



Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm



Review

Ubiquitination and endocytosis of the high affinity receptor for IgE

Rosa Molfetta, Francesca Gasparrini, Angela Santoni, Rossella Paolini *

Dept. of Experimental Medicine, Institute Pasteur-Fondazione Cenci Bolognetti, Sapienza University, 00161 Rome, Italy

ARTICLE INFO

Article history:

Received 4 May 2010
Received in revised form 31 May 2010
Accepted 9 June 2010
Available online xxx

Keywords:

Mast cells and basophils
FcεRI
Endocytosis
Ubiquitination
Lipid rafts

ABSTRACT

The high affinity receptor for IgE (FcεRI) is constitutively expressed on the surface of mast cells and basophils as a multimeric complex. Upon antigen ligation to FcεRI-bound IgE molecules, the receptor complex transduces intracellular signals leading to the release of preformed and newly synthesised inflammatory mediators.

FcεRI engagement also generates negative intracellular signals involving the coordinated action of adapters, phosphatases and ubiquitin ligases that limits the intensity and duration of positive signals. Relevant to this, antigen-induced FcεRI ubiquitination has become recognized as an important signal for the internalization and delivery of engaged receptor complexes to lysosomes for degradation.

In this article, we review recent advances in our understanding of molecular mechanisms that guarantee the clearance of antigen-stimulated FcεRI complexes from the cell surface.

A particular emphasis will be given on how lipid rafts and the ubiquitin pathway cooperate to ensure receptor internalization and sorting along the endocytic compartments.

A brief discussion regarding how ubiquitination regulates the endocytosis of Fc receptors other than FcεRI will be included.

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1. Introduction

Mast cells are the main effector cells in IgE-dependent inflammatory reactions. Indeed, upon aggregation of the high affinity receptor for IgE (FcεRI), they can produce, store, and release numerous mediators that can potentially affect the immune system and promote inflammation.

FcεRI is expressed on mast cells and basophils as a heterotetramer composed by an IgE-binding α subunit, a four transmembrane-spanning β subunit, and two identical disulphide-linked γ subunits (Metzger, 1992; Nadler et al., 2000). In rodents, all three subunits are needed for surface expression, whereas in humans a trimeric complex lacking the β-chain (FcεRIαγ₂) also exists, and is expressed not only on mast cells and basophils but also on antigen presenting cells.

Several observations obtained using a rat basophilic leukemia cell line, namely RBL-2H3, have contributed to elucidate the function of the different receptor subunits.

FcεRI α-chain contains two extracellular Ig-like domains involved in IgE binding, a transmembrane region with an aspartic acid residue, and a short cytoplasmic tail that lacks signal transduction motifs. The β and γ subunits have no role in ligand binding, but they share a conserved immunoreceptor tyrosine-based activation motif (ITAM) within their long cytoplasmic tails. Upon FcεRI aggregation, ITAMs are rapidly phosphorylated on tyrosines by the FcεRIβ-bound Src family kinase Lyn (Nadler et al., 2000; Kambayashi and Koretzky, 2007).

The phosphorylated γ-chains are competent to drive cell activation in the absence of a β-chain ITAM. However, FcεRIβ amplifies the intensity of signals from FcεRIγ (Lin et al., 1996), indicating that the two subunits can act cooperatively in promoting signal transduction.

The full activation of FcεRI requires receptor redistribution into lipid rafts, specialized regions of the plasma membrane enriched in cholesterol and glycosphingolipid that form ordered but dynamic structures floating in the less ordered surrounding membrane (Simons and Toomre, 2000). Upon FcεRI engagement, membrane rafts coalesce into larger and more stable structures where engaged receptors are concentrated (Field et al., 1997), and more easily interact with signaling molecules such as active Lyn (Sheets et al., 1999; Young et al., 2003).

Abbreviations: AP-2, adapter protein complex-2; CDE, clathrin-dependent endocytosis; CIE, clathrin-independent endocytosis; CIN85, Cbl-interacting protein of 85 kDa; FcεRI, high affinity receptor for IgE; ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibitory motif; PI3K, phosphatidylinositol-3-OH kinase; PLCγ, phospholipase Cγ; SLP-76, leukocyte protein of 76 kDa; TKB, tyrosine kinase binding domain; Ub, ubiquitin; UIM, ubiquitin interacting motif.

* Corresponding author at: Dept. of Experimental Medicine, Sapienza University, 00161 Rome, Italy. Tel.: +39 064468448.

E-mail address: rossella.paolini@uniroma1.it (R. Paolini).

Phosphorylated ITAM provides a docking site for the tandem pair of Src homology 2 (SH2) domains of the cytoplasmic kinase Syk that, in turn, is activated upon tyrosine phosphorylation.

The use of specific Syk inhibitors and Syk-negative cell lines demonstrated an obligatory role for this kinase in FcεRI-mediated signaling (Oliver et al., 1994; Costello et al., 1996; Zhang et al., 1996; Moriya et al., 1997).

In BRL-2H3 cells, a complementary pathway is initiated by Fyn, another protein tyrosine kinase of the Src family, and cooperates with the Lyn/Syk pathway in the propagation of FcεRI-mediated signal (Parravicini et al., 2002).

The functional consequences of FcεRI-mediated mast cell activation include the release of preformed mediators (e.g., histamine and β-hexosaminidase) and the *de novo* synthesis and secretion of eicosanoids, cytokines and chemokines (Galli, 2000; Kambayashi and Koretzky, 2007; Alvarez-Errico et al., 2009).

Mast cells and basophils express inhibitory receptors that counteract FcεRI-mediated responses. The extracellular domains of these receptors consist of either C-type lectin domains [e.g. mast cell-funtin-associated antigen (MAFA), now called killer cell lectin-like receptor G1 (KLRG1)] (Abramson et al., 2002) or Ig-like domains [e.g. FcγRIIB, gp49B1, paired Ig-like receptor B (PIR-B) and signal regulatory protein a (SIRPα)] (Bruhns et al., 2005; Katz, 2002). The common feature of these receptors is the presence in their cytoplasmic tail of an immunoreceptor tyrosine-based inhibitory motif (ITIM) that, once phosphorylated, recruits and activates protein and lipid phosphatases [i.e. SH2 domain-containing protein tyrosine phosphatase (SHP) and SH2 domain-containing inositol-polyphosphate 5-phosphatase (SHIP)] (Daëron et al., 2008). Thus, ITIM-bearing receptors suppress FcεRI-mediated signaling by promoting dephosphorylation events.

FcεRI engagement itself can promote a mixture of positive and negative intracellular signals whose integration determines the rate and the extent of functional mast cell and basophil responses (Molfetta et al., 2007). Concurrently, engaged receptors trigger their own endocytosis to extinguish signaling through removal of activated FcεRI complexes from the cell surface (Fattakhova et al., 2009; Molfetta et al., 2009).

Previous studies suggest that engaged receptors are mainly internalized via clathrin-mediated endocytosis (Wilson et al., 2000; Oliver et al., 2004). Accordingly, our group recently reported that scaffolding proteins regulating clathrin-mediated endocytosis are required for a rapid and efficient antigen-dependent FcεRI internalization (Molfetta et al., 2005). However, in the absence of a functional clathrin-dependent endocytic pathway, lipid rafts may alternatively drive receptor internalization (Fattakhova et al., 2006).

Despite the main endocytic route, recent evidence indicate that Fc receptor ubiquitination is required for efficient receptor entry and sorting in the endosomal compartments, where ubiquitinated receptors undergo degradation (Molfetta et al., 2009; Marois et al., 2009).

In this review, we provide insights into the endocytic pathways involved in antigen-induced FcεRI down-regulation with a particular focus on the role for the ubiquitin (Ub) system in the control of Fc receptor endocytic trafficking.

2. Endocytic routes of signaling receptors: clathrin-dependent versus lipid raft-dependent pathways

Multiple endocytic pathways mediate internalization of signaling receptors upon binding with their cognate ligands. All of them require the action of adapter proteins that recognize specific signals present within the cytoplasmic tails of the plasma membrane “cargo” proteins. Common receptor internalization signals are short

peptide motifs (Bonifacino and Traub, 2003; Sorkin, 2004). The best-characterized peptide motifs are the dileucine-based motifs and the tyrosine-based YXXPhi motif (Y represents tyrosine, X any amino acid, and Phi a bulky hydrophobic amino acid). For example, internalization of antigen-bound BCR requires tyrosine-based internalization signals present in the cytoplasmic tail of the mIg-associated Igα/Igβ heterodimer (Bonnerot et al., 1995). The FcR-associated γ chain also contains tyrosine-based internalization signals. Mutation of either of the two tyrosine residues in the γ-chain ITAM blocked both cell activation and efficient receptor internalization (Amigorena et al., 1992). Thus, it is likely that phosphorylation events promote signal recognition by components of the endocytic machinery.

More recently, ubiquitination has become recognized as a modification that generates signal for cargo internalization and transport along the endocytic machinery (Shih et al., 2000; Hicke and Dunn, 2003).

The first compartment deputed to receive incoming vesicles from plasma membrane are the early endosomes, also known as “sorting tubular endosomes”. Indeed, they receive cargo destined to recycle back to the plasma membrane or to be sorted along the endocytic machinery till the lysosomes that represent the terminal endocytic compartments responsible for cargo degradation.

Alternative fate of cargo (recycling versus degradation) may depend on which signal the cargo presents and/or which route the cargo utilizes to enter into the cell.

The best-studied cargo route is the clathrin-dependent endocytosis (CDE). This pathway requires the presence of the protein clathrin, which is the major component of the endocytic vesicle coat (Brodsky et al., 2001; Mousavi et al., 2004; Sorkin, 2004). Cargo-associated adapters recruit clathrin to the plasma membrane and promote its assembly into a polyhedral lattice leading to the formation of a clathrin-coated vesicle. The major clathrin adapter is the adapter protein complex-2 (AP-2). However, other adapters capable to bind to clathrin and to phospholipids may participate even in the absence of AP-2.

In addition to CDE, multiple clathrin-independent endocytosis (CIE) pathways have been described (Mayor and Pagano, 2007). They generally depend on lipid rafts. Currently, endocytosis is considered lipid raft-dependent if receptor uptake is sensitive to cholesterol depletion and the internalized receptors colocalize with lipid raft components including flotillins and GM1 gangliosides. Lipid rafts can also contain the protein caveolin-1 giving rise to specialized membrane invagination called caveolae (Mayor and Pagano, 2007). Although the characteristic caveolae-like structures are absent in lymphocytes (Fra et al., 1994), they have been detected in the microvilli and intracellular vesicles of cultured mouse bone marrow-derived mast cells (Shin et al., 2000). Furthermore, the expression of caveolin-1 has been recently reported in macrophages, mast cells and B cells (Kiss et al., 2000; McFarland et al., 2004; Medina et al., 2006).

Both CDE and lipid raft-dependent pathways require rearrangement of the actin cytoskeleton and the activity of the GTPase dynamin that provides the contractive force to mediate the pinch-off of the vesicles from the plasma membrane toward cytosol (Mousavi et al., 2004; Mayor and Pagano, 2007). CDE and lipid raft-dependent pathways are schematically illustrated (Fig. 1).

Several dynamin-independent endocytic pathways have been described (Mayor and Pagano, 2007). However, their contribution in receptor internalization has not been completely understood.

Growth factor receptors and immune receptors may use either CDE or CIE pathways (Di Guglielmo et al., 2003; Monjas et al., 2004; Stoddart et al., 2005; Sigismund et al., 2008). In the case of immune receptors, TCR down-modulation involves both clathrin-dependent and lipid raft-dependent endocytic routes that act differentially on directly engaged and

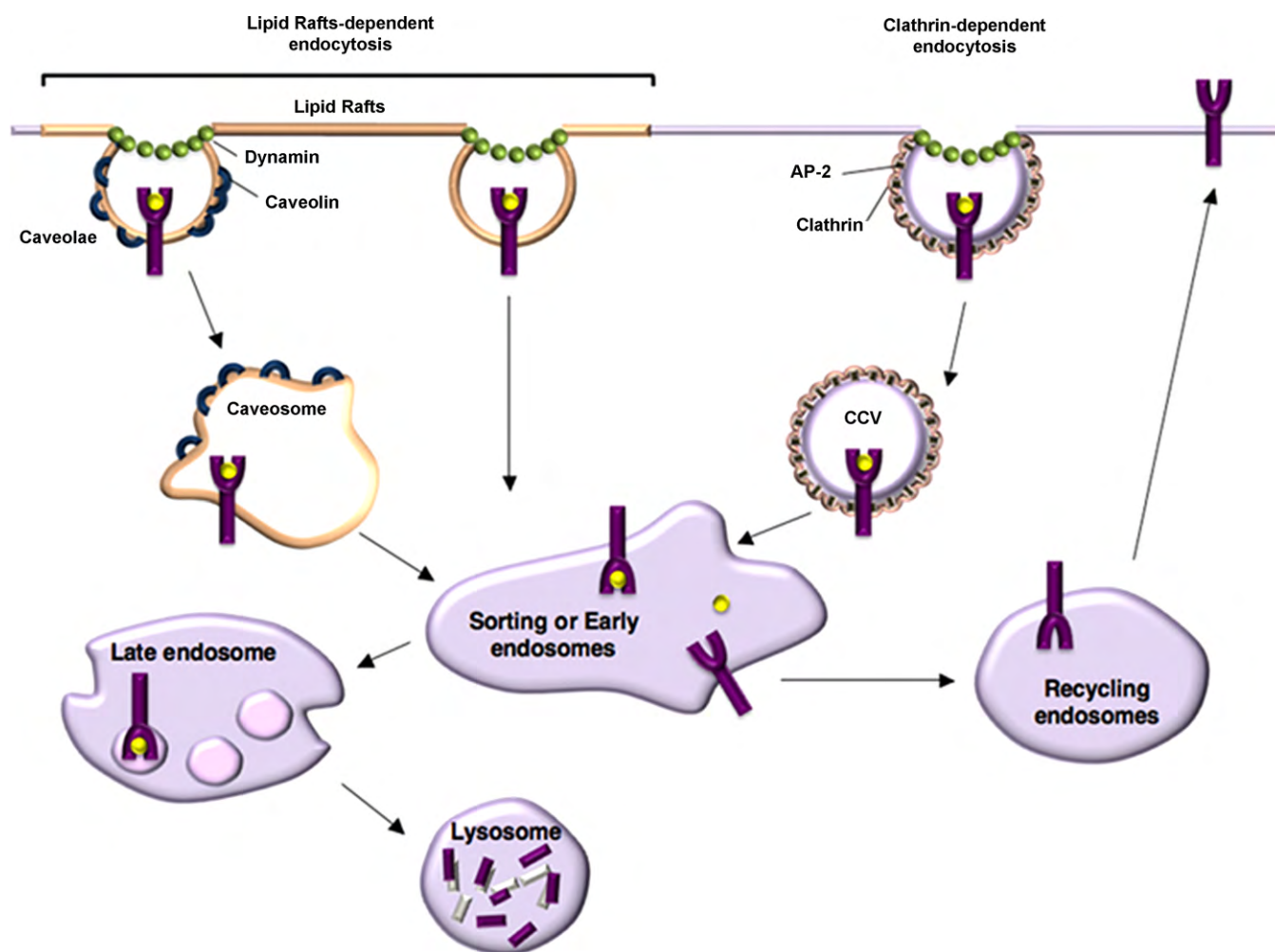


Fig. 1. Clathrin- and lipid raft-dependent endocytic routes.

Clathrin-dependent endocytosis is regulated by cargo-associated adaptors, such as AP-2. AP-2 recruits clathrin to the plasma membrane and promotes its assembly into a polyhedral lattice leading to the formation of a clathrin-coated vesicle (CCV). Clathrin-independent endocytosis generally relies cholesterol-enriched membrane domains called lipid rafts. Lipid rafts can also contain the protein caveolin-1, giving rise to specialized membrane invagination (caveolae) and vesicles (caveosome). The activity of GTPase dynamin is required to mediate the pinch-off of the vesicles which fuse with sorting endosomes (also known as early endosomes). From early endosomes, cargo recycle back to the plasma membrane or are sorted to degradative compartments.

bystander receptors (Monjas et al., 2004). Similarly, although the main endocytic route of engaged BCR is clathrin-dependent, Stoddart et al. (2005) demonstrated that BCR internalization occurs in clathrin-deficient cell lines following the clustering of glycosphingolipids.

2.1. Endocytic routes of engaged FcεRI

In the case of the FcεRI, several studies suggest that engaged receptors are mainly internalized via CDE. Although a rapid internalization of engaged FcεRI complexes does not require *de novo* formation of clathrin-coated pits (Santini and Keen, 1996), previous morphological studies showed the co-localization of cross-linked FcεRI and clathrin-coated pits, thus concluding that receptor endocytosis is mainly clathrin-mediated (Wilson et al., 2000; Oliver et al., 2004).

Accordingly, we have more recently demonstrated that the presence of a ternary complex of adapter proteins, namely Cbl/CIN85/endophilin, previously implicated in CDE of growth factor receptors (Soubeyran et al., 2002; Petrelli et al., 2002), is required for a rapid and efficient antigen-dependent FcεRI internalization (Molfetta et al., 2005).

The broadly expressed adapter protein CIN85 (Cbl-interacting protein of 85 kDa) is constitutively associated to endophilin, a regulatory component of clathrin-coated pit formation (Dikic, 2002).

In RBL-2H3 cells we found a Cbl/CIN85 interaction that increases upon antigen stimulation (Molfetta et al., 2005). Moreover, CIN85 overexpression results in a more rapid receptor internalization, trafficking into early endosomes, and delivery to the lysosomal compartment for degradation. In the same study we have also demonstrated an impairment of FcεRI-mediated degranulation in CIN85 transfectants, suggesting that the accelerated FcεRI internalization contribute to dampen functional cell responses.

Our data suggest a model in which FcεRI engagement induces the recruitment of Cbl/CIN85 complex to the plasma membrane where it drives CDE of engaged FcεRI complexes (Fig. 2).

Notably, Fattakhova et al. (2006) have recently reported that depletion of either clathrin or the clathrin adapter AP-2 by means of siRNA does not affect endocytosis of cross-linked FcεRI complexes. However, a small amount of clathrin persists after selective depletion. Thus, it remains formally possible that the residual clathrin molecules might regulate FcεRI endocytosis without the requirement of AP-2, as previously reported for other receptors (Motley et al., 2003). Alternatively, a lipid raft-dependent endocytic pathway

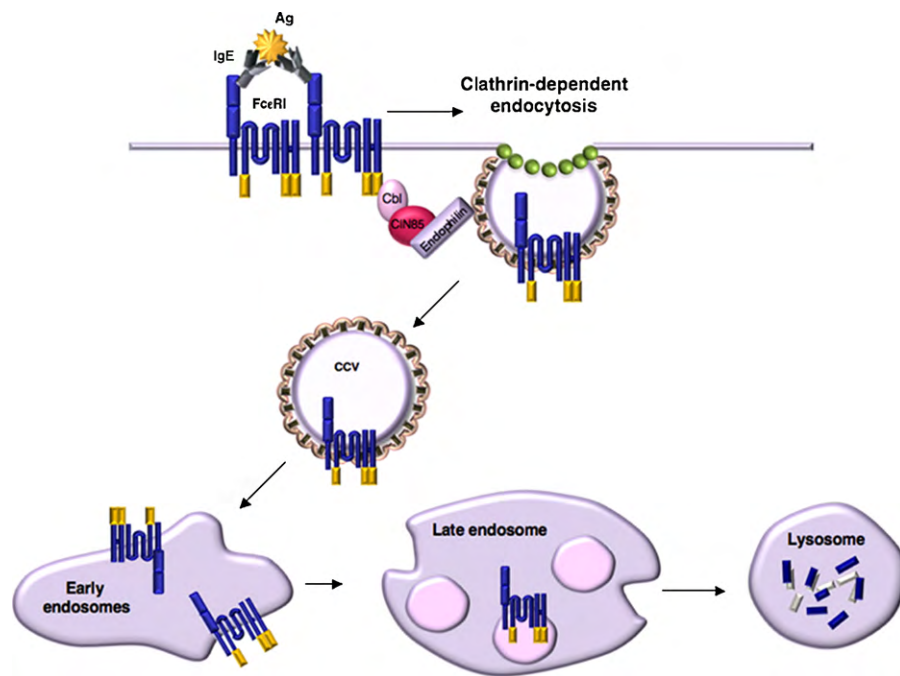


Fig. 2. Schematic model of antigen-induced FcεRI endocytosis by CIN85 and endophilin.

Upon FcεRI aggregation, Cbl recruits the preformed CIN85/endophilin complex in the proximity of engaged receptors whereby endophilin drives receptor clathrin-dependent endocytosis. Internalized FcεRI complexes are then sorted along the endocytic pathway and targeted to the lysosomal compartment for degradation.

may drive receptor internalization (Fattakhova et al., 2006; Oliver et al., 2007; Molfetta et al., 2009).

Fattakhova et al. (2006) have firstly demonstrated that the engaged FcεRI complexes remain associated with detergent insoluble structures upon their internalization. In line with this result, it was subsequently demonstrated that ligated FcεRI complexes internalize with the lipid raft-associated ganglioside GM1 (Oliver et al., 2007), and that cholesterol depletion decreases antigen-induced FcεRI internalization (Molfetta et al., 2009).

These results strongly suggest that the integrity of lipid rafts is required for efficient receptor endocytosis.

Several reports indicate the existence of a cross-talk between clathrin- and lipid raft-dependent pathways in mast cells. Proximity between clathrin and lipid rafts has been initially reported by a morphological study (Wilson et al., 2000). The presence of clathrin in lipid raft enriched fractions was subsequently described (Fattakhova et al., 2006).

Regardless of the main route (clathrin-dependent versus lipid raft-dependent endocytosis) involved in FcεRI endocytosis, several recent evidences point out that receptor ubiquitination is a key mechanism capable of regulating antigen-dependent endocytosis of receptor complexes and to control the fate of internalized receptors.

2.2. Regulation of FcεRI endocytosis by the ubiquitin pathway

Ubiquitination is a reversible post-translational modification whereby the small conserved peptide Ub is covalently attached to the ε-amino group of lysine (Lys) residues of the targeted protein (Ciechanover, 1998; Laney and Hochstrasser, 1999).

Ubiquitination is catalyzed by the sequential action of three classes of enzymes, namely E1, E2 and E3 (Fig. 3A). The Ub-activating enzyme (E1) forms a thiol-ester bond with the carboxy-terminal glycine of Ub in an ATP-dependent process. The activated Ub is subsequently accepted by the Ub-conjugating enzyme (E2), and finally transferred to the substrate through the action of the Ub protein ligase (E3). Thus, this latter class of enzymes

provides specificity to the Ub system being responsible for substrate recognition and Ub ligation to the target protein.

Ub moieties are often added to the target protein in the form of polyUb chains (Pickart and Fushman, 2004). Indeed, seven Lys residues are present in the Ub molecule, and all of them can be used for chain formation *in vivo* (Peng et al., 2003). Different topologies of polyUb chains are associated with distinct biological functions (Fig. 3B). A polyUb chain of at least four Ub molecules linked via Lys48, can trigger degradation of the target protein by the 26S proteasome (Thrower et al., 2000), whereas Lys63-linked chains serve as signal in DNA damage repair, protein trafficking, and ribosomal protein synthesis (Pickart and Fushman, 2004; Woelk et al., 2007).

Proteins may also be modified by a single Ub moiety on a single or multiple Lys residues (monoubiquitination or multiubiquitination, respectively). These modifications regulate many cellular functions, including endocytosis, virus budding, nuclear shuttling, and transcription (Mukhopadhyay and Riezman, 2007; Salmena and Pandolfi, 2007).

The role of ubiquitination in receptor tyrosine kinase (RTK) endocytosis has been extensively investigated. Indeed, platelet-derived and epidermal growth factor receptors were the first mammalian receptors found to be ubiquitinated (Mori et al., 1993; Galcheva-Gargova et al., 1995). Since these studies, several RTKs have been shown to be ubiquitinated in a ligand-dependent manner by the E3 ligase c-Cbl (Thien and Langdon, 2005; Schmidt and Dikic, 2005).

Three mammalian Cbl proteins have been identified: c-Cbl, Cbl-b and Cbl-c (also known as Cbl-3). All of them share the presence of a RING finger responsible for the E3 ligase activity and a tyrosine kinase binding (TKB) domain that determines substrate specificity and serves as a docking site for tyrosine phosphorylated proteins.

Another essential factor for the E3 activity of c-Cbl (and to a lesser extent of Cbl-b) is the tyrosine phosphorylation of its linker domain located between the TKB and RING domains. c-Cbl phosphorylation causes the conformational changes required for the release of the RING-finger associated E2, an essential step in Cbl driven ubiquitination (Kassenbrock and Anderson, 2004).

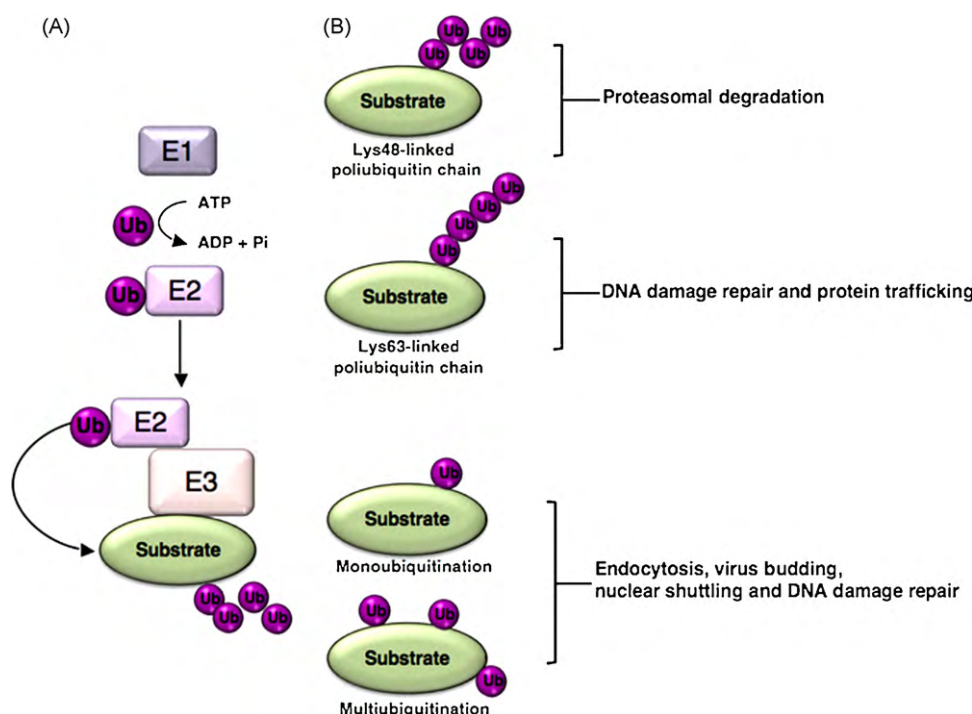


Fig. 3. Schematic representation of the ubiquitination process.

A. Three types of enzymes carry out ubiquitination: Ub is first activated by the Ub-activating enzyme E1; the activated Ub is then transferred from E1 to the ubiquitin-conjugating enzyme E2; the Ub-protein ligase E3 interacts with both the E2 and the substrate and transfers Ub to the protein substrate. B. Polyubiquitination is the modification whereby an Ub chain is attached to a target protein. Shown are the most common polyUb chains linked through Lys48 or Lys63. Mono and multiubiquitination result from the addition of a single Ub molecule to one or several Lys residues of the target protein, respectively. The different functional role of Ub modifications are indicated.

Thus, due to the presence of different protein–protein interaction domains, Cbl proteins recognize their substrates both in a phosphorylation-dependent and independent manner. *In vitro*, c-Cbl and Cbl-b show equal capacity to act as E3 ligases toward a similar substrate range. However, unlike c-Cbl, ubiquitination by Cbl-b affects protein localization (Thien and Langdon, 2005).

Several reports have firmly established that Cbl-mediated ubiquitination of RTKs is required for receptor sorting to lysosomes for degradation (Haglund et al., 2003; Duan et al., 2003; Stang et al., 2004; Sigismund et al., 2005).

Evidence collected in the past years has strongly supported a similar crucial role for the Cbl family of Ub ligases in the down-regulation of immunoreceptors, including the FcεRI (Rao et al., 2001; Wang et al., 2001; Ota and Samelson, 1997; Paolini et al., 2002).

The first evidence for a negative role played by c-Cbl in mast cells stems from experiments in which c-Cbl overexpression inhibited receptor-mediated serotonin release in RBL-2H3 cells without affecting receptor phosphorylation (Ota and Samelson, 1997).

The molecular mechanism underlying this inhibition has been subsequently elucidated. An early study from our group (Paolini and Kinet, 1993) has shown that FcεRI β and γ subunits undergo ubiquitination upon stimulation of RBL-2H3 cells with IgE and multivalent antigen. Subcellular fractionation and confocal microscopy experiments have further revealed that c-Cbl colocalizes with FcεRI β and γ subunits into lipid rafts after receptor engagement, suggesting the involvement of Cbl in receptor ubiquitination (Lafont and Simons, 2001). Our group have then identified c-Cbl as the main E3 ligase responsible for antigen-induced receptor ubiquitination in RBL-2H3 cells (Paolini et al., 2002). Indeed, overexpression of wild type c-Cbl, but not a mutant form deleted in the RING finger domain, increased antigen-induced FcεRI β and γ ubiquitination. Furthermore, proteasome inhibition resulted in persistent recep-

tor phosphorylation, supporting the involvement of Cbl-dependent ubiquitination in the down-regulation of engaged receptors.

In line with this result, we have recently shown that multiple Lys residues in the cytoplasmic tail of the FcεRI β and γ subunits are ubiquitinated by c-Cbl after antigen stimulation. We have also provided evidence that this modification controls receptor internalization. Indeed under conditions of impaired receptor ubiquitination, we observed a decrease of FcεRI down-regulation (Molfetta et al., 2009). This finding cannot be explained by an increased recycling, since a recent study in RBL-2H3 cells has demonstrated that aggregated receptor complexes do not recycle back to the cell membrane but are efficiently routed towards late endosomes and lysosomes for degradation (Fattakhova et al., 2009).

We further assessed the relationship between receptor ubiquitination and lipid rafts. We showed that the recruitment of engaged FcεRI subunits into lipid rafts precedes their ubiquitination, and that the integrity of these microdomains is required for receptor ubiquitination and endocytosis (Molfetta et al., 2009). Thus, a cohesive model is one in which lipid rafts, in addition to assemble the machinery needed for the downstream propagation of the signal from the engaged FcεRI complexes, also serve as platforms to recruit the machinery needed for receptor endocytosis.

Likely candidates for coupling ubiquitinated FcεRI to the endocytic machinery are the ubiquitous adapter proteins harboring Ub interacting motifs (UIMs) (Hofmann and Falquet, 2001; Polo et al., 2002), namely Eps15, Eps15R, epsin and Hrs. Eps15, Eps15R and Epsin display similar functions: they control the early steps of the endocytic route coupling ubiquitinated receptors with components of the budding vesicles (Carbone et al., 1997; Chen et al., 1998). Instead Hrs plays a key role in the delivery of ubiquitinated proteins to the outer membrane of the late endosomes, and in the sorting of the cargo into internal vesicles of multivesicular bodies for lysosomal degradation (Bache et al., 2003).

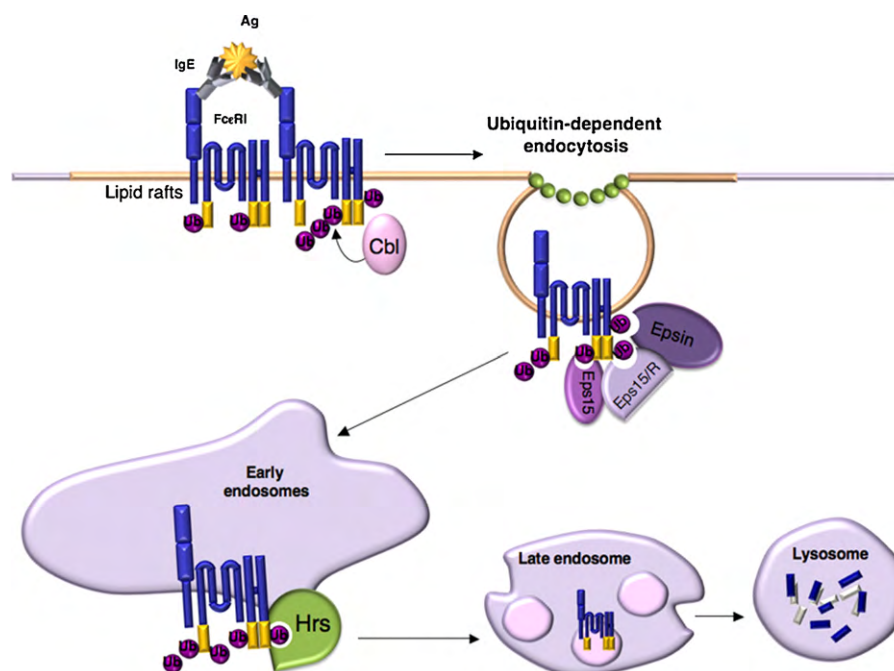


Fig. 4. Proposed model of lipid raft-dependent endocytosis of ubiquitinated FcεRI.

Lipid rafts serve as a platform to recruit engaged receptor complexes and the Ub-ligase Cbl that drives receptor ubiquitination. Ubiquitinated receptors are then recognized by specific Ub-binding adaptors that drive both receptor clearance from the plasma membrane and sorting of internalized complexes along the endosomal compartments till the lysosomes for degradation.

Only combined depletion of Eps15, Eps15R and Epsin impaired the ligand-induced FcεRI endocytosis, suggesting a partial overlapping functions of the three adaptors in the uptake of ubiquitinated receptors (Molfetta et al., 2009). Instead, a critical role for Hrs in controlling the fate of internalized receptor complexes was demonstrated: Hrs depletion retains ubiquitinated receptors into early endosomes and partially prevents their sorting into lysosomes (Molfetta et al., 2009).

Our data do not resolve the issue of whether receptor ubiquitination generates an internalization signal able to control endocytosis through a CDE or lipid raft-dependent endocytic pathway or a combination of both. However, they support a key role for the Ub-binding adapter Hrs in the delivery of ubiquitinated FcεRI to lysosomal compartments (Fig. 4).

Several lines of evidence indicate that endosomal trafficking of ubiquitinated cargo might require the action of additional Ub-binding adaptors, including the Rab5 guanine nucleotide exchange factor (GEF) RabGEF1/Rabex-5 (Raiborg et al., 2006). The small GTPase Rab5 is found at the plasma membrane and early endosomes, and together with Rabex-5 is implicated in endosomal fusion.

In addition to functioning as GEF for Rab5, Rabex-5 also acts as Ub-binding adapter and Ub ligase. Indeed, it contains two novel Ub-binding domains and the E3 ligase-encoding zing finger domain (Raiborg et al., 2006).

In mouse bone marrow-derived mast cells, Rabex-5 is required to maintain high basal levels of FcεRI surface expression, and acts as an important regulator of antigen-induced FcεRI internalization (Kalesnikoff et al., 2007). However, the mechanism responsible for this regulation remains to be identified.

3. Ubiquitination as a common feature of Fc receptor down-regulation

The role of the Ub pathway in receptor down-regulation could be extended to include other activating Fc receptors, including

the FcγRIIA and FcγRIIIA, which also become ubiquitinated upon engagement with their cognate ligands.

FcγRIIA contains in its cytoplasmic tail an ITAM motif responsible for signal transduction. FcγRIIIA is a multimeric receptor composed of a ligand binding α chain noncovalently associated with ITAM-containing γ and/or ζ dimers, originally identified as components of the FcεRI and the TCR, respectively (Ravetch and Kinetic, 1991).

Engagement of these FcγRs triggers many biological functions, including phagocytosis, degranulation, lysis of antibody-coated target cells, and the synthesis of cytokines (Daëron, 1997).

Moreover, FcγR cross-linking on phagocytes induces endocytosis of small immune complexes and phagocytosis of IgG-opsonized particles. However, the molecular mechanisms underlying endocytosis and phagocytosis differ markedly. By using a CHO-derived cell line bearing a temperature-sensitive mutation in the E1 enzyme, Booth et al. (2002) demonstrated that an active Ub conjugation system is required for endocytosis of transfected FcγRIIA, but not for phagocytosis. The same group subsequently showed that ligand-induced FcγRIIA endocytosis requires clathrin (but not the AP-2 adapter), dynamin as well as receptor mono- and polyubiquitination (Tse et al., 2003; Mero et al., 2006).

Thus, clathrin machinery and ubiquitination may cooperate in driving FcγRIIA endocytosis, as proposed for other immune receptors including the FcεRI.

More recent works in human neutrophils, showed the recruitment of ligated FcγRIIA and c-Cbl into lipid rafts followed by a rapid Cbl-dependent receptor ubiquitination and degradation (Barabé et al., 2002; Marois et al., 2009).

In addition to the clathrin-coated pit machinery and the Ub system, proteasomal activity is also required for FcγRIIA down-regulation, as proteasome inhibitors partially prevent receptor endocytosis and degradation (Booth et al., 2002; Marois et al., 2009). The reported effects of proteasome inhibitors on the degradation of FcγRIIA should include an indirect effect of these inhibitors on the lysosomal pathway, which could be suppressed

via depletion of the ubiquitin pool. However, the involvement of lysosomes in FcγRIIA degradation has not been formally demonstrated (Booth et al., 2002). Alternatively, ubiquitinated FcγRIIA itself may become a target for proteasomal degradation, as recently proposed (Marois et al., 2009).

FcγRIIIA (CD16) down-regulation on human NK cells is a consequence of both metalloprotease-induced shedding and internalization of cross-linked receptors by a mechanism that requires an intact actin cytoskeleton (Harrison et al., 1991; Borrego et al., 1994; Mota et al., 2004; Cecchetti et al., 2007) and a clathrin-coated pit machinery (Molfetta, unpublished observations).

Notably, we have demonstrated that the CD16 ζ subunit is rapidly ubiquitinated after receptor aggregation (Paolini et al., 1999), and have suggested a role for this modification in directing this subunit to lysosomal degradation. Furthermore, engaged CD16 complexes accumulate into lipid rafts (Cecchetti et al., 2007), suggesting that a lipid raft environment can also contribute in regulating CD16 ubiquitination, as demonstrated for the FcεRI on mast cells and the FcγRIIA on human neutrophils.

4. Conclusions

The highly regulated system that leads to mast cell activation is counterbalanced by an equally sophisticated series of inhibitory mechanisms.

Notably, a negative-feedback regulation of FcεRI-mediated signals is provided by the internalization and delivery of activated receptor complexes to lysosomes for degradation.

Here, we reviewed the main endocytic pathways involved in the down-regulation of engaged FcεRI complexes.

In addition to the well-characterized clathrin-dependent endocytic pathway, several findings have highlighted the important contribution of lipid raft-dependent FcεRI ubiquitination in controlling the endocytosis of engaged receptors. These results support a key role of the Ub pathway to ensure proper recognition and delivery of engaged receptors to lysosomes for degradation, thus contributing to the down-regulation of FcεRI-mediated signaling. This role of the Ub pathway could be extended to include other Fc receptors, such as the FcγRIIA and FcγRIIIA receptors, providing a more general molecular mechanism for attenuating immune responses.

Acknowledgments

Financial support was provided by the Italian Ministry of University and Research, and the Institute Pasteur-Fondazione Cenci Bolognietti.

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