Unconventional Endocytic Mechanisms

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Abstract

Endocytosis mediates the uptake of extracellular proteins, micronutrients and transmembrane cell surface proteins. Importantly, many viruses, toxins and bacteria hijack endocytosis to infect cells. The canonical pathway is Clathrin-mediated endocytosis (CME) and is active in all eukaryotic cells to support critical house-keeping functions. Unconventional mechanisms of endocytosis exit in parallel of CME, to internalize specific cargoes and support various cellular functions. These Clathrin-independent endocytic (CIE) routes use three distinct mechanisms. Acute signaling-induced membrane remodeling drives macropinocytosis, activity-dependent bulk endocytosis (ADBE), Massive Endocytosis (MEND) and EGFR Non-Clathrin Endocytosis (EGFR-NCE). Cargo capture and local membrane deformation by cytosolic proteins is used by Fast Endophilin-Mediated Endocytosis (FEME), IL-2Rβ endocytosis and Ultrafast Endocytosis (UFE) at synapses. Finally, formation of endocytic pits by clustering of extracellular lipids or cargoes according to the Glycolipid-Lectin (GL-Lect) hypothesis mediates the uptake of SV40 virus, Shiga and cholera toxins, and galectin-clustered receptors by the CLIC/GEEC and the Endophilin-A3-mediated CIE.

Highlights

- Clathrin-mediated endocytosis (CME) is the canonical pathway of entry into cells
- Unconventional mechanisms of endocytosis exist in parallel to CME
- These Clathrin-independent endocytic (CIE) routes use three distinct molecular mechanisms:
- Acute signaling-induced membrane remodeling (*e.g.* macropinocytosis)
- Cargo capture and local membrane deformation by cytosolic proteins (*e.g.* FEME)
- Extracellular lipid/cargo clustering according to the Glycolipid-Lectin hypothesis (*e.g.* CLIC/GEEC)

Introduction

Endocytosis is essential for all eukaryotic cells to internalize transmembrane proteins from the plasma membrane and to uptake extracellular material too large to diffuse through the membrane bilayer or be transported by channels and transporters [1,2]. It generates membrane-bound carriers of variable sizes (from <60nm to >5μm in diameter), which then fuse with early endosomes. By controlling the numbers of receptors, channels and transporters at the cell surface, endocytosis regulates the sensitivity of cells to their extracellular environment [3]. It also mediates synaptic vesicle recycling following synaptic transmission [4]. Finally, multitudes of viruses, bacteria and toxins exploit endocytosis to infect or poison eukaryotic cells [5].

Clathrin-mediated endocytosis (CME) is the best-characterized uptake mechanism that supports housekeeping functions in cells [6]. It is considered canonical because it is constantly active in all eukaryotic cells. In CME, cargo receptors are sorted by adaptor proteins, which then recruit Clathrin triskelia that self-polymerize into polyhedral coats [6]. CME is: 1) constitutive because it is constantly active, with or without external stimuli [6]; 2) selective, as only transmembrane proteins having specific motifs are sorted into Clathrin-coated pits (CCPs), while others are passively excluded; 3) uniform because Clathrin-coated vesicles are broadly homogeneous in sizes, shapes and sites of budding on the cell surface [6]; 4) relatively slow as it takes >30min for CME to clear an abundant receptor (>1million copies) from the cell surface [6]. The trade-off for the selectivity and reproducibility of CME is that the majority of CCPs abort before completion [6].

Unconventional mechanisms of endocytosis are used for cellular needs that are unmet by CME. For example, rapid and scalable uptake (*e.g.* receptor hyper-stimulation or compensatory uptake following synaptic vesicle release), endocytosis of cell surface proteins having no intracellular motifs (*e.g.* glycosylphosphatidylinositol-anchored proteins, GPI-APs) or internalization of receptors in specific physiological states (*e.g.* only when bound to a ligand) [1,7,8]. Several distinct mechanisms of Clathrin-independent endocytosis (CIE) exist in many *in vitro* cell lines, *ex vivo* primary cells and *in vivo* in yeast, worm, fly, mosquito, plant and mouse [1,7,8]. Over twenty viruses (including Ebola, HIV, Lassa, Herpes, Dengue and SV40), some bacteria, prions and bacterial toxins exploit CIE [1,7]. Finally, malfunctions of CIE have been reported in cancer, atherosclerosis, lysosomal storage and neurodegenerative diseases [1,7].

As exhaustive reviews already exist on each of the CIE modalities highlighted below [1,4,7-10], the focus here is to discuss recent advances in three main molecular mechanisms by which unconventional endocytic carriers form: i) acute signaling-induced membrane remodeling, ii) cargo capture and local membrane bending by cytosolic proteins and iii) extracellular lipid or cargo clustering according to the Glycolipid-Lectin (GL-Lect) hypothesis.

Acute signaling-induced membrane remodeling

Many stimuli induce large and rapid plasma membrane remodelling that is not selective, indiscriminately internalizing any lipids and proteins located on the patches of the cell surface being engulfed. Large amounts of fluid, solutes and soluble proteins are also entrapped and endocytosed [9,11]. In absence of sorting mechanisms at the cell surface, the fate of the internalized proteins is decided after entry: receptors endocytosed as 'collaterals' recycle back to the plasma membrane [9,11]. Macropinocytosis (reviewed in [7,9,11]), but also MEND and EGFR-NCE as well as ADBE in synapses, produce endocytic carriers upon stimuli.

Activity-dependent bulk endocytosis (ADBE)

At neuronal synapses, CME and UFE (*see below*) function upon regular axon depolarization. Following high stimuli, ADBE takes over in a process that resembles macropinocytosis but differs in its mechanism. ADBE is triggered by elevated concentration of calcium in synaptic terminals and high loads of membrane addition through exocytosis of synaptic vesicles [4]. In resting and weakly stimulated neurons, kinases such as Cdk5 and Glycogen Synthase Kinase 3β (GSK3β) are inhibiting ADBE by phosphorylating several endocytic proteins including Syndapin-I and the membrane scission GTPase Dynamin-1 on Ser-774 and Ser-778 [12,13] (**Figure 1**). High neuronal activity-induced Ca^{2+} rise in synaptic terminals activates the calcium-sensitive phosphatase Calcineurin which dephosphorylates and thus activates Syndapin-I and Dynamin-1 [14]. The process is further supported by the inhibition of GSK3β, upon phosophorylation by AKT, which is also activated by high stimuli [15]. Once triggered, ADBE induces actin-driven membrane invaginations at

sites of clustered synaptic vesicle cargoes, including VAMP4, but is retrieving non-specifically all molecules located on the patch of the plasma membrane generating large (up to 500nm) bulk endosomes [16] (**Figure 1**).

Massive Endocytosis (MEND)

MEND is an unconventional Clathrin- and Dynamin-independent process that mediates the rapid internalization of more ordered plasma membrane domains containing palmytoylated proteins [10]. It is activated by several transient cellular stresses, as well as Ca^{2+}, PKC or PI3K signaling [17-19] (**Figure 1**). But, unlike ADBE and macropinocytosis, it does not require local actin polymerization. This may actually hinder MEND that occurs preferably at actin-free zones [10]. Remarkably, MEND can support the internalization of >50% of the plasma membrane within seconds following acute stimuli such as cholesterol-phobic detergents (*e.g.* TritonX-100) or bacterial sphingomyelinases (generating ceramide, which favours phase separation and ordered membrane nanodomains). Internalized membrane is then handled by the endosomal pathway, most of which is recycled back to the plasma membrane within 15min [10]. Mechanistically, coalescence of lipid ordered nanodomains at the outer leaflet of the plasma membrane (often supported by palmytoylated proteins clustering or amphipathic molecule binding) is believed to promote negative (*i.e.* concave) curvature and membrane buckling towards the cytosol [18,20] (**Figure 1**). Line tension created upon membrane phase separations and local accumulation of fusogenic lipids at the rim of the nanodomains may be sufficient to trigger membrane scission in absence of Dynamin [10]. However, how these subtle lipid changes can induce such an extensive endocytosis is not clear yet and further mechanistic details will likely emerge from future studies.

EGFR Non-Clathrin Endocytosis (NCE)

At low EGF concentration (<2ng/mL), EGFR uses CME to enter cells and sustain downstream signaling following EGFR recycling back to the cell surface [21]. Higher concentrations (>2ng/mL) trigger the receptor to enter cancer cells using EGFR-NCE [21,22]. At even higher EGF concentrations (>50ng/mL), FEME and macropinocytosis also participate to the rapid EGFR clearance from the cell surface required to protect cells from excessive ERK and AKT signaling [7,23]. In all three instances of CIE uptake, EGFR ends up in lysosomes for degradation and prolonged cell desensitization. However, EGFR-NCE requires the mono-ubiquitination of EGFR and the atypical need of Reticulon-3-dependent ER-plasma membrane contact sites [24] (**Figure 1**). These are required for IP_3-mediated localized Ca^{2+} release and signaling that trigger the formation of EGFR-NCE carriers [24]. Molecular details on how they are built is still being investigated but, even though EGFR-NCE is morphologically different from macropinocytosis, it internalizes at least one receptor (CD147) as a collateral, alongside EGFR [24] (**Figure 1**).

Cargo capture and local membrane curvature by cytosolic proteins

The sorting of many transmembrane proteins into endocytic carriers requires the recognition of motifs in their cytosolic tails by adaptor proteins. As such, cargoes are enriched into forming carriers by at least one order of magnitude above their local density on the plasma membrane. Proteins lacking motifs are passively excluded. Endocytic proteins are also imposing local membrane curvature to deform the plasma membrane around the receptors being sorted. FEME, IL-2R uptake and UFE are all cargo-specific but differ in their mechanisms and regulations.

Fast-Endophilin-Mediated Endocytosis (FEME)

Unlike CME, FEME is not constitutive but rapidly triggered by the activation of receptors by their cognate ligands. Several GPCRs, RTKs, cytokine and axon guidance receptors are FEME cargoes [25,26] (**Figure 2**). It is also hijacked by the B-subunits of cholera and Shiga toxins (hereafter, CTxB and STxB) [27]. Importantly, many of these cargoes can enter cells through other endocytic pathways and, so far, only the β1-adrenergic receptor relies on FEME [28]. Atypically, FEME requires the pre-enrichment of its main component, Endophilin (*mostly A2 isoform*) into discrete clusters on the plasma membrane, prior to receptor activation [29]. Unlike CCPs, which are built at the same time as receptors are sorted into them [6], initiation sites must exist prior to receptor activation for FEME carriers to bud [29]. Following a molecular cascade starting with Cdc42-GTP recruiting CIP4 and FBP17, the 5'-phosphatases SHIP1/2, the local production of $PI(3,4)P_2$ and the recruitment of Lamellipodin, Endophilin is concentrated into distinct patches [29]. In the absence of receptor activation, the Cdc42 GTPase-activating proteins (GAPs) RICH1, SH3BP1 and Oligophrenin disassemble the Endophilin spots after 5-10s. New ones form nearby, constantly probing the membrane [29].

Upon receptor-ligand binding, the SH3 domain of Endophilin binds to proline-rich motifs that become available in cytoplasmic parts of activated receptors, or indirectly through adaptor proteins [28]. Then, in a mechanism that is not yet understood, cargo sorting transforms the initiation patches into FEME carriers that are small (0.1-1µm), pleiotropic, tubulo-vesicular Endophilin-positive assemblies. Most of them are also positive for Bin1 [28] (**Figure 2**)..Although the N-BAR domains of Endophilin and Bin1 can induce extensive membrane tubulation at high local concentration, initiation patches in resting cells did not correspond to detectable membrane invaginations [25]. Thus, either cargo capture increases local N-BAR domain levels over a critical concentration, inducing membrane curvature, or receptor clustering itself is contributing to bending. Carriers budding require the synergy between N-BAR domain-mediated friction-driven scission, Dynamin and local actin polymerization [27,30] (**Figure 2**). Unlike CME where the Clathrin coat is lost soon after budding, Endophilin remain onto FEME carriers until fusion with early endosomes. Cytosolic FEME carriers move retrogradly on microtubules, powered by Dynein, which is recruited by Bin1 [26,27]. Finally, like ADBE in neurons, FEME is negatively regulated by Cdk5 and GSK3β, which phosphorylate key residues on cargo adaptors, Dynamin and Dynein, thereby inhibiting Endophilin and Bin1 binding [26]. This explains why FEME activity differs in different cell types and why it is shut off at low concentrations of growth factors (*e.g.* serum starvation), when GSK3β activity is high.

Interleukin-2 receptor (IL-2R) uptake

Native IL-2R (IL-2Rα, β and common γ chains that trimerize upon IL-2 binding) enter T cells mostly within FEME carriers [25]. However, isolated IL-2Rβ or γc chains expressed ectopically in non-immune cells cannot bind IL-2 but constitutively internalize using a distinct, unconventional pathway [31]. It starts by the direct recruitment of the WAVE1 complex (Sra1 and Abi2 interface) to the WIRS motif in the cytoplasmic tail of IL-2Rβ [32] (**Figure 2**). Clustering of IL-2Rβ chains gather enough copies of WAVE1, followed by N-WASP, to induce local Arp2/3-mediated actin protrusions, thus building endocytic pits around them [32]. This is further supported by the recruitment of PI3K to clustered IL-2Rβ, local $PI(3,4,5)P_3$ production and Rac1-mediated WAVE and PAK-1 and -2 activation [33-35] (**Figure 2**). Although reminiscent of macropinocytosis, membrane projections around IL-2Rβ remain small (<0.5 µm) and confined, forming small and spherical carriers. Their scission is Endophilin A2-, Cortactin- and Dynamin-2-dependent [36].

Ultrafast Endocytosis (UFE)

UFE is a rapid pathway for synaptic vesicle recycling observed in motor and hippocampal neurons [4,37]. Within 100ms following a single stimulus, multiple small and uniform vesicles (~60-80nm in diameter) are

generated at the 'active zone' neighbouring the fusion sites [38]. Internalized membrane then fuses with endosomes for protein sorting, a step that requires Clathrin [39]. Mechanistically, UFE is still not fully understood. Calcium influx following axon depolarization is not sufficient to trigger UFE. However, low membrane tension is required as UFE is inhibited upon cooling of mammalian neurons (which stiffen their membranes). As the amount of membrane internalized by UFE equals that exocytosed, it is likely compensatory and requires initial addition of membrane, which would generate a sudden local decrease in membrane tension. Endophilin, Synaptojanin and Dynamin, as well as local actin polymerization, are all required for membrane curvature and vesicle budding [38,40] (**Figure 2**). Specific local protein organization and lipid composition favouring membrane fluidity, such as high levels of unsaturated fatty acids are likely supporting UFE [41]. Cargoes, especially SNARE complexes, that must be recycled following synaptic vesicle release, are likely to play a role in the mechanisms of UFE, but how they are sorted into the endocytic carriers at such a speed is not known yet.

Hybrid Extracellular Clustering - Intracellular Protein Scaffolding

The Glycolipid-Lectin (GL-Lect) hypothesis

Some CIE modalities rely on cargo clustering at the cell surface by extracellular mammalian, bacterial or viral lectins in addition to intracellular scaffolding proteins. In this case, the endocytic pit construction is promoted by a physical/mechanical (*i.e.* membrane bending) rather than biochemical (*i.e.* ligand stimulation) signal. All lectin-mediated CIE modalities share a common mechanism: 1) clustering of glycosphingolipids by lectins (plus glycosylated proteins for galectins) at the cell surface, 2) induction of an asymmetric stress in the membrane with local build-up of curvature, and 3) release of this stress by spontaneous tubulation of the membrane inwards the cell. This has been proposed as the *GL-Lect hypothesis*, according to which glycolipids and lectins cooperate to drive the formation of tubular endocytic pits without the help of cytosolic coats [42]. Of note, CIE modalities operating according to this hypothesis generally lead to the retrograde transport of cargoes to the perinuclear Golgi as a prelude to their redistribution by polarized secretion (reviewed in [43]). The different endocytic modalities described below function according to this GL-Lect hypothesis.

Clathrin-independent carriers/GPI-AP-enriched early endosomal compartments (CLIC/GEEC)

In addition to GPI-AP (*e.g* CD59, CD55, folate receptor [44] or CD90/Thy-1 [45]), other cargoes of the CLIC/GEEC route are glycosylated transmembrane proteins such as CD44 or CD98 [45], or extracellular lectins such as Galectin-3 [45] and CTxB [46] (**Figure 3**). A major portion of fluid phase endocytosis also occurs via CLIC/GEEC [45]. It is regulated by Cdc42 [44], Arf1 [47] and its GTP exchange factor (GEF) GBF1, Arp2/3 [48,49] and by the BAR domain proteins GRAF1 [50,51], IRSp53 and PICK1 [49] (**Figure 3**). A feature shared with other CIE mechanisms is its actin polymerization and cholesterol dependence, in particular for GPI-AP clustering [52,53]. Although CLIC/GEEC is Dynamin-independent, the uptake of exogenous cargoes such as CTxB is partially Dynamin-dependent [46]. Thus, a significant fraction of bacterial toxins can be endocytosed via at least two distinct CIE: the Dynamin-independent CLIC/GEEC [44,46] and the Dynamin-dependent Endophilin-A2-mediated [27] routes. CLIC/GEEC plays a central role in various biological processes, from the regulation of plasma membrane tension [54], to organ development [55] or cancer progression [56]. It can also be an entry door for viruses (*see below*).

The formation of CLIC/GEEC tubulo-vesicular invaginations of plasma membrane is driven by extracellular galectins. Galectins are a family of 15 proteins specialized in carbohydrate binding in mammals and involved in diverse physiological processes (reviewed in [57]). Galectin-3 drives the biogenesis of CLIC structures in a glycosphingolipid-dependent manner, by clustering together glycosylated proteins – such as CD44 or $\alpha5\beta1$-integrins – and glycosphingolipids at the cell surface [45,58] (**Figure 3**). Interestingly, the modulation of glycan-lectin interactions can affect cargo behavior, from stimulation to inhibition of uptake, as part of a continuum [58,59]. The fate of each cargo depends on galectin concentrations and cargo glycosylation patterns [58,59]. This may account for the differences observed between cell types. Also, different galectins can contribute to the formation of distinct CLIC subpopulations [58]. Of note, this Galectin-3-driven CLIC endocytosis works *in vivo* in transcytosis [60].

CLIC/GEEC endocytosis of viruses (SV40)

Viruses can highjack endocytic routes including CIE [61]. The non-enveloped simian virus 40 (SV40) is endocytosed in a cholesterol/GRAF1-dependent, but Clathrin-/Dynamin-/Arf6-independent manner, sharing important hallmarks of CLIC/GEEC [62,63] (**Figure 3**). Of note, CLIC/GEEC is also the entry door for other viruses, such as Adeno-Associated Virus 2 (AAV2) [64]. As for CTxB, the pentameric VP1 protein of SV40 capsid is a lectin that uses the ganglioside GM1 as a cell surface receptor [65]. Glycosphingolipid clustering by SV40 multivalent binding induces plasma membrane tubulation in a coat-independent fashion [66,67], similarly to bacterial toxins [27,68] or Galectin-3 in CLIC/GEEC [45,58].

Endophilin-A2-mediated uptake of bacterial toxins

STxB and CTxB are pentameric lectins that use, respectively, Gb3 (globotriaosylceramide) and GM1 (monosialotetrahexosylganglioside) at the surface of mammalian cells as receptors. The multivalent binding of toxins to their receptors is necessary for structured lipid clustering in the extracellular leaflet and the local induction of membrane curvature. Clustering of several toxin molecules builds up curvature until the spontaneous formation of tubular invaginations inwards the cell in a coat-independent manner [68-71]. Such invaginations are subsequently recognized by cytosolic Endophilin-A2 (**Figure 3**), which primes the membrane for pulling force-induced scission upon scaffolding its N-BAR domain around the membrane nanotubes [27,72]. The friction exerted by such scaffolds on the underlying lipids creates a diffusion barrier, favouring membrane rupture upon pulling by Dynein motors [27,30,73]. This generic scission modality, termed friction-driven scission (FDS) [30], acts additively with actin and Dynamin to increase the scission probability of toxin-containing endocytic carriers [27,68,74]. Of note, the involvement of Endophilin-A2 and Dynamin [27,68], and the absence of Galectin-3 [58], depicts this endocytic modality as distinct from the classical CLIC/GEEC route. Instead, the toxins seem to hijack FEME, although the signals initiating these endocytic processes are seemingly different.

Endophilin-A3/Galectin-8-mediated endocytosis

An additional CIE modality mediated by Endophilin-A3 isoform, distinct from FEME, has been described for the uptake of the tumor marker CD166/ALCAM (Activated Leukocyte Cell Adhesion Molecule) [75], identified as its first cargo (**Figure 3**). While Endophilin-A2 mediates the uptake of GPCRs (FEME) and bacterial toxins, Endophilin-A3 is specific to CD166/ALCAM uptake, suggesting low functional redundancy between Endophilin-A isoforms. In addition, the construction of endocytic sites from which CD166 is taken up in an Endophilin-A3-dependent manner is dependent on glycosphingolipids and driven by extracellular Galectin-8 [75], according to the GL-Lect hypothesis. Although this modality appears Dynamin-independent, it seems distinct from the classical CLIC/GEEC route, as CD166 does not colocalize with Galectin-3 [75]. Of note, CD166 is an immunoglobulin-like protein involved in cell-cell contacts that plays a role in several physiological processes, but also cancer [75]. Fine-tuning of CD166 at the cell surface by Endophilin-A3-dependent CIE may be used by cancer cells to modulate intercellular adhesiveness and ensure optimal collective migration [75].

Perspectives

The discovery of unconventional CIE mechanisms has significantly complexified our view of endocytosis. Strikingly, many molecular players – or modules – are often shared by multiple endocytic mechanisms, such as Rho GTPases, Dynamin, actin or BAR domain proteins. Therefore, a significant part of this diversity may arise from the combination of a limited number of modules. Importantly, in addition to cytosolic scaffolding proteins, extracellular lectins such as galectins are major contributors to plasma membrane bending and cargo clustering in the GL-Lect hypothesis. The observation of distinct CLIC subpopulations suggests the existence of distinct CLIC/GEEC entry doors, likely dependent on different galectin combinations. Additional studies will likely reveal the parameters explaining the diversity of CIE modalities, in particular regarding galectins and glycosylations, that are cargo- and context-dependent.

Why was such a variety of endocytic modalities conserved during evolution? Their different kinetics and specificities may have enabled cells to adapt to broader environments (*e.g.* EGFR can be taken up by EGFR-NCE, FEME and macropinocytosis in case of massive EGF stimulation). Moreover, natural selection probably favored specialized mechanisms in particular cell types/organs (*e.g.* UFE and ADBE in neurons for

rapid synaptic vesicle recycling). In this context, we should be careful when describing endocytosis. *"Pathway"* suggests that every endocytic process is strictly molecularly distinct from the other. It is actually far more complex and nuanced. The term *"pathway"* should thus be used with parsimony and could often be replaced by *"process", "mechanism"* or *"modality",* reflecting better the modularity of endocytosis. In addition, complexity also arises from the fact that most cargoes do not rely on a single portal of entry into cells. Moreover, crosstalks, competition and potential compensatory regulations between CIE mechanisms are still largely unknown. Understanding the regulation and coordination between unconventional endocytic processes will be an important milestone in the field.

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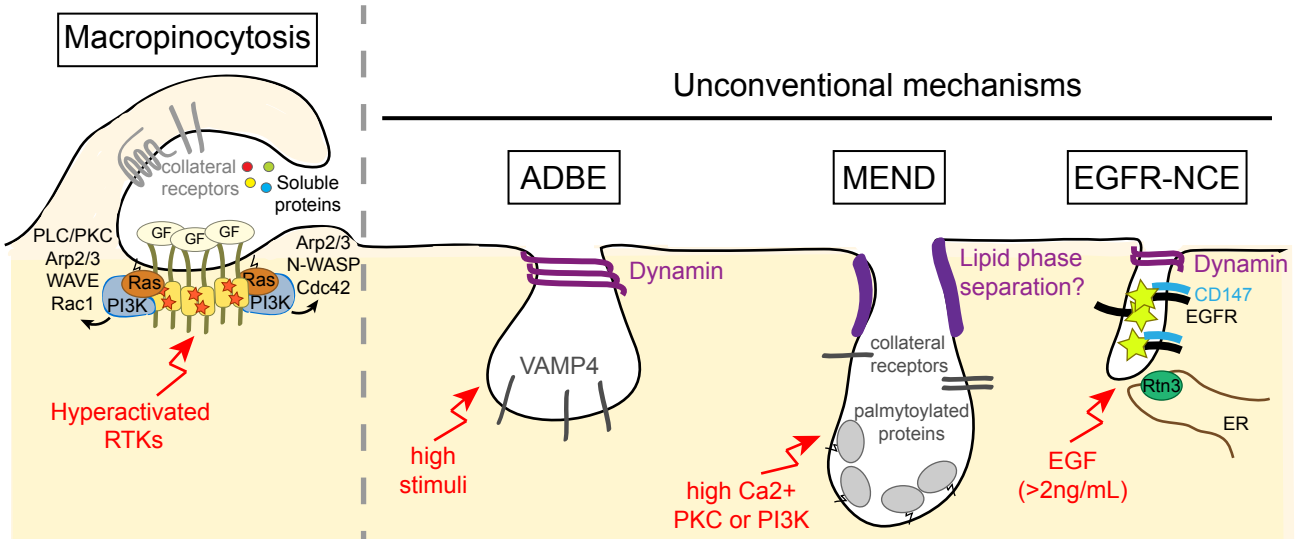
Figure legends

Figure 1. Acute signaling-induced membrane remodeling. Macropinocytosis, Activity-dependent bulk endocytosis (ADBE), Massive Endocytosis (MEND) and EGFR Non-Clathrin endocytosis (EGFR-NCE) are represented. The table compares the stimuli, markers, cargoes, signaling and cytoskeleton and scission factors. RTKs, Receptor-Tyrosine kinases; GF, growth factors, ER: endoplasmic reticulum; EGF, Epidermal Growth Factor; EGFR, EGF receptor.

Figure 2. Cargo capture and local membrane curvature. Clathrin-mediated endocytosis (CME), Fast-Endophilin-mediated endocytosis (FEME), Interleukin-2 receptor beta (IL-2Rβ) uptake and ultrafast endocytosis (UFE) are represented. The table compares the adaptors, BAR domain proteins, cargoes, Rho GTPases and cytoskeleton and scission factors. TfR, transferrin receptor; LDLR, low-density lipoprotein receptor; MI6PR, Mannose 6-phosphate receptor; EGFR, epidermal growth factor receptor; β1AR, β1-adrenergic receptor, GPCRs, G-protein coupled receptors; RTKs, Receptor-Tyrosine kinases; FDS, friction-driven scission.

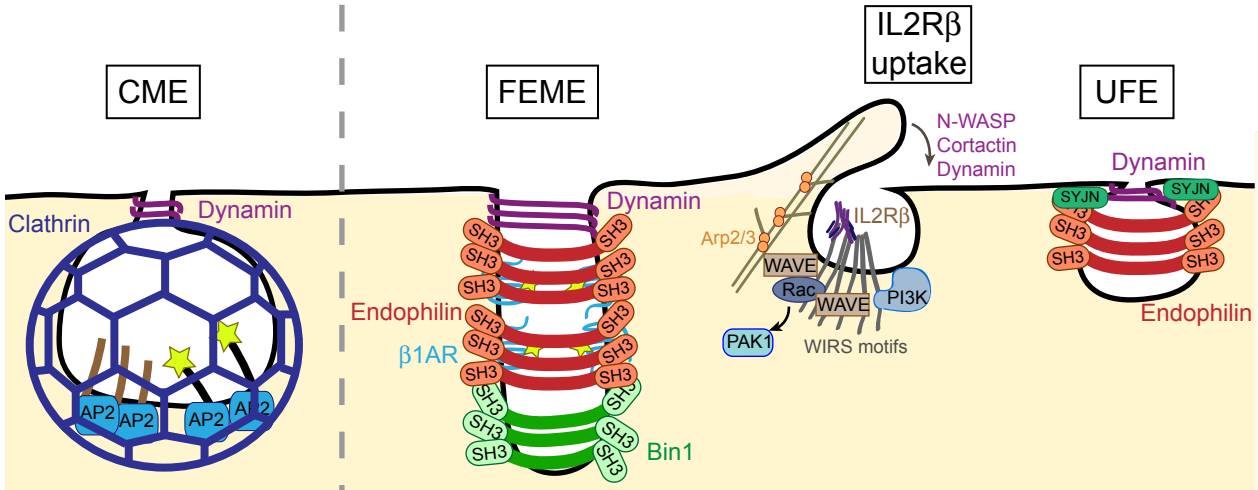
Figure 3. Hybrid CIE mechanisms relying on extracellular clustering and intracellular protein scaffolding, as compared to FEME mechanism. Endophilin-A2 (EndoA2)-dependent CIE of bacterial toxins (STxB, CTxB), Endophilin-A3 (EndoA3)/Galectin-8 (Gal8)-dependent CIE of CD166 and Galectin-3 (Gal3)-driven CLIC/GEEC are represented. The table compares the molecular actors involved in each modality: BAR domain proteins, lectins, cargoes, Rho GTPases, cytoskeleton and scission factors. Modalities operating according to the GL-Lect hypothesis are highlighted. β1AR, β1-adrenergic receptor, GPCRs, G-protein coupled receptors; RTKs, Receptor-Tyrosine kinases; EGFR, epidermal growth factor receptor; FDS, friction-driven scission, STxB, Shiga Toxin subunit B, CTxB, cholera toxin subunit B; GPI-APs, glycosylphosphatidylinositol-anchored proteins; CLIC/GEEC, clathrin-independent carriers (CLIC) GPI-AP-enriched early endosomal compartments (GEEC).



Molecular actors

	Macropinocytosis	ADBE	MEND	EGFR-NCE
Stimulus:	growth factors (high doses) mutated Ras, RTKs etc..	Axon deloplarization (high stimuli)	High Ca2+, PKC, PI3K	EGF (>2ng/mL)
Markers:	SNX5?	Syndapin-1	??	Rtn3
Cargoes:	RTKs, Integrins (+collaterals)	VAMP4, other SNAREs?	Palmytoylated protein Lipids	EGFR, CD147
Signaling:	PI3K, PLC, PKC	AKT, GSK3β, Calcineurin	Ca2+, PKC, PI3K	MAPK, AKT, Ca2+
Cytoskeleton & Scission factors:	Actin CtBP1?, Dynamin?	Actin Dynamin	no Lipid phase separation?	Actin Dynamin

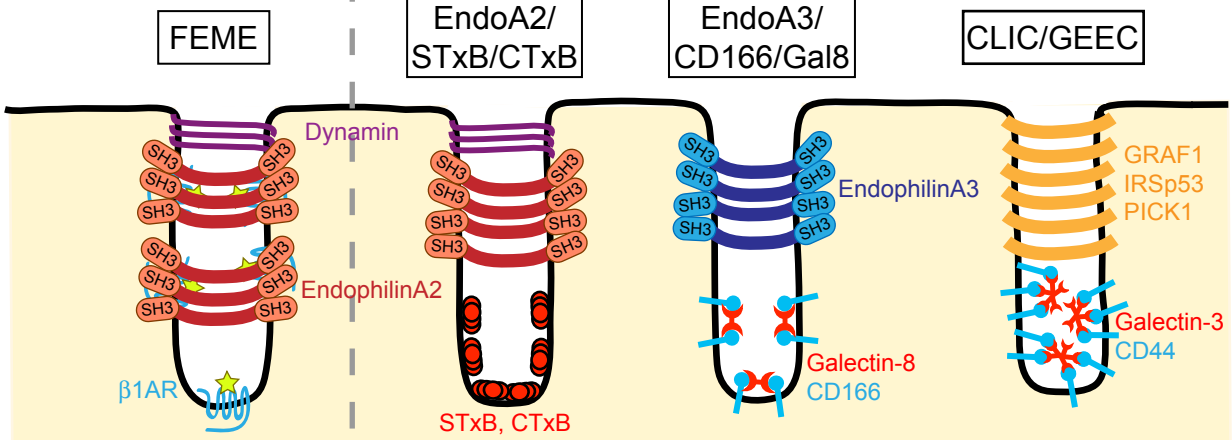
Unconventional mechanisms



Molecular actors

	CME	FEME	IL2Rβ uptake	UFE
Adaptors:	AP2, Epsin, AP180/CALM	Endophilin, Bin1	WAVE	??
BAR domain proteins:	Amphiphysin, SNX9, Endophilin	Endophilin, Bin1, FBP17, CIP4 RICH1, SH3BP1, OPHN1	Endophilin	Endophilin
Cargoes:	TfR, LDLR, EGFR, MI6PR, etc..	GPCRs (β1AR) RTKs (EGFR), PlexinA1 etc..	IL-2Rβ, IL2Rγ	SNAREs ?
Rho GTPases:	*not directly*	Cdc42, (Rac1)	Rac1	??
Cytoskeleton & Scission factors:	Actin Dynamin	Actin Microtubules (FDS) Dynamin	Actin Dynamin	Actin Dynamin

Endocytic modalities operating according to the *GL-Lect hypothesis*



Molecular actors

	FEME	EndoA2/ STxB/CTxB	EndoA3/ CD166/Gal8	CLIC/GEEC
BAR domain proteins:	Endophilin-A2, Bin1 FBP17, CIP4 RICH1, SH3BP1, OPHN1	Endophilin-A2	Endophilin-A3	GRAF1 IRSp53, PICK1
Lectins:	??	STxB, CTxB	Galectin-8	Galectin-3 SV40, CTxB
Cargoes:	GPCRs (β1AR) RTKs (EGFR), PlexinA1 etc..	STxB, CTxB SNAREs (VAMP2/3/8)	CD166/ALCAM	GPI-AP (CD59, CD55, Thy-1) Glycosylated proteins (CD44, β1-integrin, CD98)
RhoGTPases:	Cdc42, (Rac1)	??	??	Cdc42, Arf1
Cytoskeleton & Scission factors:	Actin Microtubules (FDS) Dynamin	Actin Microtubules (FDS) Dynamin	??	Actin