Endocytosis of Receptor Tyrosine Kinases

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Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

Endocytosis is the major regulator of signaling from receptor tyrosine kinases (RTKs). The canonical model of RTK endocytosis involves rapid internalization of an RTK activated by ligand binding at the cell surface and subsequent sorting of internalized ligand-RTK complexes to lysosomes for degradation. Activation of the intrinsic tyrosine kinase activity of RTKs results in autophosphorylation, which is mechanistically coupled to the recruitment of adaptor proteins and conjugation of ubiquitin to RTKs. Ubiquitination serves to mediate interactions of RTKs with sorting machineries both at the cell surface and on endosomes. The pathways and kinetics of RTK endocytic trafficking, molecular mechanisms underlying sorting processes, and examples of deviations from the standard trafficking itinerary in the RTK family are discussed in this work.

Functional activities of transmembrane proteins, including the large family of RTKs, are controlled by intracellular trafficking. RTKs are synthesized in the endoplasmic reticulum, transported to Golgi apparatus, and then delivered to the plasma membrane. At the cell surface RTKs undergo constitutive endocytosis (internalization) at a rate similar to that of other integral membrane proteins. Constitutive internalization of RTKs is much slower than their constitutive recycling from endosomes back to the cell surface. Therefore, RTKs are accumulated at the cell surface, which allows maximal accessibility to extracellular ligands. The rates of the constitutive internalization, recycling, and degradation determine the half-life of an RTK protein, which varies depending on the nature of the RTK and the cell type, and typically positively correlates with the expression level of the RTK. For example, the turnover rates range from $t_{1/2} < 1$ h for the colony stimulating factor 1 receptor (CSF-1R) in macrophages (Lee et al. 1999) to 24 h for the epidermal growth factor receptor (EGFR) overexpressed in carcinoma cells (Stoscheck and Carpenter 1984).

Activation of RTKs by growth factors elevates turnover and ultimately leads to downregulation of RTKs. First, at the cell surface, ligand binding increases the rate of RTK internalization by several folds (Fig. 1). Typically, surface RTKs can freely diffuse in the plane of the plasma membrane and, therefore, reach all endocytic sites. In some cases, however, a pool of RTKs is immobile owing to association with actin-based membrane protrusions (microvilli) or caveolae (Mineo et al. 1999; Foti et al. 2004; Hommelgaard et al. 2004). This pool is incapable of rapid internalization and retained at the

Editors: Joseph Schlessinger and Mark A. Lemmon
Additional Perspectives on Signaling by Receptor Tyrosine Kinases available at www.cshperspectives.org

 doi: 10.1101/cshperspect.a017459
Cite this article as *Cold Spring Harb Perspect Biol* 2013;5:a017459

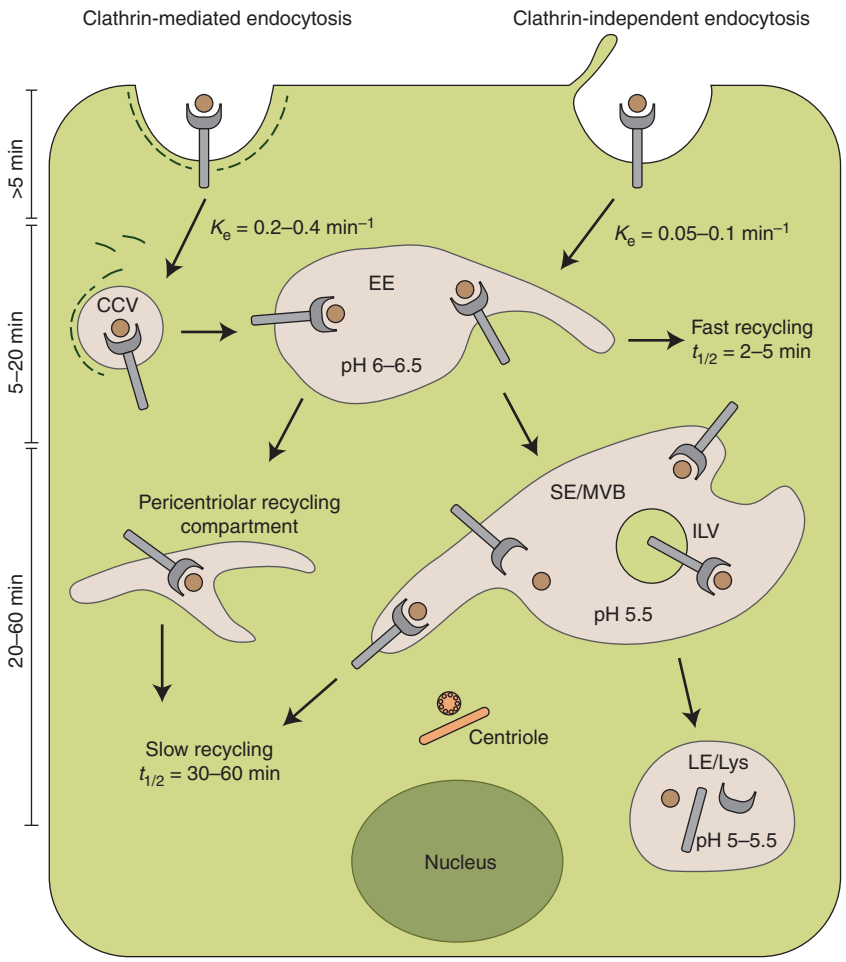

Figure 1. Pathways of RTK endocytosis. RTKs are endocytosed by clathrin-mediated and clathrin-independent mechanisms. Typical rate constants (K_e) for RTK internalization through both pathways are shown. Clathrin-coated vesicles are uncoated shortly after fission from the plasma membrane and fuse with early endosomes (EEs). In the most well-studied EGFR system, EGFR-ligand complexes are detected in these highly dynamic, morphologically heterologous compartments within 2–5 min after EGF stimulation (Haigler et al. 1979; Beguinot et al. 1984; Miller et al. 1986; Hopkins et al. 1990). Ligand-RTK complexes remain intact but certain ligands dissociate from the receptor in the acidic environment of the endosomal lumen. Released ligands remain in the vesicular parts of endosomes (most of the endosomal volume), whereas unoccupied receptors are found mainly in tubular extensions (most of the membrane area). Ligand-occupied and unoccupied RTKs can rapidly recycle from EEs through the process of back fusion of peripheral EEs with the plasma membrane or via tubular carriers derived from these endosomes (retroendocytosis). EEs mature into sorting endosomes (SE) or multivesicular bodies (MVBs) in which RTKs are incorporated into intraluminal vesicles (ILVs) by inward membrane invagination. RTKs can also be delivered to the pericentriolar Rab11-containing recycling compartment. Recycling of unoccupied and ligand-occupied RTKs is slower from the SE/MVBs and recycling compartment. SE/MVBs gradually lose early endosome components, such as Rab5 and EEA.1, and recycling cargo (such as transferrin receptors) while become enriched in resident late endosomal proteins (such as Rab7), thus maturing into late endosomes. Fusion of late endosomes with primary lysosomes carrying proteolytic enzymes results in degradation of receptors and growth factors. A typical time scale of RTK endocytosis, their accumulation in EEs, and SE/MVBs is shown in minutes.

cell surface. EGFR and its closest homolog, ErbB2, can also be retained in the plasma membrane through interactions with Na^+-K^+ exchanger and erbin, respectively (Borg et al. 2000; Lazar et al. 2004). Thus, interplay of surface retention and endocytosis-promoting mechanisms determines the extent of activation-induced acceleration of RTK internalization. The outcomes of this interplay vary within the RTK family. For instance, endocytosis of ErbB2 is not significantly increased when ErbB2 is activated by homo- or heterodimerization with other ErbBs (Yarden and Sliwkowski 2001). In contrast, some RTKs display a relatively fast constitutive internalization that is not appreciably augmented by ligand binding (McClain 1992; Burke and Wiley 1999; Jopling et al. 2011).

The second trafficking step causing down-regulation of activated RTKs is the efficient sorting of internalized RTKs to late endosomes and lysosomes for proteolytic degradation (Fig. 1). Members of all RTK subfamilies studied to date undergo accelerated lysosomal degradation on activation. Thus, activity-dependent acceleration of both sorting processes, at the cell surface and in endosomes, together resulting in RTK down-regulation, is a trademark of this receptor family. Significant advances have been made in recent years in understanding the molecular mechanisms of RTK endocytic trafficking. Yet, the specific components that mediate and regulate key internalization and sorting processes remain poorly defined. Because many conceptual findings have been made using the EGFR system and later reproduced in studies of other RTKs, EGFR has become the prototypic model system to study RTK endocytosis. Thus, we will rely on data derived in EGFR models for the broader discussion of other RTK families.

Endocytosis is the major regulator of signal transduction processes initiated by RTKs. Although the discussion of cross talk between endocytosis and signaling is outside of the scope of this article, we should emphasize that elucidation of the specific mechanisms of RTK endocytosis is essential for defining the role of endocytosis in regulation of RTK signaling. Hence our discussion will focus on various molecular mechanisms implicated in RTK endocytic trafficking. We regret that we are unable to cite many excellent studies on RTK endocytosis owing to the space constraints. We would also like to point out that a major limitation in the interpretation of RTK endocytosis literature is that methods used to analyze RTK internalization in the majority of studies fail to measure specific internalization rates because interference of receptor recycling is rarely considered. Therefore, changes in the apparent rates of RTK endocytosis observed in such studies could be owing to alterations in either internalization or recycling. We will discuss the literature in accordance to the authors' interpretation, but leave the detailed evaluation of each set of published data to reader discretion.

PATHWAYS OF RTK INTERNALIZATION

On ligand activation, increased localization of EGFR, neurotrophic tyrosine kinase receptor type 1 (TrkA), insulin receptor (IR), and a number of other RTKs within clathrin-coated pits (CCPs) has been observed (Gorden et al. 1978; Carpentier et al. 1982; Beattie et al. 2000; Bogdanovic et al. 2009). Further, internalization of EGFR and several other RTKs was blocked by small interfering RNAs (siRNAs) to clathrin heavy chain and chemical inhibitors of clathrin (Huang et al. 2004; Lampugnani et al. 2006; Zheng et al. 2008; von Kleist et al. 2011). Together, these studies show that clathrin-mediated endocytosis (CME) is the major pathway of internalization for ligand-occupied RTKs (Fig. 1).

CME is the fastest internalization pathway (rate constant $K_e \sim$ up to 0.6 min^{-1}). Kinetics analysis of EGFR endocytosis suggested that CME is saturated when a large number of surface EGFRs are activated by EGF, and the contribution of a slower clathrin-independent endocytosis (CIE) increases with the increase of EGF concentration and EGFR expression levels, leading to overall reduction in the apparent internalization rate (Wiley 1988). Indeed, siRNA depletion of clathrin significantly inhibited EGFR internalization only when low concentrations of EGF were used (Sigismund et al. 2005). Similar saturation of the rapid internalization

pathway and reduced rates of internalization were observed when IR and insulin-like growth factor 1 receptor (IGF-1R) were activated with high ligand concentrations (Backer et al. 1991; Prager et al. 1994). CIE has also been shown for fibroblast growth factor receptor (FGFR) and vascular endothelial growth factor receptor (VEGFR) (Wiedlocha and Sorensen 2004; Lanahan et al. 2010; Haugsten et al. 2011).

Mechanisms of CIE for various RTK can be roughly divided into two groups. The macropinocytosis-like endocytosis involving actin cytoskeleton rearrangements and membrane ruffling has been observed in early studies of epidermoid carcinoma A-431 cells that express very high levels of EGFR (Haigler et al. 1979), and later in other cell types (Barbieri et al. 2000; Yamazaki et al. 2002; Orth et al. 2006; Valdez et al. 2007). Another mechanism implicated in the CIE of RTKs is defined by its sensitivity to inhibitors of caveolae and cholesterol-disrupting agents (Sigismund et al. 2005; Sehat et al. 2008; Salani et al. 2010). However, the lack of experimental tools to inhibit specific CIE pathways impedes analysis of the mechanisms and functional role of CIE. Given the major role of CME in the presence of physiological concentrations of growth factors, we therefore focus our discussion on the molecular mechanisms of this pathway for RTK endocytosis.

ROLE OF TYROSINE-BASED AND DILEUCINE MOTIFS IN RTK ENDOCYTOSIS

Sequence motifs in the cytoplasmic domains of transmembrane proteins are recognized by components of the clathrin coat to trap the endocytic cargo in CCP. Several such motifs have been implicated in RTK internalization (Fig. 2). IR uses the dileucine D(E)*xxx*LL(I) ("LL") internalization motif (Haft et al. 1994; Morrison et al. 1996; Hamer et al. 1997), which directly binds to the trunk domain of the clathrin adaptor protein complex AP-2 (Fig. 3). The NP*x*Y motif (*x*, any amino acid residue) was shown to mediate internalization of IR, IGF-1R, and platelet-derived growth factor receptor (PDGFR) β (Chen et al. 1990; Backer et al. 1992; Prager et al. 1994; Wu et al. 2003a). NP*x*Y motifs interact with the phosphotyrosine-binding domains present in several monomeric adaptor proteins that, in turn, bind to the appendage domains of AP-2 and the amino-terminal domain of clathrin (Fig. 3) (Traub 2009).

Despite the importance of LL and NP*x*Y motifs for internalization of IR and IGF-1R, the interaction of these receptors with AP-2 has not been shown. Such interaction was shown only for EGFR (Sorkin and Carpenter 1993). The YRAL motif of EGFR is responsible for binding to the μ2 subunit of AP-2 (Sorkin et al. 1996). However, mutation of this motif or inactivation of the tyrosine-binding interface of μ2 did not affect EGFR internalization (Sorkin et al. 1996; Nesterov et al. 1999). The LL motif in EGFR is important for phosphorylation of Tyr6 in the β2 subunit of AP-2, suggesting the role of this LL motif in EGFR:AP-2 interaction (Huang et al. 2003). Interestingly, tyrosine phosphorylation of β2 Tyr6 was predicted to facilitate LL motif interaction with AP-2 (Kelly et al. 2008). However, mutation of this motif also did not affect clathrin-dependent EGFR internalization. Overall, the data on the role of internalization motifs and AP-2 in RTK internalization are incomplete, and the contribution of RTK:AP-2 interactions in ligand-induced RTK endocytosis remains to be defined.

MECHANISMS AND ROLE OF RTK UBIQUITINATION IN CME: CBL AND GRB2

Growth factor binding to RTKs leads to activation of the tyrosine kinase and autophosphorylation of tyrosine residues in the cytoplasmic domain of RTKs, which serve as interaction sites for proteins mediating RTK ubiquitination, a posttranslational modification by the covalent attachment of the ubiquitin polypeptide to lysine residues (Fig. 2). Ubiquitination has historically been considered as the modification that targets proteins for proteosomal degradation, but more recently the role of ubiquitin as the sorting signal in membrane trafficking has emerged (Acconcia et al. 2009). PDGFR-β and EGFR were the first RTKs found to be ubiquitinated (Mori et al. 1992; Galcheva-Gargova et al. 1995). Later, ubiquitination of all

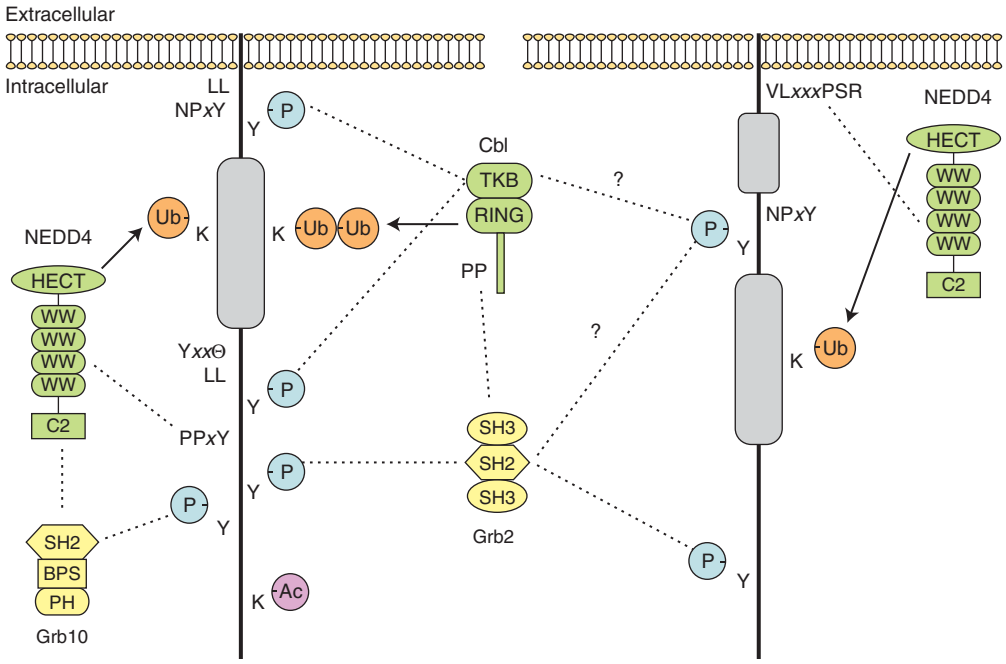

Figure 2. Sequence motifs and posttranslational modifications of RTKs involved in endocytosis. Examples of RTKs with a single kinase domain (EGFR, Met, IR/IGF-1R, Trk subtypes) and an insert-divided kinase domain (PDGFR, FGFR, kit, CSFR, VEGFR) are shown. Sequence motifs containing phosphorylated tyrosine residues (P-Y and Y-P) serve as binding sites for SH2 domains of Grb2, Grb10, and the tyrosine kinase binding (TKB) domain of c-Cbl, Cbl-b, and Cbl-3. Proline-rich motifs in Cbl-b and c-Cbl bind to SH3 domains in Grb2. WW domains of NEDD family E3 ligases bind to PP*x*Y motifs in RTKs, and in case of FGFR, to an unconventional motif in the juxtamembrane domain of this receptor. C2 domain of NEDD4-1 binds to the Grb10 SH2 domain that also binds to phosphotyrosine-containing motifs. Lysine residues are conjugated to ubiquitin in the kinase domains by Cbl, NEDD4, and other E3 ligases. Acetylation of distal lysines in the carboxyl terminus of EGFR is shown. Internalization motifs NP*x*Y, Y*xx*Θ (Θ, bulky hydrophobic residue), and LL are located in juxtamembrane domains, kinase insert, and carboxy-terminal tails of receptors.

major subtypes of RTKs has been shown; thus ubiquitination is a characteristic modification of this receptor family.

The leading model of RTK endocytosis presumes that ubiquitinated RTKs are recruited into CCP by interacting with epsin, Eps15, and Eps15R, proteins that contain ubiquitin-binding domains (UBDs) and bind to clathrin or AP-2 (Fig. 3). However, direct evidence to support this model is still lacking for most RTKs. Ubiquitin-conjugation sites were mapped in EGFR and IGF-1R, and mutational analysis has been performed to assess the importance of ubiquitination sites for the internalization of EGFR, IGF-1R, and FGFR-2 (Huang et al. 2006; Haugsten et al. 2008; Mao et al. 2011). These studies revealed that ubiquitination of EGFR and FGFR-2 is not essential for internalization. In contrast, mutation of lysines in the IGF-1R significantly decreased antibody-induced endosomal accumulation of this receptor (Mao et al. 2011).

The indirect evidence supporting the role of ubiquitination in RTK endocytosis is based on the effects of mutations or down-regulation of the components of the ubiquitination systems. Ubiquitination is mediated by the sequential action of the ubiquitin-activating (E1), ubiquitin-conjugating (E2), and ubiquitin ligase (E3) enzymes (Pickart 2004). E3 ligases determine the substrate specificity of ubiquitination. One large family of E3s that ubiquitinate RTKs is the RING finger containing ubiquitin ligases, such

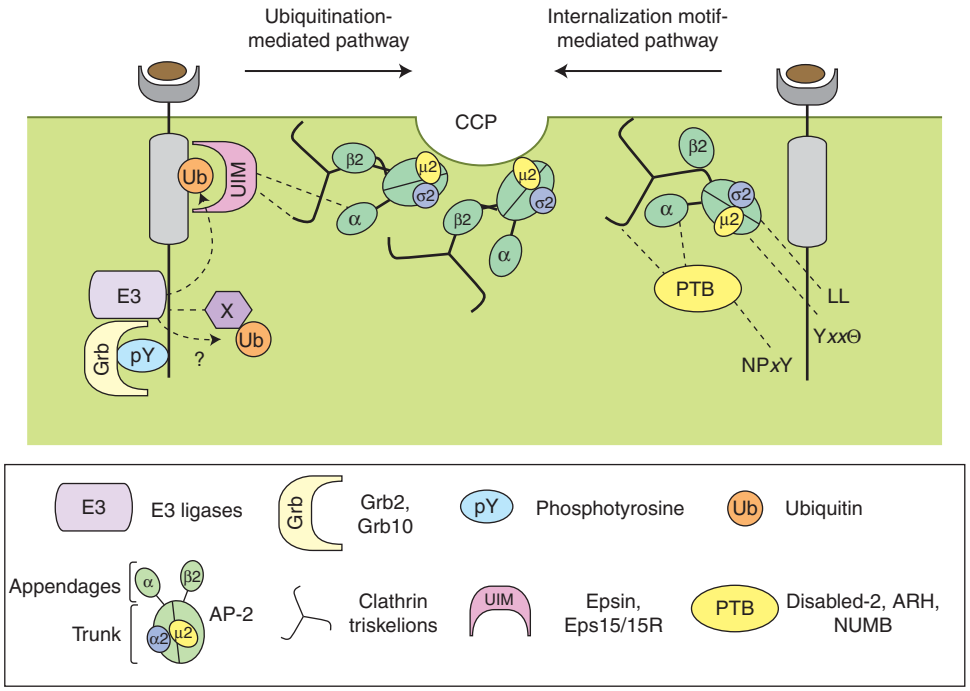

Figure 3. Hypothetic model of clathrin-mediated internalization of ligand-activated RTK. On ligand binding, RTKs are ubiquitinated by E3 ligases. Proteins, such as epsin, Eps15, and Eps15R, contain ubiquitin-interacting motifs (UIMs, a type of ubiquitin-binding domain [UBD]), and bind to ubiquitinated RTKs. UIM proteins bind to the appendage domains (mainly α) of AP-2, and epsin can also directly bind to the terminal domain of clathrin heavy chain (main components of clathrin triskelions). Interaction of RTKs with UIMs can occur before the recruitment of receptors into CCP and/or with UIMs present in assembled CCPs. The heterotetrameric AP-2 complex consists of four subunits: α, β2, μ2, and σ2. The trunk/core domain of AP-2 is formed by μ2, σ2, and the core domains of α and β2. Y*xx*Θ and LL motifs directly interact with the μ2 and σ2/β2 subunits of AP-2, respectively. NP*x*Y motifs interact with phosphotyrosine-binding domain (PTB) of adaptor proteins, such as Disabled-2, ARH, and NUMB, which themselves bind to the clathrin terminal domain and AP-2 appendage domains. The hinge region of β2 (between trunk and appendage) binds to clathrin heavy chain.

as Cbl. Cbl binds to and ubiquitinates EGFR, FGFR, PDGFR, VEGFR, CSF-1R, hepatocyte growth factor (c-Met), macrophage stimulating protein (Ron) and ephrin (EphR) receptors, as well as other RTKs (Levkowitz et al. 1999; Miyake et al. 1999; Wilhelmsen et al. 2002; Duval et al. 2003; Penengo et al. 2003; Marmor and Yarden 2004; Fasen et al. 2008). c-Cbl knockout, siRNA depletion of c-Cbl and Cbl-b, or overexpression of inactive Cbl mutants inhibit internalization of CSF-1R, EGFR, c-Kit, and several other RTKs (Lee et al. 1999; Levkowitz et al. 1999; Zeng et al. 2005; Huang et al. 2006). These studies implicate Cbl as a key mediator of RTK endocytosis. Besides Cbl, another RING family E3, Nrdp1, ubiquitinates an EGFR homolog ErbB3 in a ligand (neuregulin)-independent manner, but this ubiquitination is augmented by neuregulin stimulation through the increased stability of Nrdp1 (Cao et al. 2007).

Cbl is a multidomain protein capable of interacting with various regulatory proteins. Activity of Cbl and its turnover are regulated by a range of mechanisms involving interaction with Sprouty2 and p85Cool-1, ubiquitination by NEDD4-1, and phosphorylation by Src (Wong et al. 2002; Bao et al. 2003; Wu et al. 2003b; Marmor and Yarden 2004; Thien and Langdon 2005). Therefore, ubiquitination of RTK can be indirectly regulated by these proteins. All three

mammalian isoforms of Cbl (Cbl-b, Cbl-3, and c-Cbl) bind directly to specific phosphorylated tyrosines in RTKs, and long Cbl species (c-Cbl and Cbl-b) bind EGFR, c-Met, PDGFR-β, stem cell factor receptor (c-kit), and other RTKs indirectly through the SH2 adaptor protein Grb2 (Batzer et al. 1994; Peschard et al. 2001; Waterman et al. 2002; Jiang and Sorkin 2003; Li et al. 2007; Sun et al. 2007). The key role of Grb2 in ubiquitination and internalization of RTKs via Cbl has been shown using multiple experimental approaches in mammalian cells (Peschard 2001; Jiang 2003; Huang and Sorkin 2005; Johannessen 2006; Li 2007). In *Drosophila* and *Caenorhabditis elegans*, D-Cbl L binds to Grb2/Drk and Sli-1/Cbl binds to Grb2/SEM-5 to promote EGFR endocytosis (Yoon et al. 2000; Wang and Pai 2011). Finally, Grb2 and Cbl are colocalized with and remain bound to activated EGFR in CCPs (de Melker et al. 2001; Jiang et al. 2003; Johannessen et al. 2006). Collectively, these studies suggest that Grb2-Cbl complexes are required for the recruitment of RTKs into CCPs and, thereby, play an important role in receptor internalization.

The discrepancy between the requirement of Grb2 and Cbl for EGFR internalization and the lack of the role of EGFR ubiquitination sites might be explained by the existence of an unidentified receptor-associated protein that is (1) ubiquitinated by Cbl, and (2) mediates the internalization of the receptor (Fig. 3). It is important to note that the role of Grb2 and Cbl may be unrelated to ubiquitination. For instance, the SH2 domain of Grb2 binds to Tom1L protein that interacts with clathrin, thus linking EGFR to coated pits (Liu et al. 2009). It is unclear, however, how the SH2 domain of Grb2 binds simultaneously to both EGFR and Tom1L. Leucine repeat kinase Lrrk1 also binds to EGFR through Grb2 and promotes EGFR endocytosis (Hanafusa et al. 2011). On PDGF stimulation, Grb2 binds to PDGFR-β and the SH3 domain of Grb2 interacts with dynamin to facilitate internalization of the receptor (Kawada et al. 2009). A Cbl-interacting adaptor protein, CIN85, interacts with a plethora of partners through its five SH3 domains and is implicated in internalization of various RTKs (Petrelli et al. 2002; Soubeyran et al. 2002; Szymkiewicz et al. 2002; Kowanetz et al. 2003; Schmidt et al. 2004; Buchse et al. 2011). However, the effects of knockdown or knockout of CIN85 on clathrin-mediated RTK internalization and the localization of CIN85 in CCPs have not been shown. It can be concluded that although the key role of Cbl and Grb2 in RTK endocytosis is well established, the role of RTK ubiquitination in this process remains unclear, and ubiquitination-independent mechanisms involving Grb2 and Cbl are not fully supported by functional and localization data.

Role of NEDD4 Family and Other E3 Ligases

The second large family of E3s, the HECT domain-containing NEDD4 ligases, directly interact with substrate PP*x*Y motifs through their WW domains (Fig. 2) (Rotin and Kumar 2009). NEDD4-2 was shown to ubiquitinate TrkA receptor by binding to its carboxyl terminus (Arevalo et al. 2006). Another NEDD4 family member, AIP4/Itch, binds to the PP*x*Y motif in ErbB4 (Sundvall et al. 2008). NEDD4-1 binds to an unconventional sequence of FGFR1 and ubiquitinates this receptor (Persaud et al. 2011). siRNA depletion of NEDD4 family or overexpression of their catalytically inactive forms inhibited endocytosis of TrkA, ErbB4, and FGFR1 (Arevalo et al. 2006; Sundvall et al. 2008; Persaud et al. 2011). IGF-1R and IR ubiquitination involves an indirect binding of NEDD4-1 through the Grb10 adaptor (Fig. 2) (Vecchione et al. 2003; Monami et al. 2008). The SH2 domain of Grb10 is capable of simultaneous binding to the C2 domain of NEDD4-1 and IGF-1R (Huang and Szebenyi 2010). Expression of Grb10 and NEDD4-1 mutants significantly reduced endocytosis of IGF-1R (Vecchione et al. 2003; Monami et al. 2008). Genetic knockout of NEDD4-1 caused reduction of IGF-1R on the cell surface of mouse fibroblasts, suggesting that the role of NEDD4-1 in this system is more complex (Cao et al. 2008). The latter study proposed that NEDD4-1 regulates Grb10 levels. In the absence of NEDD4-1, Grb10 is up-regulated, which results in increased endocytosis of IGF-1R. Although NEDD4-1/Grb10 complex

appears to be involved in endocytosis of IR/IGF-1Rs, the localization of these proteins during early internalization events has not been determined.

The mechanisms by which IGF-1R is regulated by ubiquitination are convoluted because IGF-1R can also be ubiquitinated by the RING domain E3 ligase Mdm2, which is itself associated with IGF-1R through interaction with β-arrestins, and by c-Cbl (Girnita et al. 2005; Sehat et al. 2008). Indeed, siRNA knockdown of individual E3s implicated in IGF-1R ubiquitination did not affect IGF-1R endocytosis (Mao et al. 2011). The IGF-1R example of the potentially redundant role of multiple E3s in ubiquitination of a single RTK can be extended to EGFR (Cbl, Smurf2) (Levkowitz et al. 1999; Ray et al. 2011), ErbB4 (Itch, NEDD4, and WW1) (Sundvall et al. 2008; Li et al. 2009; Zeng et al. 2009), and TrkA (Cbl, NEDD4-2, and TRAF-6/UbcH7) (Geetha et al. 2005; Arevalo et al. 2006; Takahashi et al. 2011). Ubiquitination of a single RTK by different E3 ligases may result in different functional outcomes (Mao et al. 2011; Ray et al. 2011). Likely the contribution of different E3s is cell-type specific, depends on the experimental conditions, and can be indirect in some cases.

UBIQUITIN-BINDING PROTEINS IN CCPs

The model assuming that ubiquitin moieties target RTKs to CCPs implies that three UBD-containing proteins residing in CCPs (epsin-1/2, Eps15, and Eps15R) are involved in RTK internalization (Fig. 3). Binding of these proteins to ubiquitin moieties in vitro is well documented (Hawryluk et al. 2006), and coimmunoprecipitation of epsin-1 and Eps15 with activated EGFR has also been shown (Sigismund et al. 2005). There are, however, contrasting data regarding the importance of these proteins in EGFR internalization. siRNA knockdown of epsin-1 completely abolished EGF internalization in one study (Kazazic et al. 2009), whereas others report that individual or simultaneous depletion of epsin-1, Eps15, and Eps15R did not specifically block the CME of EGFR (Huang et al. 2004; Sigismund et al. 2005; Chen et al. 2009). Because epsin and Eps15/R contain multiple protein–protein interaction modules, overexpression of these proteins is not informative to their function (Sugiyama et al. 2005). Furthermore, epsin and Eps15/R may have distinct functions in cargo recruitment. Epsin is distributed throughout the entire CCP, whereas Eps15 is located at the edge of pits (Tebar et al. 1996; Kazazic et al. 2009). In general, direct interactions of epsins and Eps15/R with ubiquitinated RTKs are difficult to show, and the precise role of these three UBD-containing proteins in redundant or sequential recognition of ubiquitinated RTKs remains to be elucidated.

OTHER MECHANISMS OF RTK INTERNALIZATION

A tumor suppressor protein, RALT/Mig-6, was shown to promote EGFR endocytosis by a receptor-kinase-independent mechanism (Frosi et al. 2010). RALT/Mig6 binds to the EGFR kinase and inhibits its activity. RALT/Mig-6 is also capable of binding to AP-2 and intersectin, thus mediating internalization of EGFR through CCPs. Acetylation of three lysines at the carboxyl terminus of EGFR may also support internalization (Fig. 2). Mutation of these residues together with ubiquitination sites in the kinase domain of EGFR resulted in inefficient recruitment of EGFR into CCP, inability to phosphorylate the β2 subunit of AP-2, and significant inhibition of the EGFR CME (Goh et al. 2010). The precise role of EGFR acetylation in internalization is unknown, but could be related to the regulation of the receptor-kinase-substrate interactions. A unique Eph receptor system involves activation of Eph endocytosis by a membrane-anchored ligand (ephrin) (Pitulescu and Adams 2010). In neurons, endocytosis of ephrin-EphA4 receptor complex is mediated by the Rho family guanine exchange factor (GEF) Vav2 through an actin-dependent process (Cowan et al. 2005). Additionally, RIN1 (Ras binding and Rab5 GEF) is implicated in EphA4 endocytosis (Deininger et al. 2008). It is unclear, however, whether EphA4 endocytosis mediated by Vav2 or RIN1 is clathrin dependent.

POSTENDOCYTIC SORTING OF RTKs: MORPHOLOGY AND PATHWAYS

Endocytic carriers formed by CME and CIE deliver cargo to EEs located in the cell periphery. EEs have vesicular and tubular subcompartments; they can fuse to each other while moving along microtubules toward the centriole/Golgi area of the cell (Fig. 1). In the specialized and extreme example of neuronal cells, ligand-bound Trk receptors are internalized in the axonal terminal and transported for a long distance in EEs in a microtubule-dependent manner to the neuronal soma. The mechanisms and functions of the retrograde transport of endocytosed neurotrophin receptors were recently reviewed (Wu et al. 2009).

Movement toward the pericentriolar region is accompanied by the endosome maturation process that involves enlargement of the vesicular subcompartment, reduction in the amount of tubular extensions, and appearance of ILVs that are characteristic of MVBs. Changes in the biochemical composition of endosomes and an increasing luminal acidification also occur. Sorting of RTKs takes place in MVBs (Figs. 1 and 4). RTKs that are located in the limiting membrane of EEs and MVBs are capable of recycling back to the cell surface by moving to tubular extensions of these endosomes. RTKs that are packaged into ILVs are incapable of recycling and remain in MVBs that mature into late endosomes. Proteases and other lysosomal enzymes are delivered to MVB/late endosomes from the Golgi complex, leading to degradation of ILVs and RTKs.

MOLECULAR MECHANISMS OF RTK ENDOSOMAL SORTING

The current model of postendocytic sorting suggests that ubiquitinated RTK is recognized on the limiting membrane of a MVB by the heterodimeric complex of Hrs (hepatocyte growth factor regulated tyrosine-kinase substrate) and STAM1/2 (signal transducing adaptor molecule 1 and 2), which is the main component of ESCRT (endosomal sorting complexes required for transport)-0 (Fig. 4). UBDs in the Hrs and STAMs bind ubiquitinated RTKs and, possibly with the help of clathrin lattice associated with Hrs, retain RTKs from free diffusion in the limiting membrane and tubular extensions of MVBs, thus preventing RTK recycling. It has also been proposed that ubiquitinated EGFR interacts with GGA3, another UBD-containing adaptor, in EE before interacting with ESCRT-0 (Puertollano and Bonifacino 2004). Further, ESCRT-0 facilitates sequential recruitment of ESCRT-I, ESCRT-II, and ESCRT-III onto the MVB membrane. It is hypothesized that ubiquitinated cargo is passed along to ESCRT-I and -II, and ultimately packaged into ILV via a process of inward membrane invagination mediated by ESCRT-III (Henne et al. 2011).

The MVB sorting model is well supported by multiple lines of evidence. Degradation of EGFR, FGFR-2, and IGF-1R was highly sensitive to mutations of ubiquitin-conjugation and Cbl binding sites (Huang et al. 2006; Haugsten et al. 2008; Mao et al. 2011). Ubiquitin-deficient EGFR mutant was poorly incorporated into ILVs (Eden et al. 2011). The experimental manipulations with E3 ligases, their adaptors (Grb2 and Grb10), and other regulatory proteins cited above in the internalization section also have strong effects on the ligand-induced degradation of RTKs. For example, modulators of Cbl-mediated EGFR ubiquitination, such as Sprouty2, low-density lipoprotein receptor-related protein, Lrig1, and CIN85, also modulate the rate of lysosomal degradation of EGFR (Wong et al. 2002; Gur et al. 2004; Takayama et al. 2005; Dikic and Schmidt 2007; Ronning et al. 2011). Furthermore, the effects of overexpression of dominant–negative mutants of ESCRT components and their down-regulation by knockouts, knockdowns, and a microRNA on the lysosomal degradation of RTKs have been shown (Bache et al. 2003; Jekely and Rorth 2003; Bowers et al. 2006; Ewan et al. 2006; Wurdinger et al. 2008; Belleudi et al. 2009).

It should be emphasized that according to the ESCRT sorting model, RTK ubiquitination, and therefore, ligand binding, kinase activity, and Cbl association must be maintained until the formation of an ILV. To avoid the loss of ubiquitin, the cargo must also be deubiquiti-

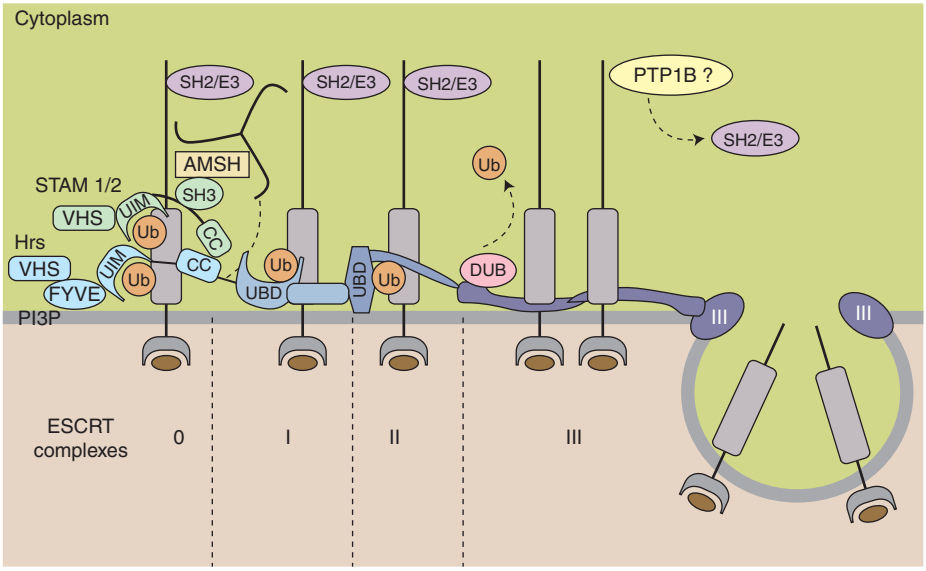

Figure 4. Hypothetic mechanism of RTK sorting into intraluminal vesicles of MVBs. Ubiquitinated RTK is recognized by UIMs of Hrs and STAM1/2 (ESCRT-0). Hrs is anchored to endosomal membrane through the interaction of its FYVE domain with phosphatidylinositol 3-phosphate (PI3P). Hrs also contains a coiled-coil domain that interacts with a similar domain of STAM, VHS domain, and a clathrin-binding motif. Binding of clathrin triskelions to Hrs nucleates an assembly of a flat clathrin lattice that further recruits additional Hrs molecules, leading to trapping of ubiquitinated cargo in the "Hrs microdomain." STAM contains the SH3 domain known to interact with AMSH. Accumulation of Hrs onto the endosomal membrane facilitates translocation of TSG101 and other components of ESCRT-I, and sequentially, components of ESCRT-II from cytosol to the MVB membrane. TSG101 and the ESCRT-II component EAP45/Vps36 have UBDs that may interact with ubiquitinated RTK. Ubiquitinated receptors appear to be transferred from ESCRT-0 to ESCRT-I and -II owing to increasing local concentrations of the latter complexes. ESCRT-III does not have ubiquitin-binding domains, and presumably traps receptors into forming ILVs by assembling into concentric hetero-oligomeric filaments and restricting diffusion of receptors. After formation of an ILV, ESCRT-III is disassembled by the Vps4 complex. The ESCRT model is reviewed by Teis et al. (2009). Before RTK entering the ILV, DUBs are proposed to remove ubiquitin from the receptor. Furthermore, receptor-associated proteins, such as SH2 adaptors and ubiquitin ligases, must also be removed before sequestration of receptors into ILV, possibly by means of receptor dephosphorylation by phosphotyrosine phosphatases like PTP1B (Eden et al. 2010). In addition, RTKs may facilitate ILV formation by phosphorylating annexin 1 (not shown) (White et al. 2006).

nated before entrance into ILVs. The requirement of such a tightly timed RTK deubiquitination for ILV incorporation, however, has not been directly shown. The deubiquitination enzyme (DUB) AMSH (associated molecule of SH3 domain of STAM) has been shown to associate with ESCRT-0 and also with the ESCRT-III component CHMP3 (Tanaka et al. 1999; Ma et al. 2007). AMSH directly deubiquitinates EGFR and PDGFR, and therefore decreases their degradation (McCullough et al. 2004, 2006; Bowers et al. 2006). Another DUB involved in cargo sorting is Usp8, which affects EGFR degradation by controlling the stability of STAM (Urbe et al. 2006) and Eps15 (Mizuno et al. 2006). However, there are contrasting effects of Usp8 knockdown on EGFR and c-Met degradation (Mizuno et al. 2005; Row et al. 2006). A conditional mouse knockout of Usp8 leads to decreased levels of EGFR, c-Met, and ErbB3 (Niendorf et al. 2007). Although it is clear that AMSH, Usp8, and possibly other DUBs play an important role in RTK sorting, further investigation is needed to distinguish between their function in deubiquitination of RTKs before ("sorting-negative" effect) or after

ubiquitin-dependent RTK-ESCRT interactions ("sorting-positive" effect) and in deubiquitination of ESCRT proteins themselves.

The importance of a specific type of ubiquitination during RTK sorting in MVBs is another unresolved issue. Proteins can be modified by attachment of a single ubiquitin to a single lysine residue (monoubiquitination) or several lysines (multimonoubiquitination). Lysines in the ubiquitin itself can further serve as substrates for ubiquitin conjugation resulting in the formation of polyubiquitin chains. Mass spectrometry studies revealed that EGFR is ubiquitinated by Lys63-linked chains (Huang et al. 2006). Similarly, TrkA was proposed to be either monoubiquitinated or Lys63-chain polyubiquitinated (Geetha et al. 2005; Arevalo et al. 2006). In contrast, IGF-1R was found to be Lys48- and Lys29-polyubiquitinated during antibody-driven down-regulation (Mao et al. 2011). Unlike Lys48-linked chains that target proteins to proteasome, Lys63-linked polyubiquitination and monoubiquitination have nonproteosomal functions (Adhikari and Chen 2009). Importantly, Lys63-linked ubiquitin chains and monoubiquitin are recognized by UBDs in a similar manner (Varadan et al. 2004). Therefore, multimonoubiquitination and Lys63-polyubiquitination provide a platform for simultaneous RTK interactions with multiple UBDs in ESCRTs and, therefore, increase the avidity of weak ubiquitin-UBD interactions and the efficiency of sorting into ILVs (Ren and Hurley 2010). Considering the ample amount of evidence supporting the role of Lys63 chains in degradation of various ubiquitinated cargo, it can be proposed that Lys63-linked polyubiquitination is the major molecular signal involved in the endocytic sorting of mammalian RTKs. However, this hypothesis and its applicability to different subtypes of RTKs require further experimentation.

Finally, it should be noted that other mechanisms that do not appear to be related to ubiquitination have been implicated in RTK endosomal sorting. The LL motif in the carboxyl terminus of EGFR is important for EGFR degradation (Huang et al. 2003). Binding of RALT/MIG-6 associated with EGFR to the endosomal SNARE Syntaxin 8 also promotes EGFR degradation (Ying et al. 2010). The precise mechanisms underlying these regulations are poorly understood, and whether ESCRTs are involved is not determined.

RTK RECYCLING

As internalized activated RTKs are efficiently targeted for lysosomal degradation, it is generally assumed that recycling of these receptors is minimal. This notion, together with technical difficulties in recycling rate measurements, translates into a scarcity of information regarding RTK recycling mechanisms. Considerable recycling of ligand-receptor complexes has been shown for IR (Huecksteadt et al. 1986), EGFR (Sorkin et al. 1991), and TrkA (Zapf-Colby and Olefsky 1998). EGF–EGFR complexes are rapidly recycled back to the cell surface from peripheral EE and with a slower kinetics from pericentriolar endosomes (Fig. 1) (Sorkin et al. 1991). The fast recycling pathway or "retroendocytosis" is mediated by constitutive formation of recycling carriers from peripheral EE, whereas a slower pathway involves a sorting step in MVBs and late recycling compartments. Studies of the kinetics of EGF–EGFR sorting showed that the degradation pathway is saturable, and therefore, the size of the recycling pool of EGF–EGFR complexes is proportional to the concentration of internalized EGF–EGFR complexes (French et al. 1994). For instance, in cells expressing high levels of EGFR and stimulated with high EGF concentrations, up to 80% of internalized EGF-receptor complexes were recycled (Sorkin et al. 1991). In addition, unoccupied EGFRs that release EGF in endosomes were recycled back to the plasma membrane (Masui et al. 1993). It is estimated that ligand-occupied receptors recycle with a slower rate than unoccupied EGFR (Resat et al. 2003). Furthermore, a pool of unoccupied, and therefore, recycled EGFR in endosomes is significantly larger when EGFR is activated by ligands like transforming growth factor α that displays a higher pH sensitivity of binding and dissociates from the receptor in endosomes, as compared with EGF that remains mostly receptor bound

(French et al. 1995; Roepstorff et al. 2009). Also, alterations that are inhibitory to the kinase activity and ubiquitination, such as threonine phosphorylation in the juxtamembrane domain of EGFR by protein kinase C (PKC) or mutations of ubiquitination sites, elevate recycling of EGFR (Bao et al. 2000; Eden et al. 2011). Likewise, activation of PKC was necessary for recycling of PDGFR-β (Hellberg et al. 2009).

The kinetic analysis of EGFR trafficking is in line with the model whereby recycling is the default pathway of the cargo that is not trapped by ESCRT-0 in MVBs. Therefore, RTKs recycle through the pathways and compartments common with nutrient carrier receptors like the transferrin receptor. These recycling pathways are regulated by Rab4 in peripheral EE and Rab11 (and homologous Rabs, such as Rab22) in pericentriolar endosomes (Grant and Donaldson 2009). Specific "recycling" sequence motifs described for other classes of receptors have not been found in RTKs (Hanyaloglu and von Zastrow 2007). Recently, however, GGA3 was shown to bind c-Met via the Crk adaptor and promote c-Met recycling, suggesting that specific "recycling targeting" mechanisms may exist (Parachoniak et al. 2011).

CONCLUDING REMARKS

An emerging common theme in the RTK endocytosis literature is that RTK internalization is a highly robust process resistant to singular perturbations (e.g., mutations or altered expression of components of the endocytosis machinery). Possible explanations behind the robustness of the endocytic process relate to the existence of clathrin-dependent and -independent mechanisms. Furthermore, CME can itself be regulated by several redundant mechanisms. In the example of EGFR, simultaneous mutation of AP-2 binding motifs, multiple ubiquitin-conjugation and acetylation sites in EGFR is necessary to substantially suppress this receptor internalization via the CME pathway (Goh et al. 2010). Similarly, participation of AP-2 binding motifs and ubiquitination has been implicated in internalization of other RTKs. Moreover, ubiquitination of a single RTK can be mediated by several E3 ligases. In contrast, lysosomal targeting is mediated by a single major mechanism—ubiquitination—and, therefore, this step of trafficking is less robust and highly sensitive to the regulation of the ubiquitination and deubiquitination systems. In light of the redundancy of endocytosis mechanisms in mammalian cell culture in vitro, it is important to gear future research toward studying the mammalian RTK endocytosis in vivo so that the mechanistic analysis of endocytic pathways that are physiological and highly relevant to human disease can be revealed.

ACKNOWLEDGMENTS

We thank Kalen Dionne for his critical reading of the manuscript. This work is supported by National Cancer Institute grants CA089151 and CA112219.

REFERENCES

Acconcia F, Sigismund S, Polo S. 2009. Ubiquitin in trafficking: The network at work. *Exp Cell Res* **315:** 1610–1618.

Adhikari A, Chen ZJ. 2009. Diversity of polyubiquitin chains. *Dev Cell* **16:** 485–486.

Arevalo JC, Waite J, Rajagopal R, Beyna M, Chen ZY, Lee FS, Chao MV. 2006. Cell survival through Trk neurotrophin receptors is differentially regulated by ubiquitination. *Neuron* **50:** 549–559.

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

Backer JM, Shoelson SE, Haring E, White MF. 1991. Insulin receptors internalize by a rapid, saturable pathway requiring receptor autophosphorylation and an intact juxtamembrane region. *J Cell Biol* **115:** 1535–1545.

Backer JM, Shoelson SE, Weiss MA, Hua QX, Cheatham RB, Haring E, Cahill DC, White MF. 1992. The insulin receptor juxtamembrane region contains two independent tyrosine/β-turn internalization signals. *J Cell Biol* **118:** 831–839.

Bao J, Alroy I, Waterman H, Schejter ED, Brodie C, Gruenberg J, Yarden Y. 2000. Threonine phosphorylation diverts internalized epidermal growth factor receptors from a degradative pathway to the recycling endosome. *J Biol Chem* **275:** 26178–26186.

Bao J, Gur G, Yarden Y. 2003. Src promotes destruction of c-Cbl: Implications for oncogenic synergy between Src and growth factor receptors. *Proc Natl Acad Sci* **100:** 2438–2443.

Barbieri MA, Roberts RL, Gumusboga A, Highfield H, Alvarez-Dominguez C, Wells A, Stahl PD. 2000. Epidermal

growth factor and membrane trafficking. EGF receptor activation of endocytosis requires Rab5a. *J Cell Biol* **151:** 539–550.

Batzer AG, Rotin D, Urena JM, Skolnik EY, Schlessinger J. 1994. Hierarchy of binding sites for Grb2 and Shc on the epidermal growth factor receptor. *Mol Cell Biol* **14:** 5192–5201.

Beattie EC, Howe CL, Wilde A, Brodsky FM, Mobley WC. 2000. NGF signals through TrkA to increase clathrin at the plasma membrane and enhance clathrin-mediated membrane trafficking. *J Neurosci* **20:** 7325–7333.

Beguinot L, Lyall RM, Willingham MC, Pastan I. 1984. Down-regulation of the epidermal growth factor receptor in KB cells is due to receptor internalization and subsequent degradation in lysosomes. *Proc Natl Acad Sci* **81:** 2384–2388.

Belleudi F, Leone L, Maggio M, Torrisi MR. 2009. Hrs regulates the endocytic sorting of the fibroblast growth factor receptor 2b. *Exp Cell Res* **315:** 2181–2191.

Bogdanovic E, Coombs N, Dumont DJ. 2009. Oligomerized Tie2 localizes to clathrin-coated pits in response to angiopoietin-1. *Histochem Cell Biol* **132:** 225–237.

Borg JP, Marchetto S, Le Bivic A, Ollendorff V, Jaulin-Bastard F, Saito H, Fournier E, Adelaide J, Margolis B, Birnbaum D. 2000. ERBIN: A basolateral PDZ protein that interacts with the mammalian ERBB2/HER2 receptor. *Nat Cell Biol* **2:** 407–414.

Bowers K, Piper SC, Edeling MA, Gray SR, Owen DJ, Lehner PJ, Luzio JP. 2006. Degradation of endocytosed epidermal growth factor and virally ubiquitinated major histocompatibility complex class I is independent of mammalian ESCRTII. *J Biol Chem* **281:** 5094–5105.

Buchse T, Horras N, Lenfert E, Krystal G, Korbel S, Schumann M, Krause E, Mikkat S, Tiedge M. 2011. CIN85 interacting proteins in B cells-specific role for SHIP-1. *Mol Cell Proteomics* **10:** M110.006239.

Burke PM, Wiley HS. 1999. Human mammary epithelial cells rapidly exchange empty EGFR between surface and intracellular pools. *J Cell Physiol* **180:** 448–460.

Cao Z, Wu X, Yen L, Sweeney C, Carraway KL 3rd. 2007. Neuregulin-induced ErbB3 downregulation is mediated by a protein stability cascade involving the E3 ubiquitin ligase Nrdp1. *Mol Cell Biol* **27:** 2180–2188.

Cao XR, Lill NL, Boase N, Shi PP, Croucher DR, Shan H, Qu J, Sweezer EM, Place T, Kirby PA, et al. 2008. Nedd4 controls animal growth by regulating IGF-1 signaling. *Sci Signal* **1:** ra5.

Carpentier JL, Gorden P, Anderson RG, Goldstein JL, Brown MS, Cohen S, Orci L. 1982. Co-localization of 125I-epidermal growth factor and ferritin-low density lipoprotein in coated pits: A quantitative electron microscopic study in normal and mutant human fibroblasts. *J Cell Biol* **95:** 73–77.

Chen WJ, Goldstein JL, Brown MS. 1990. NPXY, a sequence often found in cytoplasmic tails, is required for coated pit-mediated internalization of the low density lipoprotein receptor. *J Biol Chem* **265:** 3116–3123.

Chen H, Ko G, Zatti A, Di Giacomo G, Liu L, Raiteri E, Perucco E, Collesi C, Min W, Zeiss C, et al. 2009. Embryonic arrest at midgestation and disruption of Notch signaling produced by the absence of both epsin 1 and epsin 2 in mice. *Proc Natl Acad Sci* **106:** 13838–13843.

Cowan CW, Shao YR, Sahin M, Shamah SM, Lin MZ, Greer PL, Gao S, Griffith EC, Brugge JS, Greenberg ME. 2005. Vav family GEFs link activated Ephs to endocytosis and axon guidance. *Neuron* **46:** 205–217.

Deininger K, Eder M, Kramer ER, Zieglgansberger W, Dodt HU, Dornmair K, Colicelli J, Klein R. 2008. The Rab5 guanylate exchange factor Rin1 regulates endocytosis of the EphA4 receptor in mature excitatory neurons. *Proc Natl Acad Sci* **105:** 12539–12544.

de Melker AA, van der Horst G, Calafat J, Jansen H, 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.

Dikic I, Schmidt MH. 2007. Malfunctions within the Cbl interactome uncouple receptor tyrosine kinases from destructive transport. *Eur J Cell Biol* **86:** 505–512.

Duval M, Bedard-Goulet S, Delisle C, Gratton JP. 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.

Eden ER, White IJ, Tsapara A, Futter CE. 2010. Membrane contacts between endosomes and ER provide sites for PTP1B-epidermal growth factor receptor interaction. *Nat Cell Biol* **12:** 267–272.

Eden ER, Huang F, Sorkin A, Futter CE. 2011. The role of EGF receptor ubiquitination in regulating its intracellular traffic. *Traffic* **13:** 329–337.

Ewan LC, Jopling HM, Jia H, Mittar S, Bagherzadeh A, Howell GJ, Walker JH, Zachary IC, Ponnambalam S. 2006. Intrinsic tyrosine kinase activity is required for vascular endothelial growth factor receptor 2 ubiquitination, sorting and degradation in endothelial cells. *Traffic* **7:** 1270–1282.

Fasen K, Cerretti DP, Huynh-Do U. 2008. Ligand binding induces Cbl-dependent EphB1 receptor degradation through the lysosomal pathway. *Traffic* **9:** 251–266.

Foti M, Moukil MA, Dudognon P, Carpentier JL. 2004. Insulin and IGF-1 receptor trafficking and signalling. *Novartis Found Symp* **262:** 265–268.

French AR, Sudlow GP, Wiley HS, Lauffenburger DA. 1994. Postendocytic trafficking of epidermal growth factor-receptor complexes is mediated through saturable and specific endosomal interactions. *J Biol Chem* **269:** 15749–15755.

French AR, Tadaki DK, Niyogi SK, Lauffenburger DA. 1995. Intracellular trafficking of epidermal growth factor family ligands is directly influenced by the pH sensitivity of the receptor/ligand interaction. *J Biol Chem* **270:** 4334–4340.

Frosi Y, Anastasi S, Ballaro C, Varsano G, Castellani L, Maspero E, Polo S, Alema S, Segatto O. 2010. A two-tiered mechanism of EGFR inhibition by RALT/MIG6 via kinase suppression and receptor degradation. *J Cell Biol* **189:** 557–571.

Galcheva-Gargova Z, Theroux SJ, Davis RJ. 1995. The epidermal growth factor receptor is covalently linked to ubiquitin. *Oncogene* **11:** 2649–2655.

Geetha T, Jiang J, Wooten MW. 2005. Lysine 63 polyubiquitination of the nerve growth factor receptor TrkA directs internalization and signaling. *Mol Cell* **20:** 301–312.

Girnita L, Shenoy SK, Sehat B, Vasilcanu R, Girnita A, Lefkowitz RJ, Larsson O. 2005. β-Arrestin is crucial for ubiquitination and down-regulation of the insulin-like growth factor-1 receptor by acting as adaptor for the MDM2 E3 ligase. *J Biol Chem* **280:** 24412–24419.

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

Gorden P, Carpentier JL, Cohen S, Orci L. 1978. Epidermal growth factor: Morphological demonstration of binding, internalization, and lysosomal association in human fibroblasts. *Proc Natl Acad Sci* **75:** 5025–5029.

Grant BD, Donaldson JG. 2009. Pathways and mechanisms of endocytic recycling. *Nat Rev Mol Cell Biol* **10:** 597–608.

Gur G, Rubin C, Katz M, Amit I, Citri A, Nilsson J, Amariglio N, Henriksson R, Rechavi G, Hedman H, et al. 2004. LRIG1 restricts growth factor signaling by enhancing receptor ubiquitylation and degradation. *EMBO J* **23:** 3270–3281.

Haft CR, Klausner RD, Taylor SI. 1994. Involvement of dileucine motifs in the internalization and degradation of the insulin receptor. *J Biol Chem* **269:** 26286–26294.

Haigler HT, McKanna JA, Cohen S. 1979. Rapid stimulation of pinocytosis in human carcinoma cells A-431 by epidermal growth factor. *J Cell Biol* **83:** 82–90.

Hamer I, Haft CR, Paccaud JP, Maeder C, Taylor S, Carpentier JL. 1997. Dual role of a dileucine motif in insulin receptor endocytosis. *J Biol Chem* **272:** 21685–21691.

Hanafusa H, Ishikawa K, Kedashiro S, Saigo T, Iemura S, Natsume T, Komada M, Shibuya H, Nara A, Matsumoto K. 2011. Leucine-rich repeat kinase LRRK1 regulates endosomal trafficking of the EGF receptor. *Nat Commun* **2:** 158.

Hanyaloglu AC, von Zastrow M. 2007. A novel sorting sequence in the β2-adrenergic receptor switches recycling from default to the Hrs-dependent mechanism. *J Biol Chem* **282:** 3095–3104.

Haugsten EM, Malecki J, Bjorklund SM, Olsnes S, 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.

Haugsten EM, Zakrzewska M, Brech A, Pust S, Olsnes S, Sandvig K, Wesche J. 2011. Clathrin- and dynamin-independent endocytosis of FGFR3—Implications for signalling. *PloS ONE* **6:** e21708.

Hawryluk MJ, Keyel PA, Mishra SK, Watkins SC, Heuser JE, Traub LM. 2006. Epsin 1 is a polyubiquitin-selective clathrin-associated sorting protein. *Traffic* **7:** 262–281.

Hellberg C, Schmees C, Karlsson S, Ahgren A, Heldin CH. 2009. Activation of protein kinase C α is necessary for sorting the PDGF β-receptor to Rab4a-dependent recycling. *Mol Biol Cell* **20:** 2856–2863.

Henne WM, Buchkovich NJ, Emr SD. 2011. The ESCRT pathway. *Dev Cell* **21:** 77–91.

Hommelgaard AM, Lerdrup M, van Deurs B. 2004. Association with membrane protrusions makes ErbB2 an internalization-resistant receptor. *Mol Biol Cell* **15:** 1557–1567.

Hopkins CR, Gibson A, Shipman M, Miller K. 1990. Movement of internalized ligand-receptor complexes along a continuous endosomal reticulum. *Nature* **346:** 335–339.

Huang F, Sorkin A. 2005. Grb2-mediated recruitment of the Cbl RING domain to the EGF receptor is essential and sufficient to support receptor endocytosis. *Mol Biol Cell* **16:** 1268–1281.

Huang Q, Szebenyi DM. 2010. Structural basis for the interaction between the growth factor-binding protein GRB10 and the E3 ubiquitin ligase NEDD4. *J Biol Chem* **285:** 42130–42139.

Huang F, Jiang X, Sorkin A. 2003. Tyrosine phosphorylation of the β2 subunit of clathrin adaptor complex AP-2 reveals the role of a di-leucine motif in the epidermal growth factor receptor trafficking. *J Biol Chem* **278:** 43411–43417.

Huang F, Khvorova A, Marshall W, Sorkin A. 2004. Analysis of clathrin-mediated endocytosis of epidermal growth factor receptor by RNA interference. *J Biol Chem* **279:** 16657–16661.

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

Hueckstedt T, Olefsky JM, Brandenberg D, Heidenreich KA. 1986. Recycling of photoaffinity-labeled insulin receptors in rat adipocytes. Dissociation of insulin-receptor complexes is not required for receptor recycling. *J Biol Chem* **261:** 8655–8659.

Jekely G, Rorth P. 2003. Hrs mediates downregulation of multiple signalling receptors in *Drosophila*. *EMBO Rep* **4:** 1163–1168.

Jiang X, Sorkin A. 2003. Epidermal growth factor receptor internalization through clathrin-coated pits requires Cbl RING finger and proline-rich domains but not receptor polyubiquitylation. *Traffic* **4:** 529–543.

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

Johannessen LE, Pedersen NM, Pedersen KW, Madshus IH, Stang E. 2006. Activation of the epidermal growth factor (EGF) receptor induces formation of EGF receptor- and Grb2-containing clathrin-coated pits. *Mol Cell Biol* **26:** 389–401.

Jopling HM, Howell GJ, Gamper N, Ponnambalam S. 2011. The VEGFR2 receptor tyrosine kinase undergoes constitutive endosome-to-plasma membrane recycling. *Biochem Biophys Res Commun* **410:** 170–176.

Kawada K, Upadhyay G, Ferandon S, Janarthanan S, Hall M, Vilardaga JP, Yajnik V. 2009. Cell migration is regulated by platelet-derived growth factor receptor endocytosis. *Mol Cell Biol* **29:** 4508–4518.

Kazazic M, Bertelsen V, Pedersen KW, Vuong TT, Grandal MV, Rodland MS, Traub LM, Stang E, Madshus IH. 2009. Epsin 1 is involved in recruitment of ubiquitinated EGF receptors into clathrin-coated pits. *Traffic* **10:** 235–245.

Kelly BT, McCoy AJ, Spate K, Miller SE, Evans PR, Honing S, Owen DJ. 2008. A structural explanation for the binding of endocytic dileucine motifs by the AP2 complex. *Nature* **456:** 976–979.

Kowanetz K, Szymkiewicz I, Haglund K, Kowanetz M, Husnjak K, Taylor JD, Soubeyran P, Engstrom U, Ladbury JE, Dikic I. 2003. Identification of a novel proline-arginine motif involved in CIN85-dependent clustering of Cbl and down-regulation of epidermal growth factor receptors. *J Biol Chem* **278:** 39735–39746.

Lampugnani MG, Orsenigo F, Gagliani MC, Tacchetti C, Dejana E. 2006. Vascular endothelial cadherin controls VEGFR-2 internalization and signaling from intracellular compartments. *J Cell Biol* **174:** 593–604.

Lanahan AA, Hermans K, Claes F, Kerley-Hamilton JS, Zhuang ZW, Giordano FJ, Carmeliet P, Simons M. 2010. VEGF receptor 2 endocytic trafficking regulates arterial morphogenesis. *Dev Cell* **18:** 713–724.

Lazar CS, Cresson CM, Lauffenburger DA, Gill GN. 2004. The Na^+/H^+ exchanger regulatory factor stabilizes epidermal growth factor receptors at the cell surface. *Mol Biol Cell* **15:** 5470–5480.

Lee PS, Wang Y, Dominguez MG, Yeung YG, Murphy MA, Bowtell DD, Stanley ER. 1999. The Cbl protooncoprotein stimulates CSF-1 receptor multiubiquitination and endocytosis, and attenuates macrophage proliferation. *EMBO J* **18:** 3616–3628.

Levkowitz G, Waterman H, Ettenberg SA, Katz M, Tsygankov AY, 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.

Li N, Lorinczi M, Ireton K, Elferink LA. 2007. Specific Grb2-mediated interactions regulate clathrin-dependent endocytosis of the cMet-tyrosine kinase. *J Biol Chem* **282:** 16764–16775.

Li Y, Zhou Z, Alimandi M, Chen C. 2009. WW domain containing E3 ubiquitin protein ligase 1 targets the full-length ErbB4 for ubiquitin-mediated degradation in breast cancer. *Oncogene* **28:** 2948–2958.

Liu NS, Loo LS, Loh E, Seet LF, Hong W. 2009. Participation of Tom1L1 in EGF-stimulated endocytosis of EGF receptor. *EMBO J* **28:** 3485–3499.

Ma YM, Boucrot E, Villen J, Affar el B, Gygi SP, Gottlinger HG, Kirchhausen T. 2007. Targeting of AMSH to endosomes is required for epidermal growth factor receptor degradation. *J Biol Chem* **282:** 9805–9812.

Mao Y, Shang Y, Pham VC, Ernst JA, Lill JR, Scales SJ, Zha J. 2011. Poly-ubiquitination of the insulin-like growth factor I receptor (IGF-IR) activation loop promotes antibody-induced receptor internalization and down-regulation. *J Biol Chem* **286:** 41852–41861.

Marmor MD, Yarden Y. 2004. Role of protein ubiquitylation in regulating endocytosis of receptor tyrosine kinases. *Oncogene* **23:** 2057–2070.

Masui H, Castro L, Mendelsohn J. 1993. Consumption of EGF by A431 cells: Evidence for receptor recycling. *J Cell Biol* **120:** 85–93.

McClain DA. 1992. Mechanism and role of insulin receptor endocytosis. *Am J Med Sci* **304:** 192–201.

McCullough J, Clague MJ, Urbe S. 2004. AMSH is an endosome-associated ubiquitin isopeptidase. *J Cell Biol* **166:** 487–492.

McCullough J, Row PE, Lorenzo O, Doherty M, Beynon R, Clague MJ, Urbe S. 2006. Activation of the endosome-associated ubiquitin isopeptidase AMSH by STAM, a component of the multivesicular body-sorting machinery. *Curr Biol* **16:** 160–165.

Miller K, Beardmore J, Kanety H, Schlessinger J, Hopkins CR. 1986. Localization of the epidermal growth factor (EGF) receptor within the endosome of EGF-stimulated epidermoid carcinoma (A431) cells. *J Cell Biol* **102:** 500–509.

Mineo C, Gill GN, Anderson RG. 1999. Regulated migration of epidermal growth factor receptor from caveolae. *J Biol Chem* **274:** 30636–30643.

Miyake S, Mullane-Robinson KP, Lill NL, Douillard P, Band H. 1999. Cbl-mediated negative regulation of platelet-derived growth factor receptor-dependent cell proliferation. A critical role for Cbl tyrosine kinase-binding domain. *J Biol Chem* **274:** 16619–16628.

Mizuno E, Iura T, Mukai A, Yoshimori T, Kitamura N, Komada M. 2005. Regulation of epidermal growth factor receptor down-regulation by UBPY-mediated deubiquitination at endosomes. *Mol Biol Cell* **16:** 5163–5174.

Mizuno E, Kobayashi K, Yamamoto A, Kitamura N, Komada M. 2006. A deubiquitinating enzyme UBPY regulates the level of protein ubiquitination on endosomes. *Traffic* **7:** 1017–1031.

Monami G, Emiliozzi V, Morrione A. 2008. Grb10/Nedd4-mediated multiubiquitination of the insulin-like growth factor receptor regulates receptor internalization. *J Cell Physiol* **216:** 426–437.

Mori S, Heldin CH, Claesson-Welsh L. 1992. Ligand-induced polyubiquitination of the platelet-derived growth factor β-receptor. *J Biol Chem* **267:** 6429–6434.

Morrison P, Chung KC, Rosner MR. 1996. Mutation of Dileucine residues in the juxtamembrane region alters EGF receptor expression. *Biochemistry* **35:** 14618–14624.

Nesterov A, Carter RE, Sorkina T, Gill GN, Sorkin A. 1999. Inhibition of the receptor-binding function of clathrin adaptor protein AP-2 by dominant-negative mutant μ2 subunit and its effects on endocytosis. *EMBO J* **18:** 2489–2499.

Niendorf S, Oksche A, Kisser A, Lohler J, Prinz M, Schorle H, Feller S, Lewitzky M, Horak I, Knobeloch KP. 2007. Essential role of ubiquitin-specific protease 8 for receptor tyrosine kinase stability and endocytic trafficking in vivo. *Mol Cell Biol* **27:** 5029–5039.

Orth JD, Krueger EW, Weller SG, McNiven MA. 2006. A novel endocytic mechanism of epidermal growth factor receptor sequestration and internalization. *Cancer Res* **66:** 3603–3610.

Parachoniak CA, Luo Y, Abella JV, Keen JH, Park M. 2011. GGA3 functions as a switch to promote Met receptor recycling, essential for sustained ERK and cell migration. *Dev Cell* **20:** 751–763.

Penengo L, Rubin C, Yarden Y, Gaudino G. 2003. c-Cbl is a critical modulator of the Ron tyrosine kinase receptor. *Oncogene* **22:** 3669–3679.

Persaud A, Alberts P, Hayes M, Guettler S, Clarke I, Sicheri F, Dirks P, Ciruna B, Rotin D. 2011. Nedd4-1 binds and ubiquitylates activated FGFR1 to control its endocytosis and function. *EMBO J* **30:** 3259–3273.

Peschard P, Fournier TM, Lamorte L, Naujokas MA, Band H, Langdon WY, 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 GF, Lanzardo S, Comoglio PM, Migone N, Giordano S. 2002. The endophilin-CIN85-Cbl complex mediates ligand-dependent downregulation of c-Met. *Nature* **416:** 187–190.

Pickart CM. 2004. Back to the future with ubiquitin. *Cell* **116:** 181–190.

Pitulescu ME, Adams RH. 2010. Eph/ephrin molecules—A hub for signaling and endocytosis. *Genes Dev* **24:** 2480–2492.

Prager D, Li HL, Yamasaki H, Melmed S. 1994. Human insulin-like growth factor I receptor internalization. Role of the juxtamembrane domain. *J Biol Chem* **269:** 11934–11937.

Puertollano R, Bonifacino JS. 2004. Interactions of GGA3 with the ubiquitin sorting machinery. *Nat Cell Biol* **6:** 244–251.

Ray D, Ahsan A, Helman A, Chen G, Hegde A, Gurjar SR, Zhao L, Kiyokawa H, Beer DG, Lawrence TS, et al. 2011. Regulation of EGFR protein stability by the HECT-type ubiquitin ligase SMURF2. *Neoplasia* **13:** 570–578.

Ren X, Hurley JH. 2010. VHS domains of ESCRT-0 cooperate in high-avidity binding to polyubiquitinated cargo. *EMBO J* **29:** 1045–1054.

Resat H, Ewald JA, Dixon DA, Wiley HS. 2003. An integrated model of epidermal growth factor receptor trafficking and signal transduction. *Biophys J* **85:** 730–743.

Roepstorff K, Grandal MV, Henriksen L, Knudsen SL, Lerdrup M, Grovdal L, Willumsen BM, van Deurs B. 2009. Differential effects of EGFR ligands on endocytic sorting of the receptor. *Traffic* **10:** 1115–1127.

Ronning SB, Pedersen NM, Madshus IH, Stang E. 2011. CIN85 regulates ubiquitination and degradative endosomal sorting of the EGF receptor. *Exp Cell Res* **317:** 1804–1816.

Rotin D, Kumar S. 2009. Physiological functions of the HECT family of ubiquitin ligases. *Nat Rev Mol Cell Biol* **10:** 398–409.

Row PE, Prior IA, McCullough J, Clague MJ, Urbe S. 2006. The ubiquitin isopeptidase UBPY regulates endosomal ubiquitin dynamics and is essential for receptor down-regulation. *J Biol Chem* **281:** 12618–12624.

Salani B, Passalacqua M, Maffioli S, Briatore L, Hamoudane M, Contini P, Cordera R, Maggi D. 2010. IGF-IR internalizes with Caveolin-1 and PTRF/Cavin in HaCat cells. *PloS ONE* **5:** e14157.

Schmidt MH, Hoeller D, Yu J, Furnari FB, Cavenee WK, Dikic I, Bogler O. 2004. Alix/AIP1 antagonizes epidermal growth factor receptor downregulation by the Cbl-SETA/CIN85 complex. *Mol Cell Biol* **24:** 8981–8993.

Sehat B, Andersson S, Girnita L, 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.

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

Sorkin A, Carpenter G. 1993. Interaction of activated EGF receptors with coated pit adaptins. *Science* **261:** 612–615.

Sorkin A, Krolenko S, Kudrjavtceva N, Lazebnik J, Teslenko L, Soderquist AM, Nikolsky N. 1991. Recycling of epidermal growth factor-receptor complexes in A431 cells: Identification of dual pathways. *J Cell Biol* **112:** 55–63.

Sorkin A, Mazzotti M, Sorkina T, Scotto L, Beguinot L. 1996. Epidermal growth factor receptor interaction with clathrin adaptors is mediated by the Tyr974-containing internalization motif. *J Biol Chem* **271:** 13377–13384.

Soubeyran P, Kowanetz K, Szymkiewicz I, Langdon WY, Dikic I. 2002. Cbl-CIN85-endophilin complex mediates ligand-induced downregulation of EGF receptors. *Nature* **416:** 183–187.

Stoscheck CM, Carpenter G. 1984. Characterization of the metabolic turnover of epidermal growth factor receptor protein in A-431 cells. *J Cell Physiol* **120:** 296–302.

Sugiyama S, Kishida S, Chayama K, Koyama S, Kikuchi A. 2005. Ubiquitin-interacting motifs of Epsin are involved in the regulation of insulin-dependent endocytosis. *J Biochem* **137:** 355–364.

Sun J, Pedersen M, Bengtsson S, 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.

Sundvall M, Korhonen A, Paatero I, Gaudio E, Melino G, Croce CM, Aqeilan RI, Elenius K. 2008. Isoform-specific monoubiquitination, endocytosis, and degradation of alternatively spliced ErbB4 isoforms. *Proc Natl Acad Sci* **105:** 4162–4167.

Szymkiewicz I, Kowanetz K, Soubeyran P, Dinarina A, Lipkowitz S, 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, 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 RG, 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 β (PDGFR β). *J Biol Chem* **280:** 18504–18510.

Tanaka N, Kaneko K, Asao H, Kasai H, Endo Y, Fujita T, Takeshita T, Sugamura K. 1999. Possible involvement of a novel STAM-associated molecule "AMSH" in intracellular signal transduction mediated by cytokines. *J Biol Chem* **274:** 19129–19135.

Tebar F, Sorkina T, Sorkin A, Ericsson M, Kirchhausen T. 1996. Eps15 is a component of clathrin-coated pits and vesicles and is located at the rim of coated pits. *J Biol Chem* **271:** 28727–28730.

Teis D, Saksena S, Emr SD. 2009. SnapShot: The ESCRT machinery. *Cell* **137:** 182-182 e181.

Thien CB, Langdon WY. 2005. Negative regulation of PTK signalling by Cbl proteins. *Growth Factors* **23:** 161–167.

Traub LM. 2009. Tickets to ride: Selecting cargo for clathrin-regulated internalization. *Nat Rev Mol Cell Biol* **10:** 583–596.

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Urbe S, McCullough J, Row P, Prior IA, Welchman R, Clague MJ. 2006. Control of growth factor receptor dynamics by reversible ubiquitination. *Biochem Soc Trans* **34:** 754–756.

Valdez G, Philippidou P, Rosenbaum J, Akmentin W, Shao Y, Halegoua S. 2007. Trk-signaling endosomes are generated by Rac-dependent macroendocytosis. *Proc Natl Acad Sci* **104:** 12270–12275.

Varadan R, Assfalg M, Haririnia A, Raasi S, Pickart C, Fushman D. 2004. Solution conformation of Lys63-linked di-ubiquitin chain provides clues to functional diversity of polyubiquitin signaling. *J Biol Chem* **279:** 7055–7063.

Vecchione A, Marchese A, Henry P, Rotin D, 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.

von Kleist L, Stahlschmidt W, Bulut H, Gromova K, Puchkov D, Robertson MJ, MacGregor KA, Tomilin N, Pechstein A, Chau N, et al. 2011. Role of the clathrin terminal domain in regulating coated pit dynamics revealed by small molecule inhibition. *Cell* **146:** 471–484.

Wang PY, Pai LM. 2011. D-Cbl binding to Drk leads to dose-dependent down-regulation of EGFR signaling and increases receptor-ligand endocytosis. *PloS ONE* **6:** e17097.

Waterman H, Katz M, Rubin C, Shtiegman K, Lavi S, Elson A, Jovin T, Yarden Y. 2002. A mutant EGF-receptor defective in ubiquitylation and endocytosis unveils a role for Grb2 in negative signaling. *EMBO J* **21:** 303–313.

White IJ, Bailey LM, Aghakhani MR, Moss SE, Futter CE. 2006. EGF stimulates annexin 1-dependent inward vesiculation in a multivesicular endosome subpopulation. *EMBO J* **25:** 1–12.

Wiedlocha A, Sorensen V. 2004. Signaling, internalization, and intracellular activity of fibroblast growth factor. *Curr Top Microbiol Immunol* **286:** 45–79.

Wiley HS. 1988. Anomalous binding of epidermal growth factor to A431 cells is due to the effect of high receptor densities and a saturable endocytic system. *J Cell Biol* **107:** 801–810.

Wilhelmsen K, Burkhalter S, 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.

Wong ES, Fong CW, Lim J, Yusoff P, Low BC, Langdon WY, Guy GR. 2002. Sprouty2 attenuates epidermal growth factor receptor ubiquitylation and endocytosis, and consequently enhances Ras/ERK signalling. *EMBO J* **21:** 4796–4808.

Wu H, Windmiller DA, Wang L, Backer JM. 2003a. YXXM motifs in the PDGF-β receptor serve dual roles as phosphoinositide 3-kinase binding motifs and tyrosine-based endocytic sorting signals. *J Biol Chem* **278:** 40425–40428.

Wu WJ, Tu S, Cerione RA. 2003b. Activated Cdc42 sequesters c-Cbl and prevents EGF receptor degradation. *Cell* **114:** 715–725.

Wu C, Cui B, He L, Chen L, Mobley WC. 2009. The coming of age of axonal neurotrophin signaling endosomes. *J Proteomics* **72:** 46–55.

Wurdinger T, Tannous BA, Saydam O, Skog J, Grau S, Soutschek J, Weissleder R, Breakefield XO, Krichevsky AM. 2008. miR-296 regulates growth factor receptor overexpression in angiogenic endothelial cells. *Cancer Cell* **14:** 382–393.

Yamazaki T, Zaal K, Hailey D, Presley J, Lippincott-Schwartz J, Samelson LE. 2002. Role of Grb2 in EGF-stimulated EGFR internalization. *J Cell Sci* **115:** 1791–1802.

Yarden Y, Sliwkowski MX. 2001. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol* **2:** 127–137.

Ying H, Zheng H, Scott K, Wiedemeyer R, Yan H, Lim C, Huang J, Dhakal S, Ivanova E, Xiao Y, et al. 2010. Mig-6 controls EGFR trafficking and suppresses gliomagenesis. *Proc Natl Acad Sci* **107:** 6912–6917.

Yoon CH, Chang C, Hopper NA, Lesa GM, Sternberg PW. 2000. Requirements of multiple domains of SLI-1, a *Caenorhabditis elegans* homologue of c-Cbl, and an inhibitory tyrosine in LET-23 in regulating vulval differentiation. *Mol Biol Cell* **11:** 4019–4031.

Zapf-Colby A, Olefsky JM. 1998. Nerve growth factor processing and trafficking events following TrkA-mediated endocytosis. *Endocrinology* **139:** 3232–3240.

Zeng S, Xu Z, Lipkowitz S, Longley JB. 2005. Regulation of stem cell factor receptor signaling by Cbl family proteins (Cbl-b/c-Cbl). *Blood* **105:** 226–232.

Zeng F, Xu J, Harris RC. 2009. Nedd4 mediates ErbB4 JM-a/CYT-1 ICD ubiquitination and degradation in MDCK II cells. *FASEB J* **23:** 1935–1945.

Zheng J, Shen WH, Lu TJ, Zhou Y, Chen Q, Wang Z, Xiang T, Zhu YC, Zhang C, Duan S, et al. 2008. Clathrin-dependent endocytosis is required for TrkB-dependent Akt-mediated neuronal protection and dendritic growth. *J Biol Chem* **283:** 13280–13288.

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Cold Spring Harb Perspect Biol 2013; doi: 10.1101/cshperspect.a017459