Local actin polymerization during endocytic carrier formation

Claudia Hinze[1] and Emmanuel Boucrot[1,2,*]

[1] Institute of Structural and Molecular Biology, Division of Biosciences, University College London, London WC1E 6BT, UK
[2] Institute of Structural and Molecular Biology, Department of Biological Sciences, Birkbeck College, London WC1E 7HX, UK
*Correspondence: e.boucrot@ucl.ac.uk

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Abstract

Extracellular macromolecules, pathogens and cell surface proteins rely on endocytosis to enter cells. Key steps of endocytic carrier formation are cargo molecule selection, plasma membrane folding and detachment from the cell surface. While dedicated proteins mediate each step, the actin cytoskeleton contributes to all. However, its role can be indirect to the actual molecular events driving endocytosis. Here, we review our understanding of the molecular steps mediating local actin polymerization during the formation of endocytic carriers. Clathrin-mediated endocytosis (CME) is the least reliant on local actin polymerization, as it is only engaged to counter forces induced by membrane tension or cytoplasmic pressure. Two opposite situations are coated pit formation in yeast and at the basolateral surface of polarized mammalian cells which are respectively dependent and independent on actin polymerization. Conversely, Clathrin-independent endocytosis (CIE) forming both nanometer (CLIC/GEEC, caveolae, FEME, IL2β uptake) and micrometer carriers (macropinocytosis) are dependent on actin polymerization to power local membrane deformation and carrier budding. A variety of endocytic adaptors can recruit and activate the Cdc42/N-WASP or Rac1/WAVE complexes, which in turn engage the Arp2/3 complex, thereby mediating local actin polymerization at the membrane. However, the molecular steps for RhoA and formin-mediated actin bundling during endocytic pit formation remains unclear.

Introduction

Extracellular macromolecules and transmembrane cell surface proteins cannot diffuse across the plasma membrane and require transport by endocytic carriers to enter cells. Such carriers are formed upon wrapping of the plasma membrane around the material to be internalized, generically named ‘cargoes’. Clathrin-mediated endocytosis (henceforth, CME) is the best-characterized endocytic route and the dominant uptake pathway to sustain housekeeping functions in cells (1-3). Several Clathrin-independent endocytosis (CIE) mechanisms exist in parallel to CME to mediate bulk protein and lipid uptake and removal of specific receptors from the plasma membrane (4,5). Beside ligand and protein uptake, endocytosis controls cell spreading, polarization and migration. Consistently, endocytosis is involved in several diseases such as cancer, neurodegeneration, lysosomal storage disease and atherosclerosis. It is also hijacked by many bacteria, viruses, prions and bacterial toxins to mediate their cellular entry (6).

Once detached from the surface, endocytic carriers have three broad shapes: small spherical vesicles (50-200 nm diameter), tubules (50-500 nm diameter and up to 1-5 μm in length) and large endocytic vacuoles called macropinosomes (0.5 to >10 μm). To date, we know of three main molecular mechanisms by which endocytic carriers form: i) cargo capture and local membrane bending by cytosolic proteins; ii) extracellular lipid or cargo clustering according to the Glycolipid-Lectin (GL-Lect) hypothesis; and iii) acute signaling-induced membrane protrusions folding back onto the plasma membrane (5). Both CME and CIE processes, such as fast Endophilin-mediated endocytosis (FEME) or Interleukin-2 receptor β (IL2Rβ) uptake, rely on cytosolic proteins such as Clathrin or BAR domain-containing proteins that bend membrane upon local recruitment and polymerization. Bin1/Amphiphysin/Rvs167 (BAR) domains are dimeric curved

structures with a positively charged concave surface that senses, stabilizes and induces membrane curvature (7). Extracellular lipid or cargo clustering drives the formation of Clathrin-independent carriers / Glycosylphosphatidylinositol (GPI)-anchored protein enriched endocytic compartments (CLIC/GEEC, hereafter CLIC) and the uptake of Shiga and cholera toxins (8). Membrane protrusions folding back onto the plasma membrane create large carriers during macropinocytosis and activity-dependent bulk endocytosis (ADBE) at synapses (9,10).

The energetic cost of deforming a membrane depends mainly on its rigidity. Thus, the energy required to form an endocytic carrier is not dependent on its size but is instead the result of the local membrane rigidity. In the case of a biological membrane that has a typical bending modulus of 10-20 k_BT (where k_B is the Boltzmann constant and T the temperature), about 250-500 k_BT (being the energy produced by the hydrolysis of 10-20 ATP molecules) must be produced to form a vesicle (11). Although Clathrin and its adaptor proteins can bend the plasma membrane locally to produce coated vesicles, additional forces provided by the actin cytoskeleton are required in some physiological contexts to counter membrane tension or internal cytoplasmic pressure. Two opposite situations are coated pit formation at the basolateral surface of polarized mammalian cells (independent of actin) and endocytosis in yeast (dependent of actin) (12-14). Perhaps because of their larger sizes, their speed of formation or the absence of dense and rigid proteinaceous coats around CIE carriers, their formations rely on actin polymerization (15).

Actin transitions between a monomeric globular form (G-actin) and a polymeric filamentous form (F-actin) upon ATP loading. Actin polymerizes into helical, two-stranded filaments, the slow- and fast-growing ends of which are named 'pointed' and 'barbed' ends, respectively. Actin filaments can be crosslinked (e.g. cortical actin), bundled together (e.g. filopodia) or branch into extended network (e.g. lamellipodia) (16). This network of filaments forms the actin cytoskeleton, performing multiple functions in cells, from providing structural support to membranes to propelling organelles (17). As actin monomers are in vast excess in cells, the availability of uncapped (free) barbed ends is rate limiting for the growth of actin filaments. While actin filament capping, cross-linking (*e.g.* Fimbrin, Fascin, α-actinin, Spectrin, Filamin and Myosin), elongating (e.g. ENA/VASP), severing and depolymerizing proteins (*e.g.* Gelsolin, Severin, ADF/Cofilin) are all important regulators, the growth of the actin network is stimulated by regulated nucleation (18-20). Formins and the Arp2/3 complex are the main actin assembly-promoting factors. Branched filaments can only be nucleated by the Arp2/3 complex, which serves as a template to trigger the polymerization of a new filament at a 70 degree angle from the original one (21). As Arp2/3 has minimal basal activity, nucleation-promoting factors (NPF) such as the WASP/N-WASP and the SCAR/WAVE complexes are required to induce strong branching and nucleation (18,19,22). In the cytosol, N-WASP is auto-inhibited, while WAVE is constitutively active but inhibited in *trans* by the WAVE-regulatory complex (WRC). Both complexes need to be recruited and activated by additional proteins to induce local actin polymerization. RhoA, Rac1 and Cdc42 are membrane-bound GTPases that trigger local actin polymerization upon recruitment and activation. GTP-bound Rac1 and Cdc42 activate Arp2/3-mediated actin branching through the WASP/N-WASP and SCAR/WAVE complexes, respectively. RhoA activates formins such as mDia.

While many publications have reported a requirement for actin in endocytosis, the evidence was often limited to its perturbation upon actin depolymerization or stabilization (Cytochalasin D, Latrunculin or Jasplakinolide treatments). The effects of actin cytoskeleton perturbation on endocytosis could be very indirect (e.g. changes in membrane tension, receptor clustering and lateral diffusion or membrane fluidity). Thus, this review will focus on the discrete molecular events driving local actin polymerization during endocytic carrier formation.

Localized actin polymerization during nanometer carrier budding.

Clathrin-mediated endocytosis. Even though a functional actin cytoskeleton is present at each stage of clathrin coated pit (CCP) formation in mammalian cells(1), its function is dispensable upon low membrane tension (12,23-26). In such cases, forces generated by the polymerization of the clathrin coat, aided by membrane bending activity of adaptors such as Epsin, AP180 or BAR-domain proteins, are sufficient to form clathrin-coated vesicles (CCVs) without the assistance of local actin polymerization (1,2). However, actin is required to counteract high membrane tension at apical or adherent surfaces of cells (where the membrane is stretched by the underlying cytoskeleton), during mitosis, in hypo-osmotic environments or because of mechanical stress (12,27,28). Actin polymerization is also required for the internalization of large cargoes into CCVs, such as elongated VSV viruses (29). Finally, it is crucial for CME in yeast, as inward budding

needs to overcome the outwardly directed turgor pressure of ~1,000 pN and the stiff, ergosterol-rich, membrane (30). Actin is even more important than Clathrin for receptor-mediated endocytosis in yeast as uptake continues, albeit at reduced rate, even in the absence of Clathrin (31,32). This is not the case in mammals, where Clathrin and AP2 are obligatory for CME (33,34).

Actin is consistently found both at the periphery of the clathrin lattice during budding and at the neck of constricted CCPs, forming a ring-like collar (26,35). Thereby, local actin polymerization is believed to push budding vesicles away from the cell cortex and to assist invagination and membrane fission. Actin networks at endocytic sites are rapidly turned over (every 3-4 seconds), being assembled and dissembled concurrently upon actin nucleation, elongation, severing and depolymerisation (14,36). Although the mechanisms of carrier formation are different during yeast and mammalian CME, that of local actin polymerization around CCPs shares similarities. Complete and recent accounts of actin nucleation at yeast endocytic patches are available in excellent reviews (14,37); we focus instead on summarising the commonalities and differences in mechanisms in fungi and mammals.

In yeast, Las17 (WASP in mammals) and Pan1 (Intersectin), are kept inhibited locally by proteins arriving at early sites of CCPs, such as the F-BAR domain protein Syp1 (FCHo1 and 2), Sla1 (also related to Intersectin) and Sla2 (Hip1/Hip1R) (**Figure 1a**) (38-41). Syp1 disappears from CCPs just before the onset of actin assembly, at a time when Bzz1 (Syndapin/PACSIN) is recruited (40,42-44). Bzz1 competes with Sla1 to bind and activate Las17 (45). Ent1 (Epsin) can also activate Las17 and Pan1 to recruit Arp2/3 and initiate local actin branching and nucleation (46). Actin polymerisation emanates from a Las17 ring around the center of the budding vesicle (47). Cdc15 (PSTPIP1) assembles with Myo1 (Myosin) and remains close to the cell surface at the base of the forming tube, whereas Bzz1 and Las17 move away from the plasma membrane with the invaginating tubule (48). Thereby, Cdc15 and Bzz1 create two distinct actin polymerisation sites at CCPs.

In mammals, less is known about actin polymerization during the initiation stage, but N-WASP, Arp2/3, actin and Syndapin 2 levels at CCPs increase when that of FCHo1/2 decreases (44,49). Intersectin 1 and 2 may assume the functions of both Pan1 and Sla1 by recruiting N-WASP via their SH3 domains, in addition to acting as a guanine-nucleotide exchange factor (GEF) towards the Rho family GTPase Cdc42 (**Figure 1a**) (50,51). The SH3 domains of intersectin can also interact with the basic rich (BR) domain of Cdc42 GTPase activating proteins (GAPs) thereby inhibiting their GAP activity (**Figure 1a**) (52). Active Cdc42 recruits FBP17, CIP4 and TOCA-1, which in turn activate N-WASP via their SH3 domains (53,54) . However, as these F-BAR proteins have been detected after vesicle scission, this suggests that they may activate actin polymerization involved in the propulsion of budded CCV (44).

In both yeast and mammals, Sla2 and Hip1 proteins do not promote actin polymerisation but instead regulate the association of the actin cytoskeleton to budding CCPs (55-57). Their N-terminal ANTH domain associates with the Epsin ENTH domain at the plasma membrane, whereas their central coil-coiled region binds to clathrin light chains (CLCs) (58,59). Clathrin binding causes a conformational switch of Hip1/R, which blocks its binding to actin (**Figure 1b**) (60, 61). In addition, Hip1R (but not Hip1) associates to Cortactin via its C-terminal PR domain and inhibits local actin assembly by blocking the elongation of barbed ends (57,62,63). This suggests a mechanism whereby actin is excluded from the coat and restricted to the rim and neck of the budding vesicle, where Hip1/R is not recruited by Clathrin (**Figure 1b**) (61,64).

At the last stages, Epsin and the BAR domain proteins Amphiphysin, Endophilin (Rvs161 and Rvs167 in yeast) and SNX9 mediate the constriction of the neck by membrane curvature via their ENTH and BAR domains respectively, and local actin polymerization through their recruitment of N-WASP (**Figure 1c**) (65-69). These BAR domain proteins also concentrate Dynamin (Vps1 in yeast) at the neck, which polymerizes into ring-like structures to mediate vesicle fission upon GTP hydrolysis (70-72). Dynamin assembly is facilitated by short, gelsolin-capped actin-filaments and its polymerisation in turn enhances its GTPase activity, which removes gelsolin from barbed actin ends and allows elongation of F-actin filaments (73-75). F-actin polymerisation also stimulates dynamin binding to Cortactin in an Arp2/3-dependent manner, which allows for dynamin GTPase-mediated local actin cytoskeleton remodelling (76,77). In yeast, however, Vps1 may be less crucial for scission than Dynamin is in mammals, as actin-generated forces, Rvs161/167 and local lipid modifications appear sufficient (14). Finally, unconventional Type I Myosin motors - such as Myo3/5 in yeast and Myosin 1E and VI in mammals - generate local forces which push coated pits into the cytosol (42,44,78).

The orientation of the actin filaments around CCPs is not yet clear. Consistent with the location of the nucleating machinery, polymerization is constrained at the membrane (26,35,79-81). Electron

tomography and local actin bleaching experiments revealed that actin filaments grow towards the yeast cell cortex (**Figure 1b-c**), forming a roughly spherical 3D network at the bottom of the invagination (26,31,81). However, Las17 was also reported to move in with the membrane invagination (79,82), consistent with the late arrival of N-WASP-binding proteins in mammals, and with the branching of actin filaments transitioning towards constricted CCPs at later stages (**Figure 1c-d**) (26). Thus, actin polymerization occurs at several locations on budding CCVs and the precise details are still unclear.

Clathrin-independent endocytosis. Actin polymerization is key to the formation and budding of many nanometer-scale CIE carriers including CLICs, caveolae, FEME and IL-2Rβ-containing vesicles. Extracellular clustering of lipids and proteins by the GL-Lect mechanism drives initial CLIC formation without the need for actin polymerization (8). Instead, Arf1 recruits ARHGAP10 to disactivate Cdc42 (**Figure 2a**) (83), perhaps to release the local membrane tension that could be imposed by the underlying actin cytoskeleton. Nascent CLICs are then recognized by the cytosolic BAR domain containing proteins GRAF1 or Endophilin (84,85). Precise spatio-temporal regulation of Cdc42/N-WASP/Arp2/3-mediated actin polymerization during CLIC formation is mediated by the GAP domain of GRAF1 (**Figure 2a**) (84). Additional mechanisms may be involved in actin polymerization around Shiga and cholera toxin-containing CLICs (that are less dependent on GRAF1), as Endophilin can bind to N-WASP and to additional Cdc42 GAPs such as Oligophrenin, BPGAP1 and RICH1 (86-88). Finally, forces generated by actin polymerization (84,89,90) and by friction-driven scission generated by BAR proteins (85,91) mediate the scission of CLICs and detachment from the cell surface.

Cholesterol-binding and membrane inserted proteins Caveolin-1 to 3 associate with cytosolic proteins Cavin 1-4 to form a coat which shape caveolae into their characteristic 50-100 nm cave-like invaginations (92, 93). Caveolae bud from the plasma membrane at low frequency and traffic back and forth to early endosomes. They also function as mechano-sensing domains, whereby membrane stretching induces their flattening and buffers mechanical stress (94,95). Consistent with this, caveolae are often found aligned with some actin stress fibers (96). The molecular links are still unclear but RhoA, filamin A (which binds to Caveolin 1 (97)), Myosin 1c (which interacts with Cavin 3 (98)) and perhaps EHD2 and Pacsin 2 (99) are all required. Furthermore, reduction of actin stress fibers by Cytochalasin D or mDia1 and Abl kinases silencing decreases their association to caveolae (100).

In the case of FEME, actin-dependent initiation sites pre-exist receptor activation and carrier formation. Lamellipodin recruits and concentrates Endophilin into distinct patches on the plasma membrane (101, 102). In the absence of receptor activation, these patches disassemble after 5-10 seconds and new ones form nearby, constantly probing the membrane. It appears that active actin remodeling is required as very low doses of actin poisons (one or two orders of magnitude less than the concentration required to block CME), applied for few seconds only, are sufficient to disrupt the formation of Lamellipodin-Endophilin patches and to inhibit FEME (101). PI3K, Rac1, Cdc42, N-WASP and PAK1 and 2 all regulate the formation of FEME carriers. However, details of the coordination of membrane bending and actin polymerization are so far missing (**Figure 2b**). The N-BAR domain of Endophilin induces extensive membrane tubulation and vesicle formation at high local concentration as it contains two amphipathic helices, thereby combining protein scaffolding with hydrophobic insertion (67,103). A synergy between: (i) actin polymerization; (ii) dynein-mediated traction on microtubules; (iii) Dynamin; and (iv) friction-driven scission generated by the BAR domain or Endophilin, is required for FEME carrier scission (85,91,104).

IL-2Rβ or γc chains expressed ectopically in non-immune cells are constitutively internalized in small (200-500 nm) clathrin-independent carriers (105,106). Unlike FEME, where cytosolic adaptors recruit activated receptors, IL-2Rβ recruits the actin-nucleating complex WAVE1. Similar to several other receptors, IL-2Rβ has a WIRS motif in its cytoplasmic tail that binds to a surface composed of Sra1 and Abi2 in the WAVE1 complex (**Figure 2c**) (107,108). Clustering of IL-2Rβ chains during their concentration into nascent pits might therefore recruit enough copies of WAVE1, followed by N-WASP, to induce Arp2/3-mediated actin protrusions that fall back onto the membrane to form endocytic pits around them (**Figure 2c**) (107). PI3K recruitment by clustered IL-2Rβ, local $PI(3,4,5)P_3$ production and Rac1-mediated WAVE and PAK-1 and -2 activation are also important for local actin polymerization and endocytic carrier formation (**Figure 2c**) (109,110). Although reminiscent of macropinocytosis, actin polymerization and membrane projections

around IL-2Rβ remain localized, creating small and spherical carriers. The molecular basis for this confinement and the absence of extended actin polymerization is not yet understood.

Finally, other CIE processes that rely on RhoA- and formin-dependent actin polymerization exist both in yeast and mammalian cells (111,112). However, how linear actin cables power endocytic carrier formation remains unknown.

Local actin polymerization during micrometer carrier formation

Macropinocytosis relies critically on actin polymerization to power membrane ruffles, which then form micron-wide vacuoles. It mediates the bulk intake of extracellular fluids and molecules, as well as sizable portions of the cell surface (9,113). At highly stimulated synapses, activity-dependent bulk endocytosis (ADBE) is the dominant mode of endocytosis and shares features with macropinocytosis (10,114). In both cases, elevated and sustained signaling emanating from activated receptors induces the localized actin polymerization underneath large membrane ruffles. These fold back onto the cell surface to produce large (0.5 to >10 μm) endocytic carriers. The formation of membrane extensions relies exclusively on constant actin remodeling and low doses of actin poisons are sufficient to hinder endocytosis.

Initially, activated receptors and Ras (Ras-GTP) both recruit and activate phosphoinositides 3-kinases (PI3Ks) that produce localized patches of $PI(3,4,5)P_3$ upon phosphorylation of $PI(4,5)P_2$ (**Figure 3**) (115). $PI(3,4,5)P_3$ has a central role in triggering actin polymerization. First, electrostatic interactions between the negative charges of $PI(3,4,5)P_3$ and polybasic motifs within Rac1 and Cdc42 recruit and activate them at precise locations on the plasma membrane. Consistently, inhibiting PI3K or accumulating cytosolic H^+ (by preventing Na^+/H^+ transfer across the plasma membrane using amiloride, or its 5-(N-Ethyl-N-isopropyl) amiloride (EIPA) derivative) neutralizes negative charges of the inner membrane leaflet and blocks ruffling and macropinocytosis (116). Active Rac and Cdc42 also stimulate PAK family kinases, which support sustained actin reorganization and membrane ruffling (**Figure 3**), mostly by inactivating MLC kinase (which activates Myosin) and activating LIMK kinase (which inactivates the actin depolymerization factor Cofilin) (117). Formins (mDia) and myosins are also involved in bundling actin and forming finger-like protrusions (filopodia) at the edge of macropinocytic cups (9, 113). Second, $PI(3,4,5)P_3$ recruits phospholipases (PLC) that hydrolyze $PI(4,5)P_2$ locally into inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) (**Figure 3**). IP_3 and DAG then activate protein kinase C (PKC) (**Figure 3**), which is a potent activator of actin polymerization (118). It does so mainly by activating Src, which in turn activates p190RhoGAP and thus downregulates RhoA, thereby promoting Cdc42 and Rac1 activities (119). Third, local dephosphorylation of $PI(3,4,5)P_3$ into $PI(3,4)P_2$ by SHIP1 or 2 recruits actin remodeling proteins such as Lamellipodin and the BAR domain-containing SNX9 (**Figure 3**)(120,121). SNX9 locally recruits and activates Cdc42 and N-WASP promoting actin branching (122). Lamellipodin recruits the actin elongation factors ENA/VASP to mediate the extension of the branched filaments (120), thereby supporting the expansion of the actin network underneath membrane ruffles (**Figure 3**). GTP-loaded Rac1 and Cdc42 are switched off by several GTPase activating proteins (GAPs), including ARHGAP12, ARHGAP25 and SH3BP1 (123), thereby terminating actin polymerization. Thus, during macropinocytosis, local actin polymerization is propagated, generating membrane ruffles that form micrometer size endocytic carriers.

Perspectives and remaining challenges

Actin polymerization powers endocytic carriers that form either rapidly, under high membrane tension or without the aid of dense and rigid proteinaceous coats. The actin branching complex Arp2/3, recruited either by Cdc42/N-WASP or Rac1/WAVE, is locally recruited by a variety of endocytic adaptors, thereby linking membrane bending and actin polymerization. Upstream events such as the local recruitment and activation of Cdc42 and Rac1 by GEFs (such as the ones of the Intersectin, ELMO, VAV, DOCK families) are not well known. Arf1 and Arf6 proteins, known to take part in CME and CIE, might be involved (124). The molecular steps for RhoA- and formin-mediated actin bundling during endocytic pit formation are also not well understood. An outstanding question remains how actin polymerization is confined during nanometer endocytic carrier formation and propagated laterally on the membrane during macropinocytosis. The precise spatio-temporal mapping of actin polymerization is still missing, in particular during nanometer CIE carrier formation. It is evident that the actin networks assembled are very dynamic and turn over multiple times over the lifetime of the carrier formation. However, the mechanisms of actin network dissolution and local filament

dynamics are not well understood. The polarity of actin filaments and any potential transition from pushing to propelling forces during carrier budding are also not clear. Another important issue for further study will be to understand how endocytic carriers move in the opposite orientation to the existing actin network. Dense cross-linked cortical actin or branched extended network underlay most, if not all, of the plasma membrane and exert forces pushing towards the cell surface. Hence, any endocytic vesicle must either form at spots with no such pre-existing networks or be coupled with local actin depolymerization, to make way for the carrier to move into the cytoplasm.

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Figure Legends

Figure 1. Local actin polymerization during Clathrin-mediated endocytosis.

a, Early arriving protein FCHo1/2 (Syp1 in yeast), Eps15 and Intersectin (Pan1 and Sla1) recruit and activate AP2 and inactivate N-WASP (Las17), thereby inhibiting actin polymerization.

b, N-WASP is recruited and activated by Epsin (Ent1) and other adaptors at the rim of the clathrin coat. The binding of Hip1/Hip1R (Sla2) to Clathrin light chain and to Cortactin reduce its capacity to bind to F-actin and may trigger local actin depolymerization, facilitating the coat formation.

c, Epsin, N-BAR domain proteins Amphiphysin, Endophilin and SNX9 and possibly F-BAR proteins such as Syndapin, mediate Dynamin recruitment and induce neck constriction upon local membrane bending and actin polymerization.

d, N-WASP-mediated actin polymerization at the back of Clathrin-mediated vesicle and local actin depolymerization at the front mediate vesicle budding, and movement into the cytosol.

Figure 2. Local actin polymerization during Clathrin-independent endocytosis.

a, Extracellularly-clustered cargoes (*e.g.* CD44 by Galectin-3, GM1 by Shiga toxin or GPI-anchored protein clustered into cholesterol-rich nanodomains) induce the initial inward membrane bending into the cytosol. Local and timely Cdc42 inactivation is mediated by ARHGAP10 recruited by Arf1 to the plasma membrane (likely at initial stages) and then by Cdc42-GAP activity of GRAF1 (at later stages). Local Cdc42/N-WASP-induced actin polymerization mediate CLIC formation but the precise molecular links to the invaginations are still unknown.

b, Fast Endophilin-mediated endocytosis (FEME) requires local $PI(3,4)P_2$ production by SHIP2 and the recruitment of Lamellipodin (Lpd) and Endophilin. N-WASP and Arp2/3-mediated actin polymerization as well as Rac1/WAVE and PAK1/2 activities, are required for local actin assembly but the details are missing so far. N-WASP may be recruited and activated by the SH3 domain of Endophilin.

c, IL2Rβ clustering stimulates WAVE recruitment to WIRS motifs present in the cytoplasmic tails of IL2Rβ. IL2Rβ also recruit PI3K to produce locally $PI(3,4,5)P_3$, which recruits Rac1, thereby activating WAVE and PAK1. N-WASP, Cortactin and Dynamin are then mediating IL2Rβ endocytic pit closure and detachment from the cell surface.

Figure 3. Local actin polymerization during macropinocytosis.

Sustained and/or elevated receptor signaling trigger intense local Ras and PI3K signaling, which produce $PI(3,4,5)P_3$ and recruit Cdc42-N-WASP and Rac1-WAVE complexes as well as PAK1, thereby promoting actin polymerization-induced membrane ruffling and protrusions. $PI(3,4,5)P_3$ activates and recruits PLC, which hydrolyzes $PI(4,5)P_2$ into IP_3 and DAG, which in turn activate PKC, thus stimulating further actin polymerization. SHIP1 and 2 dephosphorylate $PI(3,4,5)P_3$ into $PI(3,4)P_2$, thereby recruiting SNX9 (which activates N-WASP) and Lamellipodin (Lpd), which mediates actin filament elongation through ENA/VASP.

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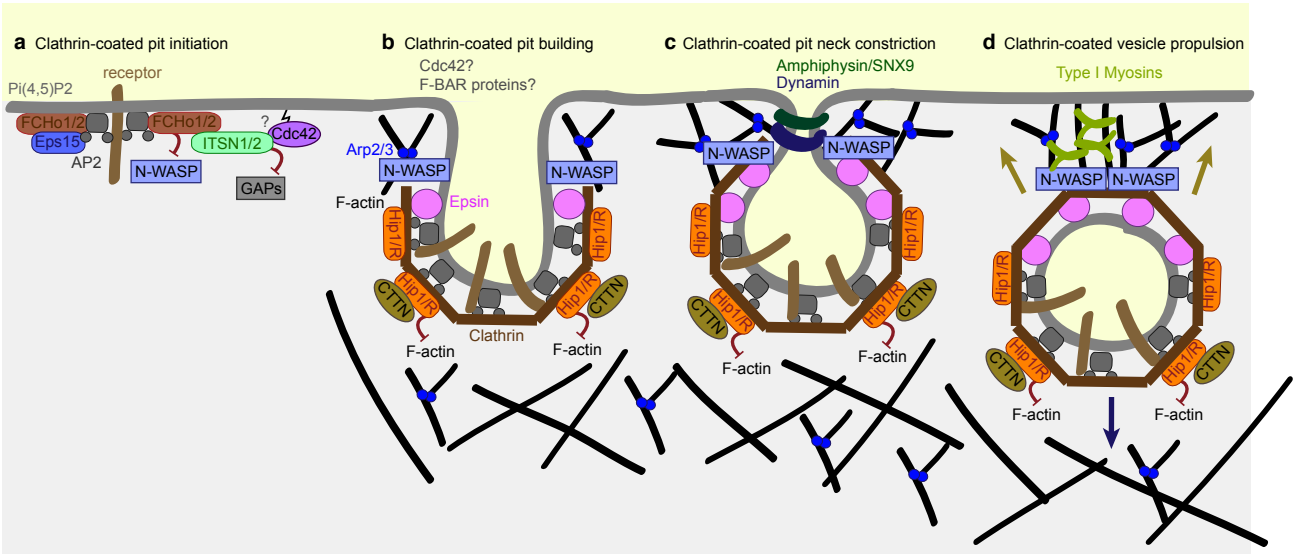

Figure 1. Local actin polymerization during Clathrin-mediated endocytosis

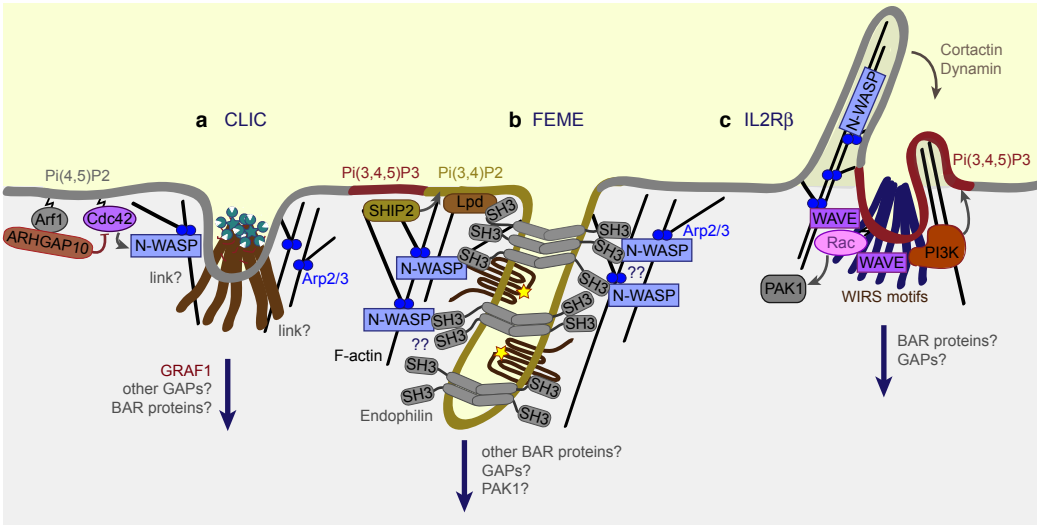

Figure 2. Local actin polymerization during Clathrin-independent endocytosis

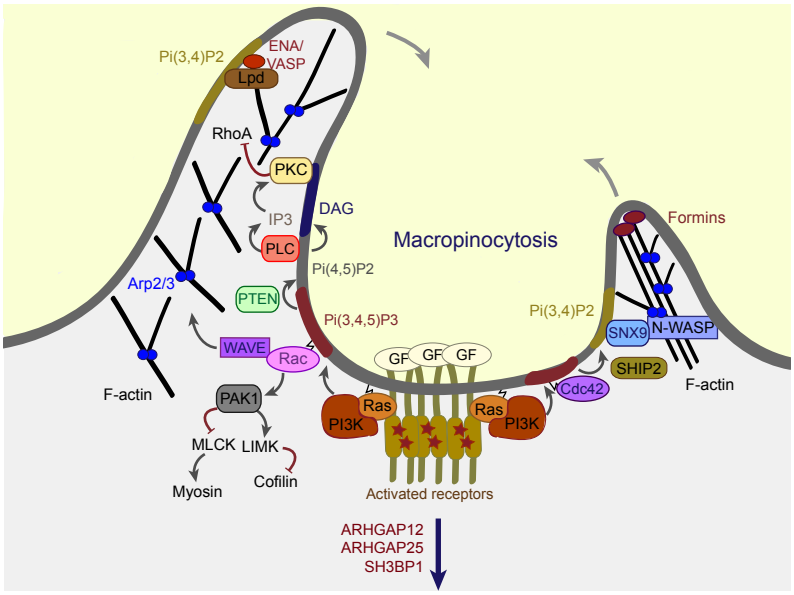

Figure 3. Local actin polymerization during macropinocytosis