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

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

53 structures with a positively charged concave surface that senses, stabilizes and induces membrane
54 curvature (7). Extracellular lipid or cargo clustering drives the formation of Clathrin-independent carriers /
55 Glycosylphosphatidylinositol (GPI)-anchored protein enriched endocytic compartments (CLIC/GEEC,
56 hereafter CLIC) and the uptake of Shiga and cholera toxins (8). Membrane protrusions folding back onto the
57 plasma membrane create large carriers during macropinocytosis and activity-dependent bulk endocytosis
58 (ADBE) at synapses (9,10).
59   The energetic cost of deforming a membrane depends mainly on its rigidity. Thus, the energy
60 required to form an endocytic carrier is not dependent on its size but is instead the result of the local
61 membrane rigidity. In the case of a biological membrane that has a typical bending modulus of 10-20 k_BT
62 (where k_B is the Boltzmann constant and T the temperature), about 250-500 k_BT (being the energy produced
63 by the hydrolysis of 10-20 ATP molecules) must be produced to form a vesicle (11). Although Clathrin and its
64 adaptor proteins can bend the plasma membrane locally to produce coated vesicles, additional forces
65 provided by the actin cytoskeleton are required in some physiological contexts to counter membrane tension
66 or internal cytoplasmic pressure. Two opposite situations are coated pit formation at the basolateral surface
67 of polarized mammalian cells (independent of actin) and endocytosis in yeast (dependent of actin) (12-14).
68 Perhaps because of their larger sizes, their speed of formation or the absence of dense and rigid
69 proteinaceous coats around CIE carriers, their formations rely on actin polymerization (15).
70 Actin transitions between a monomeric globular form (G-actin) and a polymeric filamentous form (F-actin)
71 upon ATP loading. Actin polymerizes into helical, two-stranded filaments, the slow- and fast-growing ends of
72 which are named 'pointed' and 'barbed' ends, respectively. Actin filaments can be crosslinked (e.g. cortical
73 actin), bundled together (e.g. filopodia) or branch into extended network (e.g. lamellipodia) (16). This network
74 of filaments forms the actin cytoskeleton, performing multiple functions in cells, from providing structural
75 support to membranes to propelling organelles (17). As actin monomers are in vast excess in cells, the
76 availability of uncapped (free) barbed ends is rate limiting for the growth of actin filaments. While actin
77 filament capping, cross-linking (*e.g.* Fimbrin, Fascin, α-actinin, Spectrin, Filamin and Myosin), elongating
78 (e.g. ENA/VASP), severing and depolymerizing proteins (*e.g.* Gelsolin, Severin, ADF/Cofilin) are all
79 important regulators, the growth of the actin network is stimulated by regulated nucleation (18-20). Formins
80 and the Arp2/3 complex are the main actin assembly-promoting factors. Branched filaments can only be
81 nucleated by the Arp2/3 complex, which serves as a template to trigger the polymerization of a new filament
82 at a 70 degree angle from the original one (21). As Arp2/3 has minimal basal activity, nucleation-promoting
83 factors (NPF) such as the WASP/N-WASP and the SCAR/WAVE complexes are required to induce strong
84 branching and nucleation (18,19,22). In the cytosol, N-WASP is auto-inhibited, while WAVE is constitutively
85 active but inhibited in *trans* by the WAVE-regulatory complex (WRC). Both complexes need to be recruited
86 and activated by additional proteins to induce local actin polymerization. RhoA, Rac1 and Cdc42 are
87 membrane-bound GTPases that trigger local actin polymerization upon recruitment and activation. GTP-
88 bound Rac1 and Cdc42 activate Arp2/3-mediated actin branching through the WASP/N-WASP and
89 SCAR/WAVE complexes, respectively. RhoA activates formins such as mDia.
90   While many publications have reported a requirement for actin in endocytosis, the evidence was
91 often limited to its perturbation upon actin depolymerization or stabilization (Cytochalasin D, Latrunculin or
92 Jasplakinolide treatments). The effects of actin cytoskeleton perturbation on endocytosis could be very
93 indirect (e.g. changes in membrane tension, receptor clustering and lateral diffusion or membrane fluidity).
94 Thus, this review will focus on the discrete molecular events driving local actin polymerization during
95 endocytic carrier formation.
96
97 **Localized actin polymerization during nanometer carrier budding.**
98 ***Clathrin-mediated endocytosis.*** Even though a functional actin cytoskeleton is present at each stage of
99 clathrin coated pit (CCP) formation in mammalian cells(1), its function is dispensable upon low membrane
100 tension (12,23-26). In such cases, forces generated by the polymerization of the clathrin coat, aided by
101 membrane bending activity of adaptors such as Epsin, AP180 or BAR-domain proteins, are sufficient to form
102 clathrin-coated vesicles (CCVs) without the assistance of local actin polymerization (1,2). However, actin is
103 required to counteract high membrane tension at apical or adherent surfaces of cells (where the membrane
104 is stretched by the underlying cytoskeleton), during mitosis, in hypo-osmotic environments or because of
105 mechanical stress (12,27,28). Actin polymerization is also required for the internalization of large cargoes
106 into CCVs, such as elongated VSV viruses (29). Finally, it is crucial for CME in yeast, as inward budding

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

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

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

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

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

147 At the last stages, Epsin and the BAR domain proteins Amphiphysin, Endophilin (Rvs161 and
148 Rvs167 in yeast) and SNX9 mediate the constriction of the neck by membrane curvature via their ENTH and
149 BAR domains respectively, and local actin polymerization through their recruitment of N-WASP (**Figure 1c**)
150 (65-69). These BAR domain proteins also concentrate Dynamin (Vps1 in yeast) at the neck, which
151 polymerizes into ring-like structures to mediate vesicle fission upon GTP hydrolysis (70-72). Dynamin
152 assembly is facilitated by short, gelsolin-capped actin-filaments and its polymerisation in turn enhances its
153 GTPase activity, which removes gelsolin from barbed actin ends and allows elongation of F-actin filaments
154 (73-75). F-actin polymerisation also stimulates dynamin binding to Cortactin in an Arp2/3-dependent manner,
155 which allows for dynamin GTPase-mediated local actin cytoskeleton remodelling (76,77). In yeast, however,
156 Vps1 may be less crucial for scission than Dynamin is in mammals, as actin-generated forces, Rvs161/167
157 and local lipid modifications appear sufficient (14). Finally, unconventional Type I Myosin motors - such as
158 Myo3/5 in yeast and Myosin 1E and VI in mammals - generate local forces which push coated pits into the
159 cytosol (42,44,78).

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

162 tomography and local actin bleaching experiments revealed that actin filaments grow towards the yeast cell
163 cortex (**Figure 1b-c**), forming a roughly spherical 3D network at the bottom of the invagination (26,31,81).
164 However, Las17 was also reported to move in with the membrane invagination (79,82), consistent with the
165 late arrival of N-WASP-binding proteins in mammals, and with the branching of actin filaments transitioning
166 towards constricted CCPs at later stages (**Figure 1c-d**) (26). Thus, actin polymerization occurs at several
167 locations on budding CCVs and the precise details are still unclear.
168
169 ***Clathrin-independent endocytosis.*** Actin polymerization is key to the formation and budding of many
170 nanometer-scale CIE carriers including CLICs, caveolae, FEME and IL-2Rβ-containing vesicles.
171 Extracellular clustering of lipids and proteins by the GL-Lect mechanism drives initial CLIC formation without
172 the need for actin polymerization (8). Instead, Arf1 recruits ARHGAP10 to disactivate Cdc42 (**Figure 2a**)
173 (83), perhaps to release the local membrane tension that could be imposed by the underlying actin
174 cytoskeleton. Nascent CLICs are then recognized by the cytosolic BAR domain containing proteins GRAF1
175 or Endophilin (84,85). Precise spatio-temporal regulation of Cdc42/N-WASP/Arp2/3-mediated actin
176 polymerization during CLIC formation is mediated by the GAP domain of GRAF1 (**Figure 2a**) (84). Additional
177 mechanisms may be involved in actin polymerization around Shiga and cholera toxin-containing CLICs (that
178 are less dependent on GRAF1), as Endophilin can bind to N-WASP and to additional Cdc42 GAPs such as
179 Oligophrenin, BPGAP1 and RICH1 (86-88). Finally, forces generated by actin polymerization (84,89,90) and
180 by friction-driven scission generated by BAR proteins (85,91) mediate the scission of CLICs and detachment
181 from the cell surface.
182   Cholesterol-binding and membrane inserted proteins Caveolin-1 to 3 associate with cytosolic
183 proteins Cavin 1-4 to form a coat which shape caveolae into their characteristic 50-100 nm cave-like
184 invaginations (92, 93). Caveolae bud from the plasma membrane at low frequency and traffic back and forth
185 to early endosomes. They also function as mechano-sensing domains, whereby membrane stretching
186 induces their flattening and buffers mechanical stress (94,95). Consistent with this, caveolae are often found
187 aligned with some actin stress fibers (96). The molecular links are still unclear but RhoA, filamin A (which
188 binds to Caveolin 1 (97)), Myosin 1c (which interacts with Cavin 3 (98)) and perhaps EHD2 and Pacsin 2
189 (99) are all required. Furthermore, reduction of actin stress fibers by Cytochalasin D or mDia1 and Abl
190 kinases silencing decreases their association to caveolae (100).
191   In the case of FEME, actin-dependent initiation sites pre-exist receptor activation and carrier
192 formation. Lamellipodin recruits and concentrates Endophilin into distinct patches on the plasma membrane
193 (101, 102). In the absence of receptor activation, these patches disassemble after 5-10 seconds and new
194 ones form nearby, constantly probing the membrane. It appears that active actin remodeling is required as
195 very low doses of actin poisons (one or two orders of magnitude less than the concentration required to
196 block CME), applied for few seconds only, are sufficient to disrupt the formation of Lamellipodin-Endophilin
197 patches and to inhibit FEME (101). PI3K, Rac1, Cdc42, N-WASP and PAK1 and 2 all regulate the formation
198 of FEME carriers. However, details of the coordination of membrane bending and actin polymerization are so
199 far missing (**Figure 2b**). The N-BAR domain of Endophilin induces extensive membrane tubulation and
200 vesicle formation at high local concentration as it contains two amphipathic helices, thereby combining
201 protein scaffolding with hydrophobic insertion (67,103). A synergy between: (i) actin polymerization; (ii)
202 dynein-mediated traction on microtubules; (iii) Dynamin; and (iv) friction-driven scission generated by the
203 BAR domain or Endophilin, is required for FEME carrier scission (85,91,104).
204   IL-2Rβ or γc chains expressed ectopically in non-immune cells are constitutively internalized in small
205 (200-500 nm) clathrin-independent carriers (105,106). Unlike FEME, where cytosolic adaptors recruit
206 activated receptors, IL-2Rβ recruits the actin-nucleating complex WAVE1. Similar to several other receptors,
207 IL-2Rβ has a WIRS motif in its cytoplasmic tail that binds to a surface composed of Sra1 and Abi2 in the
208 WAVE1 complex (**Figure 2c**) (107,108). Clustering of IL-2Rβ chains during their concentration into nascent
209 pits might therefore recruit enough copies of WAVE1, followed by N-WASP, to induce Arp2/3-mediated actin
210 protrusions that fall back onto the membrane to form endocytic pits around them (**Figure 2c**) (107). PI3K
211 recruitment by clustered IL-2Rβ, local PI(3,4,5)P$_3$ production and Rac1-mediated WAVE and PAK-1 and -2
212 activation are also important for local actin polymerization and endocytic carrier formation (**Figure 2c**)
213 (109,110). Although reminiscent of macropinocytosis, actin polymerization and membrane projections

214 around IL-2Rβ remain localized, creating small and spherical carriers. The molecular basis for this
215 confinement and the absence of extended actin polymerization is not yet understood.
216   Finally, other CIE processes that rely on RhoA- and formin-dependent actin polymerization exist
217 both in yeast and mammalian cells (111,112). However, how linear actin cables power endocytic carrier
218 formation remains unknown.
219

Local actin polymerization during micrometer carrier formation

221 Macropinocytosis relies critically on actin polymerization to power membrane ruffles, which then form micron-
222 wide vacuoles. It mediates the bulk intake of extracellular fluids and molecules, as well as sizable portions of
223 the cell surface (9,113). At highly stimulated synapses, activity-dependent bulk endocytosis (ADBE) is the
224 dominant mode of endocytosis and shares features with macropinocytosis (10,114). In both cases, elevated
225 and sustained signaling emanating from activated receptors induces the localized actin polymerization
226 underneath large membrane ruffles. These fold back onto the cell surface to produce large (0.5 to >10 μm)
227 endocytic carriers. The formation of membrane extensions relies exclusively on constant actin remodeling
228 and low doses of actin poisons are sufficient to hinder endocytosis.
229 Initially, activated receptors and Ras (Ras-GTP) both recruit and activate phosphoinositides 3-kinases
230 (PI3Ks) that produce localized patches of PI(3,4,5)P_3 upon phosphorylation of PI(4,5)P_2 (**Figure 3**) (115).
231 PI(3,4,5)P_3 has a central role in triggering actin polymerization. First, electrostatic interactions between the
232 negative charges of PI(3,4,5)P_3 and polybasic motifs within Rac1 and Cdc42 recruit and activate them at
233 precise locations on the plasma membrane. Consistently, inhibiting PI3K or accumulating cytosolic H^+ (by
234 preventing Na^+/H^+ transfer across the plasma membrane using amiloride, or its 5-(N-Ethyl-N-isopropyl)
235 amiloride (EIPA) derivative) neutralizes negative charges of the inner membrane leaflet and blocks ruffling
236 and macropinocytosis (116). Active Rac and Cdc42 also stimulate PAK family kinases, which support
237 sustained actin reorganization and membrane ruffling (**Figure 3**), mostly by inactivating MLC kinase (which
238 activates Myosin) and activating LIMK kinase (which inactivates the actin depolymerization factor Cofilin)
239 (117). Formins (mDia) and myosins are also involved in bundling actin and forming finger-like protrusions
240 (filopodia) at the edge of macropinocytic cups (9, 113). Second, PI(3,4,5)P_3 recruits phospholipases (PLC)
241 that hydrolyze PI(4,5)P_2 locally into inositol 1,4,5-trisphosphate (IP$_3$) and diacylglycerol (DAG) (**Figure 3**). IP$_3$
242 and DAG then activate protein kinase C (PKC) (**Figure 3**), which is a potent activator of actin polymerization
243 (118). It does so mainly by activating Src, which in turn activates p190RhoGAP and thus downregulates
244 RhoA, thereby promoting Cdc42 and Rac1 activities (119). Third, local dephosphorylation of PI(3,4,5)P_3 into
245 PI(3,4)P_2 by SHIP1 or 2 recruits actin remodeling proteins such as Lamellipodin and the BAR domain-
246 containing SNX9 (**Figure 3**)(120,121). SNX9 locally recruits and activates Cdc42 and N-WASP promoting
247 actin branching (122). Lamellipodin recruits the actin elongation factors ENA/VASP to mediate the extension
248 of the branched filaments (120), thereby supporting the expansion of the actin network underneath
249 membrane ruffles (**Figure 3**). GTP-loaded Rac1 and Cdc42 are switched off by several GTPase activating
250 proteins (GAPs), including ARHGAP12, ARHGAP25 and SH3BP1 (123), thereby terminating actin
251 polymerization. Thus, during macropinocytosis, local actin polymerization is propagated, generating
252 membrane ruffles that form micrometer size endocytic carriers.
253

Perspectives and remaining challenges

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

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

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.

References
1. Kaksonen M, Roux A. Mechanisms of clathrin-mediated endocytosis. Nat Rev Mol Cell Biol. 2018.
2. McMahon HT, Boucrot E. Molecular mechanism and physiological functions of clathrin-mediated endocytosis. Nat Rev Mol Cell Biol. 2011;12(8):517-33.
3. Bitsikas V, Correa IR, Jr., Nichols BJ. Clathrin-independent pathways do not contribute significantly to endocytic flux. Elife. 2014;3:e03970.

4. Johannes L, Parton RG, Bassereau P, Mayor S. Building endocytic pits without clathrin. Nat Rev Mol Cell Biol. 2015;16(5):311-21.

5. Ferreira APA, Boucrot E. Mechanisms of Carrier Formation during Clathrin-Independent Endocytosis. Trends Cell Biol. 2017.

6. Doherty GJ, McMahon HT. Mechanisms of endocytosis. Annu Rev Biochem. 2009;78:857-902.

7. Peter BJ, Kent HM, Mills IG, Vallis Y, Butler PJ, Evans PR, et al. BAR domains as sensors of membrane curvature: the amphiphysin BAR structure. Science. 2004;303(5657):495-9.

8. Johannes L, Wunder C, Shafaq-Zadah M. Glycolipids and Lectins in Endocytic Uptake Processes. J Mol Biol. 2016.

9. Mercer J, Helenius A. Gulping rather than sipping: macropinocytosis as a way of virus entry. Curr Opin Microbiol. 2012;15(4):490-9.

10. Cousin MA. Integration of Synaptic Vesicle Cargo Retrieval with Endocytosis at Central Nerve Terminals. Front Cell Neurosci. 2017;11:234.

11. Kozlov MM, Campelo F, Liska N, Chernomordik LV, Marrink SJ, McMahon HT. Mechanisms shaping cell membranes. Curr Opin Cell Biol. 2014;29:53-60.

12. Boulant S, Kural C, Zeeh JC, Ubelmann F, Kirchhausen T. Actin dynamics counteract membrane tension during clathrin-mediated endocytosis. Nat Cell Biol. 2011;13(9):1124-31.

13. Kaksonen M, Toret CP, Drubin DG. Harnessing actin dynamics for clathrin-mediated endocytosis. Nat Rev Mol Cell Biol. 2006;7(6):404-14.

14. Goode BL, Eskin JA, Wendland B. Actin and endocytosis in budding yeast. Genetics. 2015;199(2):315-58.

15. Romer W, Pontani LL, Sorre B, Rentero C, Berland L, Chambon V, et al. Actin dynamics drive membrane reorganization and scission in clathrin-independent endocytosis. Cell. 2010;140(4):540-53.

16. Pollard TD, Cooper JA. Actin, a central player in cell shape and movement. Science. 2009;326(5957):1208-12.

17. Pollard TD, Borisy GG. Cellular motility driven by assembly and disassembly of actin filaments. Cell. 2003;112(4):453-65.

18. Campellone KG, Welch MD. A nucleator arms race: cellular control of actin assembly. Nat Rev Mol Cell Biol. 2010;11(4):237-51.

19. Rotty JD, Wu C, Bear JE. New insights into the regulation and cellular functions of the ARP2/3 complex. Nat Rev Mol Cell Biol. 2013;14(1):7-12.

20. Krause M, Gautreau A. Steering cell migration: lamellipodium dynamics and the regulation of directional persistence. Nat Rev Mol Cell Biol. 2014;15(9):577-90.

21. Mullins RD, Kelleher JF, Xu J, Pollard TD. Arp2/3 complex from Acanthamoeba binds profilin and cross-links actin filaments. Mol Biol Cell. 1998;9(4):841-52.

22. Takenawa T, Suetsugu S. The WASP-WAVE protein network: connecting the membrane to the cytoskeleton. Nat Rev Mol Cell Biol. 2007;8(1):37-48.

23. Merrifield CJ, Feldman ME, Wan L, Almers W. Imaging actin and dynamin recruitment during invagination of single clathrin-coated pits. Nat Cell Biol. 2002;4(9):691-8.

24. Yarar D, Waterman-Storer CM, Schmid SL. A dynamic actin cytoskeleton functions at multiple stages of clathrin-mediated endocytosis. Mol Biol Cell. 2005;16(2):964-75.

25. Boucrot E, Saffarian S, Massol R, Kirchhausen T, Ehrlich M. Role of lipids and actin in the formation of clathrin-coated pits. Exp Cell Res. 2006;312(20):4036-48.

26. Collins A, Warrington A, Taylor KA, Svitkina T. Structural organization of the actin cytoskeleton at sites of clathrin-mediated endocytosis. Curr Biol. 2011;21(14):1167-75.

27. Tacheva-Grigorova SK, Santos AJ, Boucrot E, Kirchhausen T. Clathrin-mediated endocytosis persists during unperturbed mitosis. Cell Rep. 2013;4(4):659-68.

28. Kaur S, Fielding AB, Gassner G, Carter NJ, Royle SJ. An unmet actin requirement explains the mitotic inhibition of clathrin-mediated endocytosis. Elife. 2014;3:e00829.

29. Cureton DK, Massol RH, Saffarian S, Kirchhausen TL, Whelan SP. Vesicular stomatitis virus enters cells through vesicles incompletely coated with clathrin that depend upon actin for internalization. PLoS Pathog. 2009;5(4):e1000394.

30. Basu R, Munteanu EL, Chang F. Role of turgor pressure in endocytosis in fission yeast. Mol Biol Cell. 2014;25(5):679-87.

31. Kaksonen M, Toret CP, Drubin DG. A modular design for the clathrin- and actin-mediated endocytosis machinery. Cell. 2005;123(2):305-20.

32. Newpher TM, Lemmon SK. Clathrin is important for normal actin dynamics and progression of Sla2p-containing patches during endocytosis in yeast. Traffic. 2006;7(5):574-88.

33. Motley A, Bright NA, Seaman MN, Robinson MS. Clathrin-mediated endocytosis in AP-2-depleted cells. J Cell Biol. 2003;162(5):909-18.

34. Boucrot E, Saffarian S, Zhang R, Kirchhausen T. Roles of AP-2 in clathrin-mediated endocytosis. PLoS One. 2010;5(5):e10597.

35. Sochacki KA, Dickey AM, Strub MP, Taraska JW. Endocytic proteins are partitioned at the edge of the clathrin lattice in mammalian cells. Nat Cell Biol. 2017;19(4):352-61.

36. Merrifield CJ. Seeing is believing: imaging actin dynamics at single sites of endocytosis. Trends Cell Biol. 2004;14(7):352-8.

37. Merrifield CJ, Kaksonen M. Endocytic accessory factors and regulation of clathrin-mediated endocytosis. Cold Spring Harb Perspect Biol. 2014;6(11):a016733.

38. Rodal AA, Manning AL, Goode BL, Drubin DG. Negative regulation of yeast WASp by two SH3 domain-containing proteins. Curr Biol. 2003;13(12):1000-8.

39. Toshima J, Toshima JY, Duncan MC, Cope MJ, Sun Y, Martin AC, et al. Negative regulation of yeast Eps15-like Arp2/3 complex activator, Pan1p, by the Hip1R-related protein, Sla2p, during endocytosis. Mol Biol Cell. 2007;18(2):658-68.

40. Boettner DR, D'Agostino JL, Torres OT, Daugherty-Clarke K, Uygur A, Reider A, et al. The F-BAR protein Syp1 negatively regulates WASp-Arp2/3 complex activity during endocytic patch formation. Curr Biol. 2009;19(23):1979-87.

41. Feliciano D, Di Pietro SM. SLAC, a complex between Sla1 and Las17, regulates actin polymerization during clathrin-mediated endocytosis. Mol Biol Cell. 2012;23(21):4256-72.

42. Sun Y, Martin AC, Drubin DG. Endocytic internalization in budding yeast requires coordinated actin nucleation and myosin motor activity. Dev Cell. 2006;11(1):33-46.

43. Stimpson HE, Toret CP, Cheng AT, Pauly BS, Drubin DG. Early-arriving Syp1p and Ede1p function in endocytic site placement and formation in budding yeast. Mol Biol Cell. 2009;20(22):4640-51.

44. Taylor MJ, Perrais D, Merrifield CJ. A high precision survey of the molecular dynamics of mammalian clathrin-mediated endocytosis. PLoS Biol. 2011;9(3):e1000604.

45. Tonikian R, Xin X, Toret CP, Gfeller D, Landgraf C, Panni S, et al. Bayesian modeling of the yeast SH3 domain interactome predicts spatiotemporal dynamics of endocytosis proteins. PLoS Biol. 2009;7(10):e1000218.

46. Skruzny M, Brach T, Ciuffa R, Rybina S, Wachsmuth M, Kaksonen M. Molecular basis for coupling the plasma membrane to the actin cytoskeleton during clathrin-mediated endocytosis. Proc Natl Acad Sci U S A. 2012;109(38):E2533-42.

399   47.   Wang X, Galletta BJ, Cooper JA, Carlsson AE. Actin-Regulator Feedback Interactions during Endocytosis. Biophys J.
400   2016;110(6):1430-43.
401   48.   Arasada R, Pollard TD. Distinct roles for F-BAR proteins Cdc15p and Bzz1p in actin polymerization at sites of endocytosis in
402   fission yeast. Curr Biol. 2011;21(17):1450-9.
403   49.   Henne WM, Boucrot E, Meinecke M, Evergren E, Vallis Y, Mittal R, et al. FCHo proteins are nucleators of clathrin-mediated
404   endocytosis. Science. 2010;328(5983):1281-4.
405   50.   Hussain NK, Jenna S, Glogauer M, Quinn CC, Wasiak S, Guipponi M, et al. Endocytic protein intersectin-l regulates actin
406   assembly via Cdc42 and N-WASP. Nat Cell Biol. 2001;3(10):927-32.
407   51.   Novokhatska O, Dergai M, Houssin N, Tsyba L, Moreau J, Rynditch A. Intersectin 2 nucleotide exchange factor regulates
408   Cdc42 activity during Xenopus early development. Biochem Biophys Res Commun. 2011;408(4):663-8.
409   52.   Primeau M, Ben Djoudi Ouadda A, Lamarche-Vane N. Cdc42 GTPase-activating protein (CdGAP) interacts with the SH3D
410   domain of Intersectin through a novel basic-rich motif. FEBS Lett. 2011;585(6):847-53.
411   53.   Giuliani C, Troglio F, Bai Z, Patel FB, Zucconi A, Malabarba MG, et al. Requirements for F-BAR proteins TOCA-1 and TOCA-
412   2 in actin dynamics and membrane trafficking during Caenorhabditis elegans oocyte growth and embryonic epidermal
413   morphogenesis. PLoS Genet. 2009;5(10):e1000675.
414   54.   Watson JR, Fox HM, Nietlispach D, Gallop JL, Owen D, Mott HR. Investigation of the Interaction between Cdc42 and Its
415   Effector TOCA1: HANDOVER OF Cdc42 TO THE ACTIN REGULATOR N-WASP IS FACILITATED BY DIFFERENTIAL
416   BINDING AFFINITIES. J Biol Chem. 2016;291(26):13875-90.
417   55.   Kaksonen M, Sun Y, Drubin DG. A pathway for association of receptors, adaptors, and actin during endocytic internalization.
418   Cell. 2003;115(4):475-87.
419   56.   Henry KR, D'Hondt K, Chang J, Newpher T, Huang K, Hudson RT, et al. Scd5p and clathrin function are important for cortical
420   actin organization, endocytosis, and localization of sla2p in yeast. Mol Biol Cell. 2002;13(8):2607-25.
421   57.   Engqvist-Goldstein AE, Zhang CX, Carreno S, Barroso C, Heuser JE, Drubin DG. RNAi-mediated Hip1R silencing results in
422   stable association between the endocytic machinery and the actin assembly machinery. Mol Biol Cell. 2004;15(4):1666-79.
423   58.   Bennett EM, Chen CY, Engqvist-Goldstein AE, Drubin DG, Brodsky FM. Clathrin hub expression dissociates the actin-binding
424   protein Hip1R from coated pits and disrupts their alignment with the actin cytoskeleton. Traffic. 2001;2(11):851-8.
425   59.   Skruzny M, Desfosses A, Prinz S, Dodonova SO, Gieras A, Uetrecht C, et al. An organized co-assembly of clathrin adaptors
426   is essential for endocytosis. Dev Cell. 2015;33(2):150-62.
427   60.   Chen CY, Brodsky FM. Huntingtin-interacting protein 1 (Hip1) and Hip1-related protein (Hip1R) bind the conserved sequence
428   of clathrin light chains and thereby influence clathrin assembly in vitro and actin distribution in vivo. J Biol Chem.
429   2005;280(7):6109-17.
430   61.   Wilbur JD, Chen CY, Manalo V, Hwang PK, Fletterick RJ, Brodsky FM. Actin binding by Hip1 (huntingtin-interacting protein 1)
431   and Hip1R (Hip1-related protein) is regulated by clathrin light chain. J Biol Chem. 2008;283(47):32870-9.
432   62.   Brady RJ, Damer CK, Heuser JE, O'Halloran TJ. Regulation of Hip1r by epsin controls the temporal and spatial coupling of
433   actin filaments to clathrin-coated pits. J Cell Sci. 2010;123(Pt 21):3652-61.
434   63.   Le Clainche C, Pauly BS, Zhang CX, Engqvist-Goldstein AE, Cunningham K, Drubin DG. A Hip1R-cortactin complex
435   negatively regulates actin assembly associated with endocytosis. EMBO J. 2007;26(5):1199-210.
436   64.   Boettner DR, Friesen H, Andrews B, Lemmon SK. Clathrin light chain directs endocytosis by influencing the binding of the
437   yeast Hip1R homologue, Sla2, to F-actin. Mol Biol Cell. 2011;22(19):3699-714.
438   65.   Ferguson SM, Raimondi A, Paradise S, Shen H, Mesaki K, Ferguson A, et al. Coordinated actions of actin and BAR proteins
439   upstream of dynamin at endocytic clathrin-coated pits. Dev Cell. 2009;17(6):811-22.
440   66.   Messa M, Fernandez-Busnadiego R, Sun EW, Chen H, Czapla H, Wrasman K, et al. Epsin deficiency impairs endocytosis by
441   stalling the actin-dependent invagination of endocytic clathrin-coated pits. Elife. 2014;3:e03311.
442   67.   Boucrot E, Pick A, Camdere G, Liska N, Evergren E, McMahon HT, et al. Membrane fission is promoted by insertion of
443   amphipathic helices and is restricted by crescent BAR domains. Cell. 2012;149(1):124-36.
444   68.   Gallop JL, Walrant A, Cantley LC, Kirschner MW. Phosphoinositides and membrane curvature switch the mode of actin
445   polymerization via selective recruitment of toca-1 and Snx9. Proc Natl Acad Sci U S A. 2013;110(18):7193-8.
446   69.   Schoneberg J, Lehmann M, Ullrich A, Posor Y, Lo WT, Lichtner G, et al. Lipid-mediated PX-BAR domain recruitment couples
447   local membrane constriction to endocytic vesicle fission. Nat Commun. 2017;8:15873.
448   70.   Taylor MJ, Lampe M, Merrifield CJ. A feedback loop between dynamin and actin recruitment during clathrin-mediated
449   endocytosis. PLoS Biol. 2012;10(4):e1001302.
450   71.   Palmer SE, Smaczynska-de R, II, Marklew CJ, Allwood EG, Mishra R, Johnson S, et al. A dynamin-actin interaction is
451   required for vesicle scission during endocytosis in yeast. Curr Biol. 2015;25(7):868-78.
452   72.   Antonny B, Burd C, De Camilli P, Chen E, Daumke O, Faelber K, et al. Membrane fission by dynamin: what we know and
453   what we need to know. EMBO J. 2016;35(21):2270-84.
454   73.   Gu C, Yaddanapudi S, Weins A, Osborn T, Reiser J, Pollak M, et al. Direct dynamin-actin interactions regulate the actin
455   cytoskeleton. EMBO J. 2010;29(21):3593-606.
456   74.   Gu C, Chang J, Shchedrina VA, Pham VA, Hartwig JH, Suphamungmee W, et al. Regulation of dynamin oligomerization in
457   cells: the role of dynamin-actin interactions and its GTPase activity. Traffic. 2014;15(8):819-38.
458   75.   Grassart A, Cheng AT, Hong SH, Zhang F, Zenzer N, Feng Y, et al. Actin and dynamin2 dynamics and interplay during
459   clathrin-mediated endocytosis. J Cell Biol. 2014;205(5):721-35.
460   76.   Merrifield CJ, Perrais D, Zenisek D. Coupling between clathrin-coated-pit invagination, cortactin recruitment, and membrane
461   scission observed in live cells. Cell. 2005;121(4):593-606.
462   77.   Mooren OL, Kotova TI, Moore AJ, Schafer DA. Dynamin2 GTPase and cortactin remodel actin filaments. J Biol Chem.
463   2009;284(36):23995-4005.
464   78.   Spudich G, Chibalina MV, Au JS, Arden SD, Buss F, Kendrick-Jones J. Myosin VI targeting to clathrin-coated structures and
465   dimerization is mediated by binding to Disabled-2 and PtdIns(4,5)P2. Nat Cell Biol. 2007;9(2):176-83.
466   79.   Idrissi FZ, Blasco A, Espinal A, Geli MI. Ultrastructural dynamics of proteins involved in endocytic budding. Proc Natl Acad Sci
467   U S A. 2012;109(39):E2587-94.
468   80.   Kukulski W, Schorb M, Kaksonen M, Briggs JA. Plasma membrane reshaping during endocytosis is revealed by time-resolved
469   electron tomography. Cell. 2012;150(3):508-20.
470   81.   Picco A, Mund M, Ries J, Nedelec F, Kaksonen M. Visualizing the functional architecture of the endocytic machinery. Elife.
471   2015;4.
472   82.   Idrissi FZ, Grotsch H, Fernandez-Golbano IM, Presciatto-Baschong C, Riezman H, Geli MI. Distinct acto/myosin-I structures
473   associate with endocytic profiles at the plasma membrane. J Cell Biol. 2008;180(6):1219-32.
474   83.   Kumari S, Mayor S. ARF1 is directly involved in dynamin-independent endocytosis. Nat Cell Biol. 2008;10(1):30-41.
475   84.   Lundmark R, Doherty GJ, Howes MT, Cortese K, Vallis Y, Parton RG, et al. The GTPase-activating protein GRAF1 regulates
476   the CLIC/GEEC endocytic pathway. Curr Biol. 2008;18(22):1802-8.

85. Renard HF, Simunovic M, Lemiere J, Boucrot E, Garcia-Castillo MD, Arumugam S, et al. Endophilin-A2 functions in membrane scission in clathrin-independent endocytosis. Nature. 2015;517(7535):493-6.

86. Richnau N, Aspenstrom P. Rich, a rho GTPase-activating protein domain-containing protein involved in signaling by Cdc42 and Rac1. J Biol Chem. 2001;276(37):35060-70.

87. Lua BL, Low BC. Activation of EGF receptor endocytosis and ERK1/2 signaling by BPGAP1 requires direct interaction with EEN/endophilin II and a functional RhoGAP domain. J Cell Sci. 2005;118(Pt 12):2707-21.

88. Nakano-Kobayashi A, Kasri NN, Newey SE, Van Aelst L. The Rho-linked mental retardation protein OPHN1 controls synaptic vesicle endocytosis via endophilin A1. Curr Biol. 2009;19(13):1133-9.

89. Sabharanjak S, Sharma P, Parton RG, Mayor S. GPI-anchored proteins are delivered to recycling endosomes via a distinct cdc42-regulated, clathrin-independent pinocytic pathway. Dev Cell. 2002;2(4):411-23.

90. Howes MT, Kirkham M, Riches J, Cortese K, Walser PJ, Simpson F, et al. Clathrin-independent carriers form a high capacity endocytic sorting system at the leading edge of migrating cells. J Cell Biol. 2010;190(4):675-91.

91. Simunovic M, Manneville JB, Renard HF, Evergren E, Raghunathan K, Bhatia D, et al. Friction Mediates Scission of Tubular Membranes Scaffolded by BAR Proteins. Cell. 2017;170(1):172-84 e11.

92. Parton RG, del Pozo MA. Caveolae as plasma membrane sensors, protectors and organizers. Nat Rev Mol Cell Biol. 2013;14(2):98-112.

93. Cheng JP, Nichols BJ. Caveolae: One Function or Many? Trends Cell Biol. 2016;26(3):177-89.

94. Sinha B, Koster D, Ruez R, Gonnord P, Bastiani M, Abankwa D, et al. Cells respond to mechanical stress by rapid disassembly of caveolae. Cell. 2011;144(3):402-13.

95. Lamaze C, Tardif N, Dewulf M, Vassilopoulos S, Blouin CM. The caveolae dress code: structure and signaling. Curr Opin Cell Biol. 2017;47:117-25.

96. Echarri A, Del Pozo MA. Caveolae - mechanosensitive membrane invaginations linked to actin filaments. J Cell Sci. 2015;128(15):2747-58.

97. Muriel O, Echarri A, Hellriegel C, Pavon DM, Beccari L, Del Pozo MA. Phosphorylated filamin A regulates actin-linked caveolae dynamics. J Cell Sci. 2011;124(Pt 16):2763-76.

98. Hernandez VJ, Weng J, Ly P, Pompey S, Dong H, Mishra L, et al. Cavin-3 dictates the balance between ERK and Akt signaling. Elife. 2013;2:e00905.

99. Moren B, Shah C, Howes MT, Schieber NL, McMahon HT, Parton RG, et al. EHD2 regulates caveolar dynamics via ATP-driven targeting and oligomerization. Mol Biol Cell. 2012;23(7):1316-29.

100. Echarri A, Muriel O, Pavon DM, Azegrouz H, Escolar F, Terron MC, et al. Caveolar domain organization and trafficking is regulated by Abl kinases and mDia1. J Cell Sci. 2012;125(Pt 13):3097-113.

101. Boucrot E, Ferreira AP, Almeida-Souza L, Debard S, Vallis Y, Howard G, et al. Endophilin marks and controls a clathrin-independent endocytic pathway. Nature. 2015;517(7535):460-5.

102. Vehlow A, Soong D, Vizcay-Barrena G, Bodo C, Law AL, Perera U, et al. Endophilin, Lamellipodin, and Mena cooperate to regulate F-actin-dependent EGF-receptor endocytosis. EMBO J. 2013;32(20):2722-34.

103. Gallop JL, Jao CC, Kent HM, Butler PJ, Evans PR, Langen R, et al. Mechanism of endophilin N-BAR domain-mediated membrane curvature. EMBO J. 2006;25(12):2898-910.

104. Renard HF, Johannes L, Morsomme P. Increasing Diversity of Biological Membrane Fission Mechanisms. Trends Cell Biol. 2018.

105. Lamaze C, Dujeancourt A, Baba T, Lo CG, Benmerah A, Dautry-Varsat A. Interleukin 2 receptors and detergent-resistant membrane domains define a clathrin-independent endocytic pathway. Mol Cell. 2001;7(3):661-71.

106. Sauvonnet N, Dujeancourt A, Dautry-Varsat A. Cortactin and dynamin are required for the clathrin-independent endocytosis of gammac cytokine receptor. J Cell Biol. 2005;168(1):155-63.

107. Basquin C, Trichet M, Vihinen H, Malarde V, Lagache T, Ripoll L, et al. Membrane protrusion powers clathrin-independent endocytosis of interleukin-2 receptor. EMBO J. 2015;34(16):2147-61.

108. Chen B, Brinkmann K, Chen Z, Pak CW, Liao Y, Shi S, et al. The WAVE regulatory complex links diverse receptors to the actin cytoskeleton. Cell. 2014;156(1-2):195-207.

109. Grassart A, Dujeancourt A, Lazarow PB, Dautry-Varsat A, Sauvonnet N. Clathrin-independent endocytosis used by the IL-2 receptor is regulated by Rac1, Pak1 and Pak2. EMBO Rep. 2008;9(4):356-62.

110. Basquin C, Malarde V, Mellor P, Anderson DH, Meas-Yedid V, Olivo-Marin JC, et al. The signalling factor PI3K is a specific regulator of the clathrin-independent dynamin-dependent endocytosis of IL-2 receptors. J Cell Sci. 2013;126(Pt 5):1099-108.

111. Prosser DC, Drivas TG, Maldonado-Baez L, Wendland B. Existence of a novel clathrin-independent endocytic pathway in yeast that depends on Rho1 and formin. J Cell Biol. 2011;195(4):657-71.

112. Soykan T, Kaempf N, Sakaba T, Vollweiter D, Goerdeler F, Puchkov D, et al. Synaptic Vesicle Endocytosis Occurs on Multiple Timescales and Is Mediated by Formin-Dependent Actin Assembly. Neuron. 2017;93(4):854-66 e4.

113. Marques PE, Grinstein S, Freeman SA. SnapShot:Macropinocytosis. Cell. 2017;169(4):766- e1.

114. Nicholson-Fish JC, Kokotos AC, Gillingwater TH, Smillie KJ, Cousin MA. VAMP4 Is an Essential Cargo Molecule for Activity-Dependent Bulk Endocytosis. Neuron. 2015;88(5):973-84.

115. Siempelkamp BD, Rathinaswamy MK, Jenkins ML, Burke JE. Molecular mechanism of activation of class IA phosphoinositide 3-kinases (PI3Ks) by membrane-localized HRas. J Biol Chem. 2017;292(29):12256-66.

116. Koivusalo M, Welch C, Hayashi H, Scott CC, Kim M, Alexander T, et al. Amiloride inhibits macropinocytosis by lowering submembranous pH and preventing Rac1 and Cdc42 signaling. J Cell Biol. 2010;188(4):547-63.

117. Rane CK, Minden A. P21 activated kinases: structure, regulation, and functions. Small GTPases. 2014;5.

118. Rosse C, Linch M, Kermorgant S, Cameron AJ, Boeckeler K, Parker PJ. PKC and the control of localized signal dynamics. Nat Rev Mol Cell Biol. 2010;11(2):103-12.

119. Brandt D, Gimona M, Hillmann M, Haller H, Mischak H. Protein kinase C induces actin reorganization via a Src- and Rho-dependent pathway. J Biol Chem. 2002;277(23):20903-10.

120. Krause M, Leslie JD, Stewart M, Lafuente EM, Valderrama F, Jagannathan R, et al. Lamellipodin, an Ena/VASP ligand, is implicated in the regulation of lamellipodial dynamics. Dev Cell. 2004;7(4):571-83.

121. Posor Y, Eichhorn-Gruenig M, Puchkov D, Schoneberg J, Ullrich A, Lampe A, et al. Spatiotemporal control of endocytosis by phosphatidylinositol-3,4-bisphosphate. Nature. 2013;499(7457):233-7.

122. Bendris N, Schmid SL. Endocytosis, Metastasis and Beyond: Multiple Facets of SNX9. Trends Cell Biol. 2017;27(3):189-200.

123. Schlam D, Bagshaw RD, Freeman SA, Collins RF, Pawson T, Fairn GD, et al. Phosphoinositide 3-kinase enables phagocytosis of large particles by terminating actin assembly through Rac/Cdc42 GTPase-activating proteins. Nat Commun. 2015;6:8623.

124. Singh V, Davidson AC, Hume PJ, Humphreys D, Koronakis V. Arf GTPase interplay with Rho GTPases in regulation of the actin cytoskeleton. Small GTPases. 2017:1-8.

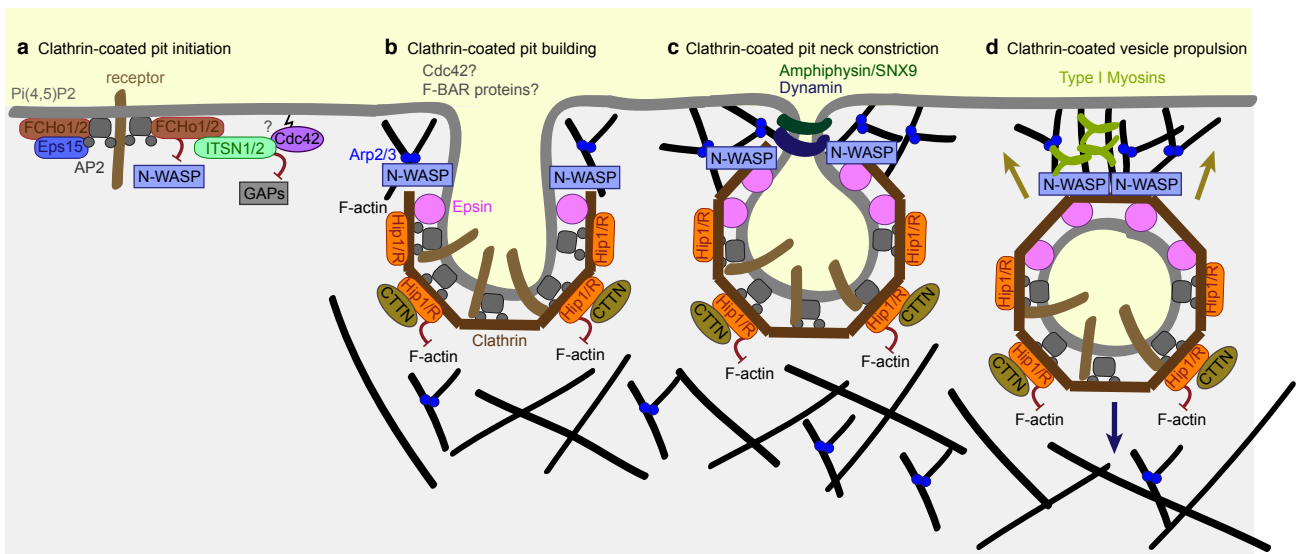**Figure 1.** Local actin polymerization during Clathrin-mediated endocytosis
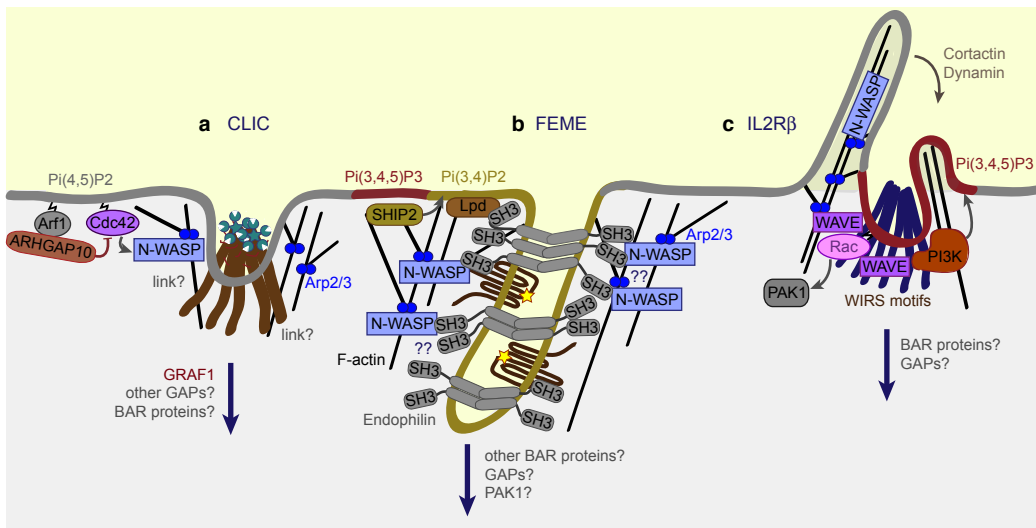
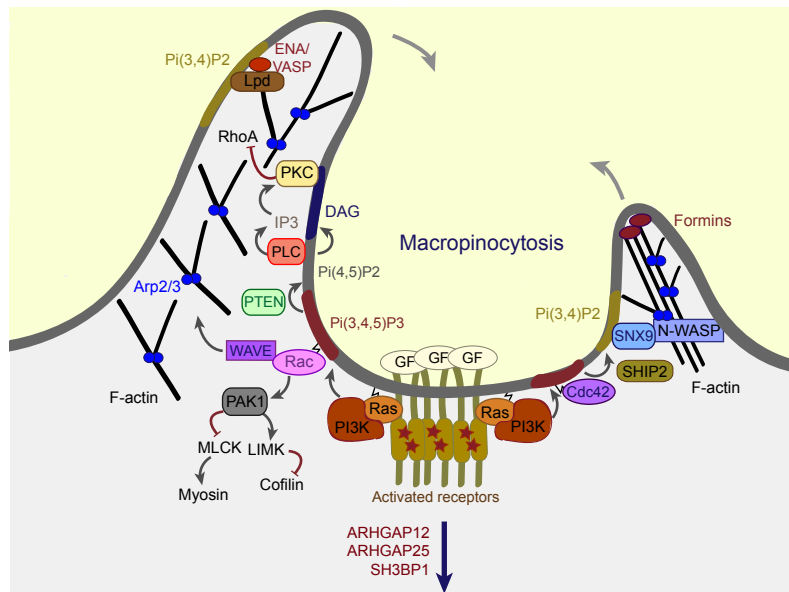**Figure 2.** Local actin polymerization during Clathrin-independent endocytosis

Figure 3. Local actin polymerization during macropinocytosis