interaction that might alter the function and localization of the UBD-containing protein and possibly affect its ability to interact with ubiquitylated cargo during endosomal trafficking.

the UBDs of these proteins (Fig. 2) (Hoeller et al., 2007). Alternatively, the UBD can recruit a monoubiquitylated E3 ubiquitin ligase or interact with a ubiquitin-like domain within the E3 ligase, which in turn mediates monoubiquitylation of the UBD-containing protein, as has been shown for NEDD4- and Parkin-mediated monoubiquitylation of EPS15, respectively (Fig. 2) (Fallon et al., 2006; Woelk et al., 2006).

Functional consequences of coupled monoubiquitylation are now being elucidated, and numerous studies indicate that this modification can negatively regulate protein function during endosomal receptor trafficking (Fig. 2). For example, an EPS15–ubiquitin chimera, created by fusion of ubiquitin to the C-terminus of EPS15 to mimic its permanent monoubiquitylation, fails to localize properly to EGFR-positive endosomes and does not interact with ubiquitylated EGFRs, and thus does not promote efficient EGFR endocytosis and degradation (Fallon et al., 2006; Hoeller et al., 2006). Moreover, an HRS–ubiquitin chimera loses its ability to recognize and sort ubiquitylated cargo (Hoeller et al., 2006). A scenario that is functionally equivalent to the mechanism that inactivates HRS through monoubiquitylation was recently described for the FYVE-domain-containing negative regulator of EGFR signaling, ZFYVE28 (for zinc finger FYVE domain-containing 28, also known as LST2). Non-ubiquitylated ZFYVE28 localizes to PtdIns3*P*-positive endosomes through its FYVE domain and promotes degradative endosomal receptor sorting (Mosesson et al., 2009). However, monoubiquitylation of ZFYVE28 inhibits its endosomal localization

and thereby its ability to target EGFRs for lysosomal degradation (Mosesson et al., 2009). Similarly, the guanine exchange factor for RAB5, RABGEF1, which cooperates with RAB5 to control endosomal fusion and contains two UBDs, fails to localize to endosomes following its monoubiquitylation, which results in defective endosomal cargo trafficking (Mattera and Bonifacino, 2008). Another example is provided by the UBA-domain-containing inhibitor of RTK endocytosis, STS2. An STS2–ubiquitin chimera was found to be substantially less active than its wild-type counterpart in stabilizing activated EGFRs, whereas an STS2 mutant lacking the ubiquitin acceptor site was more active (Hoeller et al., 2006).

Taken together, these studies indicate that covalent attachment of monoubiquitin to UBD-containing endocytic regulators can have an autoinhibitory effect by influencing the localization and/or function of these proteins during receptor endocytosis and sorting. It has to be taken into account that many of these studies were performed with chimeric proteins and that these mechanisms might not apply to all monoubiquitylated proteins. The physiological consequences of coupled monoubiquitylation need to be further studied using mutant proteins lacking the ubiquitin acceptor sites. Nevertheless, it is possible that coupled monoubiquitylation represents a molecular mechanism that can promote the transfer of ubiquitylated cargo between different UBD-containing endocytic proteins during endosomal receptor trafficking along the endocytic pathway.

Biological importance of receptor ubiquitylation

During development and adult life of multicellular organisms, intercellular communication and signal transmission through cell surface receptors, including RTKs, are tightly controlled in order to provide fidelity in cellular, biological and physiological processes (Schlessinger, 2000; Yarden and Sliwkowski, 2001). A remarkable example of modulation of RTK signaling by endocytosis occurs during *Drosophila melanogaster* oogenesis, when a cluster of cells, called the border cells, undergoes directed migration that is dependent on signals by two major RTKs, the EGFR and the PDGF- and VEGF-receptor related (PVR) protein (Rorth, 2009). During this process, regulators of endocytosis, such as CBL (and its ubiquitin ligase activity) and RAB5, as well as regulators of recycling (e.g. RAB11) are involved in maintaining localized RTK signaling to promote guided cell migration. This emphasizes the importance of RTK endocytosis for fine-tuning receptor signals in vivo (Assaker et al., 2010; Jekely et al., 2005). Moreover, recent studies indicate that CBL controls endocytic sorting of FGF8 and thus controls morphogen gradient interpretation during embryonic development in zebrafish (Nowak et al., 2011). The importance of accurate RTK signaling is also highlighted by the fact that uncontrolled RTK signaling, caused by, for example, activating RTK mutations or deletions, RTK overexpression or autocrine growth factor loops, have been associated with a variety of diseases, such as developmental syndromes and cancer (Abella and Park, 2009; Lemmon and Schlessinger, 2010; Wesche et al., 2011; Witsch et al., 2010).

Accumulating evidence also suggests that defects in the machinery that controls negative regulation of RTKs by ubiquitylation and receptor endocytosis and endosomal sorting might contribute to constitutive RTK signaling (Abella and Park, 2009; Bache et al., 2004; Haglund et al., 2007; Mosesson et al., 2008). Mutations in components of this machinery have indeed been implicated in carcinogenesis. For example, mutations of either CBL-binding sites in RTKs or mutations of CBL that abolish its ubiquitin ligase activity, have been reported in many oncogenic forms of both RTKs and CBL, and these result in defective CBL-mediated receptor downregulation (Haglund et al., 2007; Peschard and Park, 2003). Moreover, it has been shown that interfering with regulators of endocytosis and endosomal sorting, including components of the ESCRT machinery, causes the accumulation of ubiquitylated receptors, increased receptor signaling and tumor overgrowth in *Drosophila melanogaster,* and mutations in components of the endocytic machinery have been detected in human tumors (Abella and Park, 2009; Bache et al., 2004; Haglund et al., 2007; Hariharan and Bilder, 2006; Mosesson et al., 2008; Vaccari and Bilder, 2009). Taken together, these findings highlight the importance of accurate ubiquitin-mediated degradative endosomal receptor sorting for maintaining of cellular homeostasis.

Conclusions and perspectives

Since the discovery of ubiquitin as a signal that governs endocytic receptor trafficking, ubiquitylation has emerged as a major post-translational modification that controls downregulation and degradative endosomal sorting of diverse types of receptors and transmembrane proteins. Among the multiple types of possible ubiquitin modifications, three major types have been shown to modify receptors: monoubiquitylation, multiple monoubiquitylation and Lys63-linked polyubiquitylation. A key challenge will be to elucidate the functional roles of the different types of ubiquitin modifications during receptor endocytosis and endosomal sorting, and to characterize the identity, contribution and function of ubiquitin modifications for different types of receptors and in response to specific cellular stimuli. In the case of EGFR, mass spectrometry has been crucial in revealing both the relative contribution, and to identity the different types, of its ubiquitin modifications in response to EGF stimulation (Huang et al., 2006), and further insight into the potential role of Lys63-linked ubiquitin chains in MVE sorting will be of great importance (Barriere et al., 2006; Lauwers et al., 2009; Paiva et al., 2009; Ren and Hurley, 2010).

During the last decade, different machineries that are responsible for ubiquitin-dependent receptor trafficking, including the ESCRT machinery and various UBD-containing endocytic adaptor proteins have been identified. Furthermore, functional and structural studies have given essential insight into the molecular mechanisms of ubiquitin-dependent membrane trafficking (Dikic et al., 2009; Hurley and Stenmark, 2011; Raiborg and Stenmark, 2009). However, in order to increase further the understanding of the specificity of these processes, structural studies in the context of full-length ubiquitylated cargo are needed. Moreover, increased insight into the dynamics of sorting of ubiquitylated cargo along the endocytic pathway is required. For example, we still do not fully understand how cargo can be transferred between different ubiquitin-binding proteins along the endocytic route, how multivalent interactions contribute to cargo sorting, to what extent monoubiquitylation of UBD-containing proteins participates in this process and how the emerging cross-talk between E3 ubiquitin ligases, DUBs and ubiquitin-binding proteins can affect receptor trafficking. As the field is currently developing at great speed, there is no doubt that exciting discoveries and novel insight into these and other fundamentally important issues concerning ubiquitin-dependent receptor trafficking will be gained in the near future.

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References

Abella, J. V. and Park, M. (2009). Breakdown of endocytosis in the oncogenic activation of receptor tyrosine kinases. *Am. J. Physiol. Endocrinol. Metab.* **296**, E973-E984.

Acconcia, F., Sigismund, S. and Polo, S. (2009). Ubiquitin in trafficking: the network at work. *Exp. Cell Res.* **315**, 1610-1618.

Akutsu, M., Kawasaki, M., Katoh, Y., Shiba, T., Yamaguchi, Y., Kato, R., Kato, K., Nakayama, K. and Wakatsuki, S. (2005). Structural basis for recognition of ubiquitinated cargo by Tom1-GAT domain. *FEBS Lett.* **579**, 5385-5391.

Alam, S. L., Sun, J., Payne, M., Welch, B. D., Blake, B. K., Davis, D. R., Meyer, H. H., Emr, S. D. and Sundquist, W. I. (2004). Ubiquitin interactions of NZF zinc fingers. *EMBO J.* **23**, 1411-1421.

Arevalo, J. C., Waite, J., Rajagopal, R., Beyna, M., Chen, Z. Y., Lee, F. S. and Chao, M. V. (2006). Cell survival through Trk neurotrophin receptors is differentially regulated by ubiquitination. *Neuron* **50**, 549-559.

Assaker, G., Ramel, D., Wculek, S. K., Gonzalez-Gaitan, M. and Emery, G. (2010). Spatial restriction of receptor tyrosine kinase activity through a polarized endocytic cycle controls border cell migration. *Proc. Natl. Acad. Sci. USA* **107**, 22558-22563.

Babst, M., Odorizzi, G., Estepa, E. J. and Emr, S. D. (2000). Mammalian tumor susceptibility gene 101 (TSG101) and the yeast homologue, Vps23p, both function in late endosomal trafficking. *Traffic* **1**, 248-258.

Bache, K. G., Raiborg, C., Mehlum, A. and Stenmark, H. (2003). STAM and Hrs are subunits of a multivalent ubiquitin-binding complex on early endosomes. *J. Biol. Chem.* **278**, 12513-12521.

Bache, K. G., Slagsvold, T. and Stenmark, H. (2004). Defective downregulation of receptor tyrosine kinases in cancer. *EMBO J.* **23**, 2707-2712.

Barriere, H., Nemes, C., Lechardeur, D., Khan-Mohammad, M., Fruh, K. and Lukacs, G. L. (2006). Molecular basis of oligoubiquitin-dependent internalization of membrane proteins in Mammalian cells. *Traffic* **7**, 282-297.

Benmerah, A., Lamaze, C., Begue, B., Schmid, S. L., Dautry-Varsat, A. and Cerf-Bensussan, N. (1998). AP-2/Eps15 interaction is required for receptor-mediated endocytosis. *J. Cell Biol.* **140**, 1055-1062.

Bergink, S. and Jentsch, S. (2009). Principles of ubiquitin and SUMO modifications in DNA repair. *Nature* **458**, 461-467.

Bezsonova, I., Bruce, M. C., Wiesner, S., Lin, H., Rotin, D. and Forman-Kay, J. D. (2008). Interactions between the three CIN85 SH3 domains and ubiquitin: implications for CIN85 ubiquitination. *Biochemistry* **47**, 8937-8949.

Bilodeau, P. S., Urbanowski, J. L., Winistorfer, S. C. and Piper, R. C. (2002). The Vps27p Hse1p complex binds ubiquitin and mediates endosomal protein sorting. *Nat. Cell Biol.* **4**, 534-539.

Bishop, N., Horman, A. and Woodman, P. (2002). Mammalian class E vps proteins recognize ubiquitin and act in the removal of endosomal protein-ubiquitin conjugates. *J. Cell Biol.* **157**, 91-101.

Burd, C. G. and Emr, S. D. (1998). Phosphatidylinositol(3)-phosphate signaling mediated by specific binding to RING FYVE domains. *Mol. Cell* **2**, 157-162.

Chen, H., Fre, S., Slepnev, V. I., Capua, M. R., Takei, K., Butler, M. H., Di Fiore, P. P. and De Camilli, P. (1998). Epsin is an EH-domain-binding protein implicated in clathrin-mediated endocytosis. *Nature* **394**, 793-797.

Chu, T., Sun, J., Saksena, S. and Emr, S. D. (2006). New component of ESCRT-I regulates endosomal sorting complex assembly. *J. Cell Biol.* **175**, 815-823.

Clague, M. J. and Urbe, S. (2006). Endocytosis: the DUB version. *Trends Cell Biol.* **16**, 551-559.

Davies, B. A., Topp, J. D., Sfeir, A. J., Katzmann, D. J., Carney, D. S., Tall, G. G., Friedberg, A. S., Deng, L., Chen, Z. and Horazdovsky, B. F. (2003). Vps9p CUE domain ubiquitin binding is required for efficient endocytic protein traffic. *J. Biol. Chem.* **278**, 19826-19833.

de Melker, A. A., van Der Horst, G., Calafat, J., Jansen, H. and Borst, J. (2001). c-Cbl ubiquitinates the EGF receptor at the plasma membrane and remains receptor associated throughout the endocytic route. *J. Cell Sci.* **114**, 2167-2178.

de Melker, A. A., van der Horst, G. and Borst, J. (2004). Ubiquitin ligase activity of c-Cbl guides the epidermal growth factor receptor into clathrin-coated pits by two distinct modes of Eps15 recruitment. *J. Biol. Chem.* **279**, 55465-55473.

Di Fiore, P. P., Polo, S. and Hofmann, K. (2003). When ubiquitin meets ubiquitin receptors: a signalling connection. *Nat. Rev. Mol. Cell Biol.* **4**, 491-497.

Dikic, I., Wakatsuki, S. and Walters, K. J. (2009). Ubiquitin-binding domains-from structures to functions. *Nat. Rev. Mol. Cell. Biol.* **10**, 659-671.

Doherty, G. J. and McMahon, H. T. (2009). Mechanisms of endocytosis. *Annu. Rev. Biochem.* **78**, 857-902.

Dores, M. R., Schnell, J. D., Maldonado-Baez, L., Wendland, B. and Hicke, L. (2010). The function of yeast epsin and Ede1 ubiquitin-binding domains during receptor internalization. *Traffic* **11**, 151-160.

Duval, M., Bedard-Goulet, S., Delisle, C. and Gratton, J. P. (2003). Vascular endothelial growth factor-dependent down-regulation of Flk-1/KDR involves Cbl-mediated ubiquitination. Consequences on nitric oxide production from endothelial cells. *J. Biol. Chem.* **278**, 20091-20097.

Fallon, L., Belanger, C. M., Corera, A. T., Kontogiannea, M., Regan-Klapisz, E., Moreau, F., Voortman, J., Haber, M., Rouleau, G., Thorarinsdottir, T. et al. (2006). A regulated interaction with the UIM protein Eps15 implicates parkin in EGF receptor trafficking and PI(3)K-Akt signalling. *Nat. Cell Biol.* **8**, 834-842.

Fasen, K., Cerretti, D. P. and Huynh-Do, U. (2008). Ligand binding induces Cbl-dependent EphB1 receptor degradation through the lysosomal pathway. *Traffic* **9**, 251-266.

Fisher, R. D., Wang, B., Alam, S. L., Higginson, D. S., Robinson, H., Sundquist, W. I. and Hill, C. P. (2003). Structure and ubiquitin binding of the ubiquitin interacting motif. *J. Biol. Chem.* **14**, 14.

Futter, C. E., Pearse, A., Hewlett, L. J. and Hopkins, C. R. (1996). Multivesicular endosomes containing internalized EGF-EGF receptor complexes mature and then fuse directly with lysosomes. *J. Cell Biol.* **132**, 1011-1023.

Galan, J. M. and Haguenauer-Tsapis, R. (1997). Ubiquitin lys63 is involved in ubiquitination of a yeast plasma membrane protein. *EMBO J.* **16**, 5847-5854.

Gaullier, J. M., Simonsen, A., D'Arrigo, A., Bremnes, B., Stenmark, H. and Aasland, R. (1998). FYVE fingers bind PtdIns(3)P. *Nature* **394**, 432-433.

Geetha, T. and Wooten, M. W. (2008). TrkA receptor endolysosomal degradation is both ubiquitin and proteasome dependent. *Traffic* **9**, 1146-1156.

Geetha, T., Jiang, J. and Wooten, M. W. (2005). Lysine 63 polyubiquitination of the nerve growth factor receptor TrkA directs internalization and signaling. *Mol. Cell* **20**, 301-312.

Gill, D. J., Teo, H., Sun, J., Perisic, O., Veprintsev, D. B., Emr, S. D. and Williams, R. L. (2007). Structural insight into the ESCRT-I/-II link and its role in MVB trafficking. *EMBO J.* **26**, 600-612.

Goh, L. K., Huang, F., Kim, W., Gygi, S. and Sorkin, A. (2010). Multiple mechanisms collectively regulate clathrin-mediated endocytosis of the epidermal growth factor receptor. *J. Cell Biol.* **189**, 871-883.

Grovdal, L. M., Stang, E., Sorkin, A. and Madshus, I. H. (2004). Direct interaction of Cbl with pTyr 1045 of the EGF receptor (EGFR) is required to sort the EGFR to lysosomes for degradation. *Exp. Cell Res.* **300**, 388-395.

Haglund, K. and Dikic, I. (2005). Ubiquitylation and cell signaling. *EMBO J.* **24**, 3353-3359.

Haglund, K., Shimokawa, N., Szymkiewicz, I. and Dikic, I. (2002). Cbl-directed monoubiquitination of CIN85 is involved in regulation of ligand-induced degradation of EGF receptors. *Proc. Natl. Acad. Sci. USA* **99**, 12191-12196.

Haglund, K., Sigismund, S., Polo, S., Szymkiewicz, I., Di Fiore, P. P. and Dikic, I. (2003). Multiple monoubiquitination of RTKs is sufficient for their endocytosis and degradation. *Nat. Cell Biol.* **5**, 461-466.

Haglund, K., Rusten, T. E. and Stenmark, H. (2007). Aberrant receptor signaling and trafficking as mechanisms in oncogenesis. *Crit. Rev. Oncog.* **13**, 1-34.

Hansen, C. G. and Nichols, B. J. (2009). Molecular mechanisms of clathrin-independent endocytosis. *J. Cell Sci.* **122**, 1713-1721.

Hanson, P. I., Roth, R., Lin, Y. and Heuser, J. E. (2008). Plasma membrane deformation by circular arrays of ESCRT-III protein filaments. *J. Cell Biol.* **180**, 389-402.

Hariharan, I. K. and Bilder, D. (2006). Regulation of imaginal disc growth by tumor-suppressor genes in Drosophila. *Annu. Rev. Genet.* **40**, 335-361.

Haugsten, E. M., Malecki, J., Bjorklund, S. M., Olsnes, S. and Wesche, J. (2008). Ubiquitination of fibroblast growth factor receptor 1 is required for its intracellular sorting but not for its endocytosis. *Mol. Biol. Cell* **19**, 3390-3403.

Hershko, A. and Ciechanover, A. (1998). The ubiquitin system. *Annu. Rev. Biochem.* **67**, 425-479.

Hicke, L. and Riezman, H. (1996). Ubiquitination of a yeast plasma membrane receptor signals its ligand- stimulated endocytosis. *Cell* **84**, 277-287.

Hirano, S., Kawasaki, M., Ura, H., Kato, R., Raiborg, C., Stenmark, H. and Wakatsuki, S. (2006). Double-sided ubiquitin binding of Hrs-UIM in endosomal protein sorting. *Nat. Struct. Mol. Biol.* **13**, 272-277.

Hislop, J. N. and von Zastrow, M. (2011). Role of ubiquitination in endocytic trafficking of G-protein-coupled receptors. *Traffic* **12**, 137-148.

Hoeller, D., Crosetto, N., Blagoev, B., Raiborg, C., Tikkanen, R., Wagner, S., Kowanetz, K., Breitling, R., Mann, M., Stenmark, H. et al. (2006). Regulation of ubiquitin-binding proteins by monoubiquitination. *Nat. Cell Biol.* **8**, 163-169.

Hoeller, D., Hecker, C. M., Wagner, S., Rogov, V., Dotsch, V. and Dikic, I. (2007). E3-independent monoubiquitination of ubiquitin-binding proteins. *Mol. Cell* **26**, 891-898.

Hong, Y. H., Ahn, H. C., Lim, J., Kim, H. M., Ji, H. Y., Lee, S., Kim, J. H., Park, E. Y., Song, H. K. and Lee, B. J. (2009). Identification of a novel ubiquitin binding site of STAM1 VHS domain by NMR spectroscopy. *FEBS Lett.* **583**, 287-292.

Huang, F. and Sorkin, A. (2005). Growth factor receptor binding protein 2-mediated recruitment of the RING domain of Cbl to the epidermal growth factor receptor is essential and sufficient to support receptor endocytosis. *Mol. Biol. Cell* **16**, 1268-1281.

Huang, F., Kirkpatrick, D., Jiang, X., Gygi, S. and Sorkin, A. (2006). Differential regulation of EGF receptor internalization and degradation by multiubiquitination within the kinase domain. *Mol. Cell* **21**, 737-748.

Huang, F., Goh, L. K. and Sorkin, A. (2007). EGF receptor ubiquitination is not necessary for its internalization. *Proc. Natl. Acad. Sci. USA* **104**, 16904-16909.

Hurley, J. H. (2010). The ESCRT complexes. *Crit. Rev. Biochem. Mol. Biol.* **45**, 463-487.

Hurley, J. H. and Hanson, P. I. (2010). Membrane budding and scission by the ESCRT machinery: it's all in the neck. *Nat. Rev. Mol. Cell Biol.* **11**, 556-566.

Hurley, J. H. and Stenmark, H. (2011). Molecular mechanisms of ubiquitin-dependent membrane traffic. *Annu. Rev. Biophys.* **40**, 119-142.

Ikeda, F. and Dikic, I. (2008). Atypical ubiquitin chains: new molecular signals. 'Protein modifications: beyond the usual suspects' review series. *EMBO Rep.* **9**, 536-542.

Im, Y. J. and Hurley, J. H. (2008). Integrated structural model and membrane targeting mechanism of the human ESCRT-II complex. *Dev. Cell* **14**, 902-913.

Im, Y. J., Wollert, T., Boura, E. and Hurley, J. H. (2009). Structure and function of the ESCRT-II-III interface in multivesicular body biogenesis. *Dev. Cell* **17**, 234-243.

Jekely, G., Sung, H. H., Luque, C. M. and Rorth, P. (2005). Regulators of endocytosis maintain localized receptor tyrosine kinase signaling in guided migration. *Dev. Cell* **9**, 197-207.

Jiang, X., Huang, F., Marusyk, A. and Sorkin, A. (2003). Grb2 regulates internalization of EGF receptors through clathrin-coated pits. *Mol. Biol. Cell* **14**, 858-870.

Joazeiro, C. A., Wing, S. S., Huang, H., Leverson, J. D., Hunter, T. and Liu, Y. C. (1999). The tyrosine kinase negative regulator c-Cbl as a RING-type, E2- dependent ubiquitin-protein ligase. *Science* **286**, 309-312.

Kanazawa, C., Morita, E., Yamada, M., Ishii, N., Miura, S., Asao, H., Yoshimori, T. and Sugamura, K. (2003). Effects of deficiencies of STAMs and Hrs, mammalian class E Vps proteins, on receptor downregulation. *Biochem. Biophys. Res. Commun.* **309**, 848-856.

Katoh, Y., Shiba, Y., Mitsuhashi, H., Yanagida, Y., Takatsu, H. and Nakayama, K. (2004). Tollip and Tom1 form a complex and recruit ubiquitin-conjugated proteins onto early endosomes. *J. Biol. Chem.* **279**, 24435-24443.

Katz, M., Shtiegman, K., Tal-Or, P., Yakir, L., Mosesson, Y., Harari, D., Machluf, Y., Asao, H., Jovin, T., Sugamura, K. et al. (2002). Ligand-independent degradation of epidermal growth factor receptor involves receptor ubiquitylation and Hgs, an adaptor whose ubiquitin- interacting motif targets ubiquitylation by Nedd4. *Traffic* **3**, 740-751.

Katzmann, D. J., Babst, M. and Emr, S. D. (2001). Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I. *Cell* **106**, 145-155.

Katzmann, D. J., Odorizzi, G. and Emr, S. D. (2002). Receptor downregulation and multivesicular-body sorting. *Nat. Rev. Mol. Cell Biol.* **3**, 893-905.

Katzmann, D. J., Stefan, C. J., Babst, M. and Emr, S. D. (2003). Vps27 recruits ESCRT machinery to endosomes during MVB sorting. *J. Cell Biol.* **162**, 413-423.

Kawasaki, M., Shiba, T., Shiba, Y., Yamaguchi, Y., Matsugaki, N., Igarashi, N., Suzuki, M., Kato, R., Kato, K., Nakayama, K. et al. (2005). Molecular mechanism of ubiquitin recognition by GGA3 GAT domain. *Genes Cells* **10**, 639-654.

Kazazic, M., Bertelsen, V., Pedersen, K. W., Vuong, T. T., Grandal, M. V., Rodland, M. S., Traub, L. M., Stang, E. and Madshus, I. H. (2009). Epsin 1 is involved in recruitment of ubiquitinated EGF receptors into clathrin-coated pits. *Traffic* **10**, 235-245.

Kjenseth, A., Fykerud, T., Rivedal, E. and Leithe, E. (2010). Regulation of gap junction intercellular communication by the ubiquitin system. *Cell Signal.* **22**, 1267-1273.

Klapisz, E., Sorokina, I., Lemeer, S., Pijnenburg, M., Verkleij, A. J. and van Bergen en Henegouwen, P. M. (2002). A ubiquitin-interacting motif (UIM) is essential for Eps15 and Eps15R ubiquitination. *J. Biol. Chem.* **277**, 30746-30753.

Kobayashi, S., Sawano, A., Nojima, Y., Shibuya, M. and Maru, Y. (2004). The c-Cbl/CD2AP complex regulates VEGF-induced endocytosis and degradation of Flt-1 (VEGFR-1). *FASEB J.* **18**, 929-931.

Kolling, R. and Hollenberg, C. P. (1994). The ABC-transporter Ste6 accumulates in the plasma membrane in a ubiquitinated form in endocytosis mutants. *EMBO J.* **13**, 3261-3271.

Komander, D., Clague, M. J. and Urbe, S. (2009). Breaking the chains: structure and function of the deubiquitinases. *Nat. Rev. Mol. Cell Biol.* **10**, 550-563.

Kostelansky, M. S., Schluter, C., Tam, Y. Y., Lee, S., Ghirlando, R., Beach, B., Conibear, E. and Hurley, J. H. (2007). Molecular architecture and functional model of the complete yeast ESCRT-I heterotetramer. *Cell* **129**, 485-498.

Kowanetz, K., Crosetto, N., Haglund, K., Schmidt, M. H., Heldin, C. H. and Dikic, I. (2004). Suppressors of T-cell receptor signaling Sts-1 and Sts-2 bind to Cbl and inhibit endocytosis of receptor tyrosine kinases. *J. Biol. Chem.* **279**, 32786-32795.

Lauwers, E., Jacob, C. and Andre, B. (2009). K63-linked ubiquitin chains as a specific signal for protein sorting into the multivesicular body pathway. *J. Cell Biol.* **185**, 493-502.

Le Bras, S., Loyer, N. and Le Borgne, R. (2011). The multiple facets of ubiquitination in the regulation of notch signaling pathway. *Traffic* **12**, 149-161.

Lee, P. S., Wang, Y., Dominguez, M. G., Yeung, Y. G., Murphy, M. A., Bowtell, D. D. and Stanley, E. R. (1999). The Cbl protooncoprotein stimulates CSF-1 receptor multiubiquitination and endocytosis, and attenuates macrophage proliferation. *EMBO J.* **18**, 3616-3628.

Lee, S., Tsai, Y. C., Mattera, R., Smith, W. J., Kostelansky, M. S., Weissman, A. M., Bonifacino, J. S. and Hurley, J. H. (2006). Structural basis for ubiquitin recognition and autoubiquitination by Rabex-5. *Nat. Struct. Mol. Biol.* **13**, 264-271.

Lemmon, M. A. and Schlessinger, J. (2010). Cell signaling by receptor tyrosine kinases. *Cell* **141**, 1117-1134.

Lennartsson, J., Wardega, P., Engstrom, U., Hellman, U. and Heldin, C. H. (2006). Alix facilitates the interaction between c-Cbl and platelet-derived growth factor beta-receptor and thereby modulates receptor down-regulation. *J. Biol. Chem.* **281**, 39152-39158.

Levkowitz, G., Waterman, H., Zamir, E., Kam, Z., Oved, S., Langdon, W. Y., Beguinot, L., Geiger, B. and Yarden, Y. (1998). c-Cbl/Sli-1 regulates endocytic sorting and ubiquitination of the epidermal growth factor receptor. *Genes Dev.* **12**, 3663-3674.

Levkowitz, G., Waterman, H., Ettenberg, S. A., Katz, M., Tsygankov, A. Y., Alroy, I., Lavi, S., Iwai, K., Reiss, Y., Ciechanover, A. et al. (1999). Ubiquitin ligase activity and tyrosine phosphorylation underlie suppression of growth factor signaling by c-Cbl/Sli-1. *Mol. Cell* **4**, 1029-1040.

Longva, K. E., Blystad, F. D., Stang, E., Larsen, A. M., Johannessen, L. E. and Madshus, I. H. (2002). Ubiquitination and proteasomal activity is required for transport of the EGF receptor to inner membranes of multivesicular bodies. *J. Cell Biol.* **156**, 843-854.

Lu, Q., Hope, L. W., Brasch, M., Reinhard, C. and Cohen, S. N. (2003). TSG101 interaction with HRS mediates endosomal trafficking and receptor down-regulation. *Proc. Natl. Acad. Sci. USA* **100**, 7626-7631.

Madshus, I. H. and Stang, E. (2009). Internalization and intracellular sorting of the EGF receptor: a model for understanding the mechanisms of receptor trafficking. *J. Cell Sci.* **122**, 3433-3439.

Masson, K., Heiss, E., Band, H. and Ronnstrand, L. (2006). Direct binding of Cbl to Tyr568 and Tyr936 of the stem cell factor receptor/c-Kit is required for ligand-induced ubiquitination, internalization and degradation. *Biochem. J.* **399**, 59-67.

Mattera, R. and Bonifacino, J. S. (2008). Ubiquitin binding and conjugation regulate the recruitment of Rabex-5 to early endosomes. *EMBO J.* **27**, 2484-2494.

Mayor, S. and Pagano, R. E. (2007). Pathways of clathrin-independent endocytosis. *Nat. Rev. Mol. Cell Biol.* **8**, 603-612.

Miaczynska, M. and Bar-Sagi, D. (2010). Signaling endosomes: seeing is believing. *Curr. Opin. Cell Biol.* **22**, 535-540.

Miller, S. L., Malotky, E. and O'Bryan, J. P. (2004). Analysis of the role of ubiquitin-interacting motifs in ubiquitin binding and ubiquitylation. *J. Biol. Chem.* **279**, 33528-33537.

Miranda, M. and Sorkin, A. (2007). Regulation of receptors and transporters by ubiquitination: new insights into surprisingly similar mechanisms. *Mol. Interv.* **7**, 157-167.

Miyake, S., Lupher, M. L., Jr, Druker, B. and Band, H. (1998). The tyrosine kinase regulator Cbl enhances the ubiquitination and degradation of the platelet-derived growth factor receptor alpha. *Proc. Natl. Acad. Sci. USA* **95**, 7927-7932.

Miyake, S., Mullane-Robinson, K. P., Lill, N. L., Douillard, P. and Band, H. (1999). Cbl-mediated negative regulation of platelet-derived growth factor receptor- proliferation. A critical role for Cbl tyrosine kinase-binding domain. *J. Biol. Chem.* **274**, 16619-16628.

Miyazawa, K., Toyama, K., Gotoh, A., Hendrie, P. C., Mantel, C. and Broxmeyer, H. E. (1994). Ligand-dependent polyubiquitination of c-kit gene product: a possible mechanism of receptor down modulation in M07e cells. *Blood* **83**, 137-145.

Mizuno, E., Kawahata, K., Kato, M., Kitamura, N. and Komada, M. (2003). STAM proteins bind ubiquitinated proteins on the early endosome via the VHS domain and ubiquitin-interacting motif. *Mol. Biol. Cell* **14**, 3675-3689.

Mori, S., Heldin, C. H. and Claesson-Welsh, L. (1992). Ligand-induced polyubiquitination of the platelet-derived growth factor beta-receptor. *J. Biol. Chem.* **267**, 6429-6434.

Mori, S., Heldin, C. H. and Claesson-Welsh, L. (1993). Ligand-induced ubiquitination of the platelet-derived growth factor beta-receptor plays a negative regulatory role in its mitogenic signaling. *J. Biol. Chem.* **268**, 577-583.

Mori, S., Claesson-Welsh, L., Okuyama, Y. and Saito, Y. (1995). Ligand-induced polyubiquitination of receptor tyrosine kinases. *Biochem. Biophys. Res. Commun.* **213**, 32-39.

Morita, E., Sandrin, V., Alam, S. L., Eckert, D. M., Gygi, S. P. and Sundquist, W. I. (2007). Identification of human MVB12 proteins as ESCRT-I subunits that function in HIV budding. *Cell Host Microbe* **2**, 41-53.

Mosesson, Y., Shtiegman, K., Katz, M., Zwang, Y., Vereb, G., Szollosi, J. and Yarden, Y. (2003). Endocytosis of receptor tyrosine kinases is driven by monoubiquitylation, not polyubiquitylation. *J. Biol. Chem.* **278**, 21323-21326.

Mosesson, Y., Mills, G. B. and Yarden, Y. (2008). Derailed endocytosis: an emerging feature of cancer. *Nat. Rev. Cancer* **8**, 835-850.

Mosesson, Y., Chetrit, D., Schley, L., Berghoff, J., Ziv, T., Carvalho, S., Milanezi, F., Admon, A., Schmitt, F., Ehrlich, M. et al. (2009). Monoubiquitinylation regulates endosomal localization of Lst2, a negative regulator of EGF receptor signaling. *Dev. Cell* **16**, 687-698.

Moustakas, A., Souchelnytskyi, S. and Heldin, C. H. (2001). Smad regulation in TGF-beta signal transduction. *J. Cell Sci.* **114**, 4359-4369.

Murdaca, J., Treins, C., Monthouel-Kartmann, M. N., Pontier-Bres, R., Kumar, S., Van Obberghen, E. and Giorgetti-Peraldi, S. (2004). Grb10 prevents Nedd4-mediated vascular endothelial growth factor receptor-2 degradation. *J. Biol. Chem.* **279**, 26754-26761.

Nakatsu, F., Sakuma, M., Matsuo, Y., Arase, H., Yamasaki, S., Nakamura, N., Saito, T. and Ohno, H. (2000). A di-leucine signal in the ubiquitin moiety. Possible involvement in ubiquitination-mediated endocytosis. *J. Biol. Chem.* **275**, 26213-26219.

Nowak, M., Machate, A., Yu, S. R., Gupta, M. and Brand, M. (2011). Interpretation of the FGF8 morphogen gradient is regulated by endocytic trafficking. *Nat. Cell Biol.* **13**, 153-158.

Oestreich, A. J., Davies, B. A., Payne, J. A. and Katzmann, D. J. (2007). Mvb12 is a novel member of ESCRT-I involved in cargo selection by the multivesicular body pathway. *Mol. Biol. Cell* **18**, 646-657.

Oldham, C. E., Mohney, R. P., Miller, S. L., Hanes, R. N. and O'Bryan, J. P. (2002). The ubiquitin-interacting motifs target the endocytic adaptor protein epsin for ubiquitination. *Curr. Biol.* **12**, 1112-1126.

Paiva, S., Vieira, N., Nondier, I., Haguenauer-Tsapis, R., Casal, M. and Urban-Grimal, D. (2009). Glucose-induced ubiquitylation and endocytosis of the yeast Jen1 transporter: role of lysine 63-linked ubiquitin chains. *J. Biol. Chem.* **284**, 19228-19236.

Penengo, L., Mapelli, M., Murachelli, A. G., Confalonieri, S., Magri, L., Musacchio, A., Di Fiore, P. P., Polo, S. and Schneider, T. R. (2006). Crystal structure of the ubiquitin binding domains of rabex-5 reveals two modes of interaction with ubiquitin. *Cell* **124**, 1183-1195.

Peschard, P. and Park, M. (2003). Escape from Cbl-mediated downregulation: a recurrent theme for oncogenic deregulation of receptor tyrosine kinases. *Cancer Cell* **3**, 519-523.

Peschard, P., Fournier, T. M., Lamorte, L., Naujokas, M. A., Band, H., Langdon, W. Y. and Park, M. (2001). Mutation of the c-Cbl TKB domain binding site on the Met receptor tyrosine kinase converts it into a transforming protein. *Mol. Cell* **8**, 995-1004.

Petrelli, A., Gilestro, G. F., Lanzardo, S., Comoglio, P. M., Migone, N. and Giordano, S. (2002). The endophilin-CIN85-Cbl complex mediates ligand-dependent downregulation of c-Met. *Nature* **416**, 187-190.

Pickart, C. M. and Eddins, M. J. (2004). Ubiquitin: structures, functions, mechanisms. *Biochim. Biophys. Acta* **1695**, 55-72.

Polo, S., Sigismund, S., Faretta, M., Guidi, M., Capua, M. R., Bossi, G., Chen, H., De Camilli, P. and Di Fiore, P. P. (2002). A single motif responsible for ubiquitin recognition and monoubiquitination in endocytic proteins. *Nature* **416**, 451-455.

Pornillos, O., Alam, S. L., Rich, R. L., Myszka, D. G., Davis, D. R. and Sundquist, W. I. (2002). Structure and functional interactions of the Tsg101 UEV domain. *EMBO J.* **21**, 2397-2406.

Prag, G., Misra, S., Jones, E. A., Ghirlando, R., Davies, B. A., Horazdovsky, B. F. and Hurley, J. H. (2003). Mechanism of ubiquitin recognition by the CUE domain of Vps9p. *Cell* **113**, 609-620.

Prag, G., Lee, S., Mattera, R., Arighi, C. N., Beach, B. M., Bonifacino, J. S. and Hurley, J. H. (2005). Structural mechanism for ubiquitinated-cargo recognition by the Golgi-localized, gamma-ear-containing, ADP-ribosylation-factor-binding proteins. *Proc. Natl. Acad. Sci. USA* **102**, 2334-2339.

Puertollano, R. and Bonifacino, J. S. (2004). Interactions of GGA3 with the ubiquitin sorting machinery. *Nat. Cell Biol.* **6**, 244-251.

Raguz, J., Wagner, S., Dikic, I. and Hoeller, D. (2007). Suppressor of T-cell receptor signalling 1 and 2 differentially regulate endocytosis and signalling of receptor tyrosine kinases. *FEBS Lett.* **581**, 4767-4772.

Raiborg, C. and Stenmark, H. (2009). The ESCRT machinery in endosomal sorting of ubiquitylated membrane proteins. *Nature* **458**, 445-452.

Raiborg, C., Bache, K. G., Mehlum, A., Stang, E. and Stenmark, H. (2001a). Hrs recruits clathrin to early endosomes. *EMBO J.* **20**, 5008-5021.

Raiborg, C., Bremnes, B., Mehlum, A., Gillooly, D. J., D'Arrigo, A., Stang, E. and Stenmark, H. (2001b). FYVE and coiled-coil domains determine the specific localisation of Hrs to early endosomes. *J. Cell Sci.* **114**, 2255-2263.

Raiborg, C., Bache, K. G., Gillooly, D. J., Madshus, I. H., Stang, E. and Stenmark, H. (2002). Hrs sorts ubiquitinated proteins into clathrin-coated microdomains of early endosomes. *Nat. Cell Biol.* **4**, 394-398.

Reddi, A. L., Ying, G., Duan, L., Chen, G., Dimri, M., Douillard, P., Druker, B. J., Naramura, M., Band, V. and Band, H. (2007). Binding of Cbl to a phospholipase Cgamma1-docking site on platelet-derived growth factor receptor beta provides a dual mechanism of negative regulation. *J. Biol. Chem.* **282**, 29336-29347.

Ren, X. and Hurley, J. H. (2010). VHS domains of ESCRT-0 cooperate in high-avidity binding to polyubiquitinated cargo. *EMBO J.* **29**, 1045-1054.

Rorth, P. (2009). Collective cell migration. *Annu. Rev. Cell Dev. Biol.* **25**, 407-429.

Roxrud, I., Raiborg, C., Pedersen, N. M., Stang, E. and Stenmark, H. (2008). An endosomally localized isoform of Eps15 interacts with Hrs to mediate degradation of epidermal growth factor receptor. *J. Cell Biol.* **180**, 1205-1218.

Sachse, M., Urbe, S., Oorschot, V., Strous, G. J. and Klumperman, J. (2002). Bilayered clathrin coats on endosomal vacuoles are involved in protein sorting toward lysosomes. *Mol. Biol. Cell* **13**, 1313-1328.

Schlessinger, J. (2000). Cell signaling by receptor tyrosine kinases. *Cell* **103**, 211-225.

Schmidt, M. H. and Dikic, I. (2005). The Cbl interactome and its functions. *Nat. Rev. Mol. Cell Biol.* **6**, 907-919.

Scita, G. and Di Fiore, P. P. (2010). The endocytic matrix. *Nature* **463**, 464-473.

Scott, R. P., Eketjall, S., Aineskog, H. and Ibanez, C. F. (2005). Distinct turnover of alternatively spliced isoforms of the RET kinase receptor mediated by differential recruitment of the Cbl ubiquitin ligase. *J. Biol. Chem.* **280**, 13442-13449.

Sehat, B., Andersson, S., Girnita, L. and Larsson, O. (2008). Identification of c-Cbl as a new ligase for insulin-like growth factor-I receptor with distinct roles from Mdm2 in receptor ubiquitination and endocytosis. *Cancer Res.* **68**, 5669-5677.

Shekhtman, A. and Cowburn, D. (2002). A ubiquitin-interacting motif from Hrs binds to and occludes the ubiquitin surface necessary for polyubiquitination in monoubiquitinated proteins. *Biochem. Biophys. Res. Commun.* **296**, 1222-1227.

Shiba, Y., Katoh, Y., Shiba, T., Yoshino, K., Takatsu, H., Kobayashi, H., Shin, H. W., Wakatsuki, S. and Nakayama, K. (2004). GAT (GGA and Tom1) domain responsible for ubiquitin binding and ubiquitination. *J. Biol. Chem.* **279**, 7105-7111.

Shields, S. B. and Piper, R. C. (2011). How ubiquitin functions with ESCRTs. *Traffic* **12**, 1306-1317.

Shields, S. B., Oestreich, A. J., Winistorfer, S., Nguyen, D., Payne, J. A., Katzmann, D. J. and Piper, R. (2009). ESCRT ubiquitin-binding domains function cooperatively during MVB cargo sorting. *J. Cell Biol.* **185**, 213-224.

Shih, S. C., Katzmann, D. J., Schnell, J. D., Sutanto, M., Emr, S. D. and Hicke, L. (2002). Epsins and Vps27p/Hrs contain ubiquitin-binding domains that function in receptor endocytosis. *Nat. Cell Biol.* **4**, 389-393.

Shih, S. C., Prag, G., Francis, S. A., Sutanto, M. A., Hurley, J. H. and Hicke, L. (2003). A ubiquitin-binding motif required for intramolecular monoubiquitylation, the CUE domain. *EMBO J.* **22**, 1273-1281.

Sigismund, S., Woelk, T., Puri, C., Maspero, E., Tacchetti, C., Transidico, P., Di Fiore, P. P. and Polo, S. (2005). Clathrin-independent endocytosis of ubiquitinated cargos. *Proc. Natl. Acad. Sci. USA* **102**, 2760-2765.

Sigismund, S., Argenzio, E., Tosoni, D., Cavallaro, E., Polo, S. and Di Fiore, P. P. (2008). Clathrin-mediated internalization is essential for sustained EGFR signaling but dispensable for degradation. *Dev. Cell* **15**, 209-219.

Slagsvold, T., Aasland, R., Hirano, S., Bache, K. G., Raiborg, C., Trambaiolo, D., Wakatsuki, S. and Stenmark, H. (2005). Eap45 in mammalian ESCRT-II binds ubiquitin via a phosphoinositide-interacting GLUE domain. *J. Biol. Chem.* **280**, 19600-19606.

Sorkin, A. and von Zastrow, M. (2009). Endocytosis and signalling: intertwining molecular networks. *Nat. Rev. Mol. Cell Biol.* **10**, 609-622.

Soubeyran, P., Kowanetz, K., Szymkiewicz, I., Langdon, W. Y. and Dikic, I. (2002). Cbl-CIN85-endophilin complex mediates ligand-induced downregulation of EGF receptors. *Nature* **416**, 183-187.

Stamenova, S. D., French, M. E., He, Y., Francis, S. A., Kramer, Z. B. and Hicke, L. (2007). Ubiquitin binds to and regulates a subset of SH3 domains. *Mol. Cell* **25**, 273-284.

Stang, E., Johannessen, L. E., Knardal, S. L. and Madshus, I. H. (2000). Polyubiquitination of the epidermal growth factor receptor occurs at the plasma membrane upon ligand-induced activation. *J. Biol. Chem.* **275**, 13940-13947.

Stang, E., Blystad, F. D., Kazazic, M., Bertelsen, V., Brodahl, T., Raiborg, C., Stenmark, H. and Madshus, I. H. (2004). Cbl-dependent ubiquitination is required for progression of EGF receptors into clathrin-coated pits. *Mol. Biol. Cell* **15**, 3591-3604.

Stefani, F., Zhang, L., Taylor, S., Donovan, J., Rollinson, S., Doyotte, A., Brownhill, K., Bennion, J., Pickering-Brown, S. and Woodman, P. (2011). UBAP1 is a component of an endosome-specific ESCRT-I complex that is essential for MVB sorting. *Curr. Biol.* **21**, 1245-1250.

Strous, G. J., van Kerkhof, P., Govers, R., Ciechanover, A. and Schwartz, A. L. (1996). The ubiquitin conjugation system is required for ligand-induced endocytosis and degradation of the growth hormone receptor. *EMBO J.* **15**, 3806-3812.

Stuchell, M. D., Garrus, J. E., Muller, B., Stray, K. M., Ghaffarian, S., McKinnon, R., Krausslich, H. G., Morham, S. G. and Sundquist, W. I. (2004). The human endosomal sorting complex required for transport (ESCRT-I) and its role in HIV-1 budding. *J. Biol. Chem.* **279**, 36059-36071.

Sun, J., Pedersen, M., Bengtsson, S. and Ronnstrand, L. (2007). Grb2 mediates negative regulation of stem cell factor receptor/c-Kit signaling by recruitment of Cbl. *Exp. Cell Res.* **313**, 3935-3942.

Sundquist, W. I., Schubert, H. L., Kelly, B. N., Hill, G. C., Holton, J. M. and Hill, C. P. (2004). Ubiquitin recognition by the human TSG101 protein. *Mol. Cell* **13**, 783-789.

Swanson, K. A., Kang, R. S., Stamenova, S. D., Hicke, L. and Radhakrishnan, I. (2003). Solution structure of Vps27 UIM-ubiquitin complex important for endosomal sorting and receptor downregulation. *EMBO J.* **22**, 4597-4606.

Swanson, K. A., Hicke, L. and Radhakrishnan, I. (2006). Structural basis for monoubiquitin recognition by the Ede1 UBA domain. *J. Mol. Biol.* **358**, 713-724.

Szymkiewicz, I., Kowanetz, K., Soubeyran, P., Dinarina, A., Lipkowitz, S. and Dikic, I. (2002). CIN85 participates in Cbl-b-mediated down-regulation of receptor tyrosine kinases. *J. Biol. Chem.* **277**, 39666-39672.

Takahashi, Y., Shimokawa, N., Esmaeili-Mahani, S., Morita, A., Masuda, H., Iwasaki, T., Tamura, J., Haglund, K. and Koibuchi, N. (2011). Ligand-induced downregulation of TrkA is partly regulated through ubiquitination by Cbl. *FEBS Lett.* **585**, 1741-1747.

Takayama, Y., May, P., Anderson, R. G. and Herz, J. (2005). Low density lipoprotein receptor-related protein 1 (LRP1) controls endocytosis and c-CBL-mediated ubiquitination of the platelet-derived growth factor receptor beta (PDGFR beta). *J. Biol. Chem.* **280**, 18504-18510.

Teo, H., Veprintsev, D. B. and Williams, R. L. (2004). Structural insights into endosomal sorting complex required for transport (ESCRT-I) recognition of ubiquitinated proteins. *J. Biol. Chem.* **279**, 28689-28696.

Thien, C. B. and Langdon, W. Y. (2001). Cbl: many adaptations to regulate protein tyrosine kinases. *Nat. Rev. Mol. Cell Biol.* **2**, 294-307.

Umebayashi, K., Stenmark, H. and Yoshimori, T. (2008). Ubc4/5 and c-Cbl continue to ubiquitinate EGF receptor after internalization to facilitate polyubiquitination and degradation. *Mol. Biol. Cell* **19**, 3454-3462.

Urbe, S., Sachse, M., Row, P. E., Preisinger, C., Barr, F. A., Strous, G., Klumperman, J. and Clague, M. J. (2003). The UIM domain of Hrs couples receptor sorting to vesicle formation. *J. Cell Sci.* **116**, 4169-4179.

Vaccari, T. and Bilder, D. (2009). At the crossroads of polarity, proliferation and apoptosis: the use of Drosophila to unravel the multifaceted role of endocytosis in tumor suppression. *Mol. Oncol.* **3**, 354-365.

van Delft, S., Govers, R., Strous, G. J., Verkleij, A. J. and van Bergen en Henegouwen, P. M. (1997). Epidermal growth factor induces ubiquitination of Eps15. *J. Biol. Chem.* **272**, 14013-14016.

Varshavsky, A. (1997). The ubiquitin system. *Trends Biochem. Sci.* **22**, 383-387.

Vecchione, A., Marchese, A., Henry, P., Rotin, D. and Morrione, A. (2003). The Grb10/Nedd4 complex regulates ligand-induced ubiquitination and stability of the insulin-like growth factor I receptor. *Mol. Cell. Biol.* **23**, 3363-3372.

Wang, Y., Yeung, Y. G. and Stanley, E. R. (1999). CSF-1 stimulated multiubiquitination of the CSF-1 receptor and of Cbl follows their tyrosine phosphorylation and association with other signaling proteins. *J. Cell Biochem.* **72**, 119-134.

Wesche, J., Haglund, K. and Haugsten, E. M. (2011). Fibroblast growth factors and their receptors in cancer. *Biochem. J.* **437**, 199-213.

Wilhelmsen, K., Burkhalter, S. and van der Geer, P. (2002) C-Cbl binds the CSF-1 receptor at tyrosine 973, a novel phosphorylation site in the receptor's carboxy-terminus. *Oncogene* **21**, 1079-1089.

Witsch, E., Sela, M. and Yarden, Y. (2010). Roles for growth factors in cancer progression. *Physiology (Bethesda)* **25**, 85-101.

Woelk, T., Oldrini, B., Marspero, E., Confalonieri, S., Cavallaro, E., di Fiore, P. and Polo, S. (2006). Molecular mechanisms of coupled monoubiquitination. *Nat. Cell Biol.* **8**, 1246-1254.

Wollert, T. and Hurley, J. H. (2010). Molecular mechanism of multivesicular body biogenesis by ESCRT complexes. *Nature* **464**, 864-869.

Wollert, T., Wunder, C., Lippincott-Schwartz, J. and Hurley, J. H. (2009). Membrane scission by the ESCRT-III complex. *Nature* **458**, 172-177.

Wong, A., Lamothe, B., Lee, A., Schlessinger, J. and Lax, I. (2002a). FRS2 alpha attenuates FGF receptor signaling by Grb2-mediated recruitment of the ubiquitin ligase Cbl. *Proc. Natl. Acad. Sci. USA* **99**, 6684-6689.

Wong, E. S., Fong, C. W., Lim, J., Yusoff, P., Low, B. C., Langdon, W. Y. and Guy, G. R. (2002b). Sprouty2 attenuates epidermal growth factor receptor ubiquitylation and endocytosis, and consequently enhances Ras/ERK signalling. *EMBO J.* **21**, 4796-4808.

Yamakami, M., Yoshimori, T. and Yokosawa, H. (2003). Tom1, a VHS domain-containing protein, interacts with tollip, ubiquitin, and clathrin. *J. Biol. Chem.* **278**, 52865-52872.

Yarden, Y. and Sliwkowski, M. X. (2001). Untangling the ErbB signalling network. *Nat. Rev. Mol. Cell Biol.* **2**, 127-137.

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