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The mammalian endocytic cytoskeleton

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ABSTRACT

Clathrin-mediated endocytosis (CME) is the major route through which cells internalise various substances and recycle membrane components. Via the coordinated action of many proteins, the membrane bends and invaginates to form a vesicle that buds off—along with its contents—into the cell. The contribution of the actin cytoskeleton to this highly dynamic process in mammalian cells is not well understood. Unlike in yeast, where there is a strict requirement for actin in CME, the significance of the actin cytoskeleton to mammalian CME is variable. However, a growing number of studies have established the actin cytoskeleton as a core component of mammalian CME, and our understanding of its contribution has been increasing at a rapid pace. In this review, we summarise the state-of-the-art regarding our understanding of the endocytic cytoskeleton, its physiological significance, and the questions that remain to be answered.

1. Introduction

Endocytosis is the process by which cells internalise nutrients, pathogens, and membrane components (Schmid et al., 2014). Multiple types of internalisation strategies exist in multicellular organisms (Doherty and McMahon, 2009; Mayor and Pagano, 2007), but clathrin-mediated endocytosis (CME) is arguably the predominant endocytic route (Bitsikas et al., 2014; Kaksonen and Roux, 2018; McMahon and Boucrot, 2011). The distinguishing feature of CME is the formation of membrane invaginations covered by polygonal clathrin cages termed clathrin-coated pits (CCPs). The process of CME can be roughly divided into four stages: initiation, maturation, scission, and uncoating (for more details, see Kaksonen and Roux, 2018). These steps are orchestrated by a machinery composed of more than 60 proteins (Bhave et al., 2020; McMahon and Boucrot, 2011; Schmid and McMahon, 2007) performing a variety of tasks such as membrane bending, cargo recruitment, scaffolding, lipid modification, and cytoskeletal regulation, to name a few.

The actin cytoskeleton is a major component of multiple cellular processes involving rapid morphological changes (Blanchoin et al., 2014). The association of actin filaments with CCPs was suggested over 40 years ago (Heuser and Evans, 1980). Indeed, the actin cytoskeleton is essential for CME in yeast, where a large body of research has elucidated the role of actin in CCP formation and vesicle scission (Goode et al., 2015; Idrissi et al., 2012; Kaksonen et al., 2003; Kukulski et al., 2012; Skruzny et al., 2012; Sun et al., 2015). However, the details of actin's participation in mammalian CME are still unclear. In this review, we summarise our current knowledge of the role of the endocytic cytoskeleton in mammalian cells, examine some of the unanswered questions in the biology of this structure, and discuss its emerging role in physiology and diseases. The presence of other cytoskeletal structures (microtubules and intermediate filaments) in mammalian CME has been reported (Franck et al., 2019; Montagnac et al., 2013). While their role in CME progression is still unclear, evidence from other fields have firmly established that cytoskeletal elements cross-regulate each other (Seetharaman and Etienne-Manneville, 2020). For this reason, despite focusing exclusively on the actin cytoskeleton in this review, we opted to use the term endocytic cytoskeleton, rather than endocytic actin cytoskeleton, to leave an open door in the future for the inclusion of other cytoskeletal elements.

2. The dynamics and appearance of the endocytic cytoskeleton

Actin polymerisation during CME can be readily visualised by live-cell imaging. Initial studies using cells expressing fluorescently tagged actin and clathrin revealed that actin signal rises as the intensity of clathrin falls, corresponding to the internalisation of the endocytic vesicle (Merrifield et al., 2002). The rise in actin signal also coincides

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with a peak in the intensity of dynamin in the majority of endocytic events (Merrifield et al., 2002). Endogenously tagged actin shows an identical trend, with more than 85% of the dynamin fluorescence intensity peaks coinciding with a transient rise in the intensity of endogenously tagged actin (Grassart et al., 2014). This rise in fluorescence intensity of tagged actin indicates active polymerisation at CCPs during late CME. Moreover, correlative light electron microscopy (EM) experiments have revealed that some CCPs were associated with actin patches that were not detectable in fluorescence microscopy, indicating that the use of fluorescence microscopy may lead to underestimating the association of actin filaments with CCPs (Collins et al., 2011).

Platinum replica EM revealed patches of short, branched actin filaments peripherally associated with CCPs. These actin patches associate with CCPs of various shapes and sizes, including small, flat structures corresponding to early CME. Occasionally, actin also forms a collar-like structure encircling CCPs (Collins et al., 2011). In addition, the interaction between actin patches and endocytic structures happens mainly at the bases of shallow CCPs and the necks of late CCPs, although additional interactions may have been destroyed by the sample preparation procedure (Collins et al., 2011). Indeed, a recent study using in situ cryo-electron tomography (cryo-ET) reported that actin filaments are not exclusively present at the base of CCPs, but also around and below them (Serwas et al., 2021). Interestingly, this study found both branched and unbranched actin filaments at all CME stages, and that unbranched actin filaments also form bundles adjacent to CCPs (Serwas et al., 2021). The average length of actin filaments around CCPs (branched: 81.74 ± 74 nm; unbranched: 141 ± 112 nm) indicates that at least some of these filaments are newly polymerised and may contribute to force generation at CME site (Serwas et al., 2021).

3. The functional relevance of the endocytic cytoskeleton in mammalian cells

Early studies employing pharmacological inhibitors of actin polymerisation reported conflicting results, leading to ambiguity about the role of the actin cytoskeleton in mammalian CME. Gottlieb et al. (1993) found that treating MDCK cells using cytochalasin D (CytoD; an actin-disrupting drug that caps barbed ends, inducing depolymerisation of dynamic filaments) led to an inhibition of CME in the apical, but not basolateral, surface. This effect was also observed in Caco-2 cells (Jackman et al., 1994). Interestingly, electron micrographs of cells treated with CytoD showed an increased number of pits on the apical surface, possibly due to inefficient scission (Gottlieb et al., 1993). Similarly, thymosin β4 and DNase I—both capable of sequestering actin monomers, thus inhibiting actin polymerisation—suppressed the uptake of transferrin in a cell-free system, indicating inhibition of CME, and latrunculin A inhibited receptor-mediated endocytosis in intact cells (Lamaze et al., 1997). Importantly, the effects of the perturbation of the actin cytoskeleton on endocytosis appeared to be the result of inefficient budding of coated vesicles from the membrane, as electron micrographs showed an increased abundance of deeply invaginated pits (Lamaze et al., 1997). This effect was also observed in other studies employing latrunculin or jasplakinolide to disrupt actin dynamics in intact cells (Taylor et al., 2011; Yarar et al., 2005). However, these and other studies also reported a dramatic reduction in the rate of formation of CCPs, suggesting that actin dynamics are involved at multiple CME stages (Grassart et al., 2014; Merrifield et al., 2005; Moskowitz et al., 2005; Taylor et al., 2012; Yamada et al., 2007; Yarar et al., 2005; Yoshida et al., 2018). Indeed, live-cell imaging combined with atomic force microscopy provided evidence that inhibiting actin polymerisation significantly hinders the closing of CCPs, while inhibiting actin depolymerisation affected the frequency of CCP formation (Yoshida et al., 2018).

Conversely, other reports have indicated that CME is insensitive to perturbations of the actin cytoskeleton in mammalian cells, suggesting that the function of actin is not critical. The extent of transferrin uptake by African green monkey kidney (Vero) cells in culture was not affected by incubation with various concentrations of CytoD for 15 minutes (Sandvig and Van Deurs, 1990). Indeed, Fujimoto et al. (2000) reported that the effects of actin-disrupting drugs were dependent on cell type and on whether the cells were growing in suspension or as adherent cultures. The authors monitored the rate and extent of transferrin uptake as an indicator of the rate of CME in their preparations, and the effect of disrupting actin dynamics ranged from a 70% reduction in transferrin uptake to no effect at all (Fujimoto et al., 2000). Another study concluded that the presence of latrunculins or CytoD following alcohol-mediated synchronisation does not affect neither vesicle dynamics nor *de novo* formation of CCPs (Boucrot et al., 2006). The authors, however, did observe a marked reduction in transferrin uptake as well as an increase in the lateral mobility and velocity of AP2 puncta, and noted that the extent of these effects was a function of exposure time to the actin-disrupting drugs (Boucrot et al., 2006). Similarly, CytoD showed little to no effect on transferrin uptake in MDCK cells in another study (Maples et al., 1997), and the dynamics of CCPs in Swiss 3T3 cells were not affected by latrunculin A (Saffarian et al., 2009).

It is not clear to us where this discrepancy stems from. Yarar et al. (2005) argue that attempting to evaluate the contribution of the actin cytoskeleton to CME through bulk biochemical assays disallows observing the presence of different sub-populations of CCPs that may have different requirements and levels of involvement with the actin cytoskeleton. Furthermore, the contribution of actin to CME appears to be multi-faceted and unique at every stage (Grassart et al., 2014; Merrifield et al., 2005; Moskowitz et al., 2005; Taylor et al., 2012; Yarar et al., 2005). Consequently, any perturbation of the actin cytoskeleton will differently affect its function at each specific CME stage, which necessitates more careful evaluation. In addition, commonly used actin-disrupting drugs do not completely abolish actin dynamics nor destroy all actin structures. For example, CytoD disrupts the actin cytoskeleton by capping the barbed ends of actin filaments (thus inhibiting polymerisation), but it also nucleates new filaments and leaves an abundance of short filaments that are nonetheless able to form networks (Goddette and Frieden, 1986; Schliwa, 1982). Accordingly, while CytoD may effectively destroy bulky dynamic structures like actin stress fibres, the short filaments and network fragments created in the aftermath may potentially be sufficient for CME. Indeed, platinum replica EM and fluorescence microscopy showed that actin filaments at CME sites are insensitive to actin-disrupting drugs (Collins et al., 2011). Moreover, as we discuss below, perturbing the function of a number of endocytic actin-binding proteins or their regulators has a dramatic effect on CME initiation and progression. In our view, careful re-interpretation of CME phenotypes caused by actin-disrupting drugs is advisable. Furthermore, the consistent endocytic slow down observed upon genetic manipulations of actin regulators firmly places the endocytic cytoskeleton as a core CME component with a kinetic function. In fact, a core kinetic function of the endocytic cytoskeleton in CME provides a simple and elegant explanation to the presence of active actin polymerisation in the vast majority of CME events in multiple cell lines (Almeida-Souza et al., 2018; Grassart et al., 2014; Taylor et al., 2011).

4. The role of the endocytic cytoskeleton in CME progression

The mechanism through which the actin cytoskeleton contributes to CME is not fully clear, and appears to be multi-faceted. In yeast, actin polymerisation and filament bundling generate force that is necessary to overcome high cell turgor, mediating invagination and budding (Aghamohammadzadeh and Ayscough, 2009; Kukulski et al., 2012; Picco et al., 2018). On the other hand, mammalian cell membranes are typically not under the same pressure and actin polymerisation at CCPs peaks at the later stages of CCP formation (Ferguson et al., 2017). Interestingly, under elevated membrane tension, latrunculin- (Boulant et al., 2011; Yarar et al., 2005) and jasplakinolide- (Boulant et al., 2011; Ferguson et al., 2017; Yarar et al., 2005) mediated perturbation of actin

polymerisation leads to a dramatic increase in the lifetimes of CCPs and the proportion of stalled 'U-shaped' pits. This effect is readily reversible by restoring membrane tension (Boulant et al., 2011; Ferguson et al., 2017). A similar effect is also seen upon inhibiting the nucleation of branched actin filaments through inhibiting ARP2/3 function under high membrane tension conditions (Kaplan et al., 2021). Notably, a recent study suggests that the actin polymerisation machinery is preferentially recruited to stalled CCPs, probably helping to provide the force required to carry these events to completion (Jin et al., 2021). Together, these data suggest that, in yeast and mammalian cells alike, actin polymerisation plays a role in late CME by generating the force necessary to overcome membrane tension. This notion is also supported by mathematical modelling (Akamatsu et al., 2020; Hassinger et al., 2017) and in experiments with double dynamin knockout cells, where the scission defect leads to the formation of elongated tubular clathrin structures that are dependent on an intact actin cytoskeleton (Ferguson et al., 2009).

In addition to its role in force generation at late-stage CME, a few reports suggest a role of the endocytic cytoskeleton also at earlier CME stages. In yeast, the initiation of CME requires remodelling the actin cytoskeleton, involving first severing actin filaments, followed by actin polymerisation (Chen and Pollard, 2013). There is not enough evidence to suggest this to similarly be the case in mammalian cells, but disrupting actin cytoskeleton dynamics inhibits the formation of new CCPs, as well as reduces the lateral motility of CCPs in Swiss 3T3 cells (Yarar et al., 2005). Moreover, 'CCP exclusion zones' around individual CCPs have been reported in a variety of cell types (Boucrot et al., 2006; Ehrlich et al., 2004; Fujiwara et al., 2002; Santini et al., 2002), possibly because of the local rearrangement of the cortical actin cytoskeleton (Boucrot et al., 2006; Santini et al., 2002). Electron micrographs also show that CCPs lie in 'islands' largely devoid of, and surrounded by, cortical actin (Fujimoto et al., 2000), although it is possible that actin filaments in these regions were lost during sample preparation. In any case, actin filaments surrounding CCPs have a distinct appearance compared to the surrounding cortical actin (Collins et al., 2011). Moreover, electron micrographs show that actin filaments are indeed associated with early, shallow CCPs (Collins et al., 2011). The role these actin filaments play—if any—during early CME remains to be elucidated. Nevertheless, it is plausible that the initiation of CME in mammalian cells requires remodelling the cortical actin cytoskeleton, although this needs to be experimentally verified.

5. Endocytic actin-associated proteins

In this section we will summarise the contribution of a number of key actin-associated proteins in the regulation of the mammalian endocytic cytoskeleton. An overview of where these proteins are located at CCPs is shown in Fig. 1. In Box 1, we highlight some of the studies in *Drosophila* and *C. elegans* that have produced key insights into the understanding of the endocytic cytoskeleton.

The ARP2/3 complex—composed of 7 subunits—is the only actin nucleator capable of nucleating actin branches from existing actin filaments (Goley and Welch, 2006). Branched actin networks likely constitute the majority of the endocytic cytoskeleton (Collins et al., 2011; Serwas et al., 2021), making ARP2/3 an essential component. Indeed, inhibiting ARP2/3 function by knocking down ARP3 leads to a dramatic increase in the lifetimes of CME events accompanied by a ~ 43% reduction in transferrin uptake (Almeida-Souza et al., 2018). A similar increase of CME lifetimes also happens upon pharmacological inhibition of ARP2/3, and the effect is augmented when the cells are incubated in slightly hypotonic conditions (Kaplan et al., 2021). Live-cell imaging showed that recruitment of the ARP2/3 complex follows recruitment of the ARP2/3 activator neuronal Wiskott-Aldrich syndrome protein (N-WASP) and coincides with an increase in F-actin concentration (Taylor et al., 2011).

The ARP2/3-mediated nucleation of branched actin requires activation by a nucleation promoting factor (NPF) (Goley and Welch, 2006). Two of these factors have been identified in CME: N-WASP (Benesch et al., 2005; Kessels and Qualmann, 2002; Merrifield et al., 2004) and cortactin (Cao et al., 2003). Interestingly, while the recruitment of N-WASP precedes the recruitment of ARP2/3—consistent with its role in ARP2/3 activation—cortactin shows similar recruitment dynamics to ARP2/3, with both peaking around the same time as dynamin (Taylor et al., 2011). Indeed, cortactin binds to the proline rich domain (PRD) of dynamin (McNiven et al., 2000), and the affinity of this interaction is ~ 8-fold stronger in the presence of ARP2/3 and actin filaments (Zhu et al., 2005). Moreover, compared to cortactin, N-WASP has a higher affinity to the ARP2/3 complex and is a significantly more potent NPF (Uruno et al., 2001). This suggests that N-WASP, not cortactin, is the major ARP2/3 activator in the endocytic cytoskeleton. However, perturbing cortactin function leads to a reduction in transferrin uptake (Cao et al., 2003), indicating it plays an important role in CME. Disrupting the interaction of cortactin with HIP1R leads to the stable association of the endocytic cytoskeletal machinery with CCPs and their cargo (Engqvist-Goldstein et al., 2004; Le Clainche et al., 2007). Importantly, in the

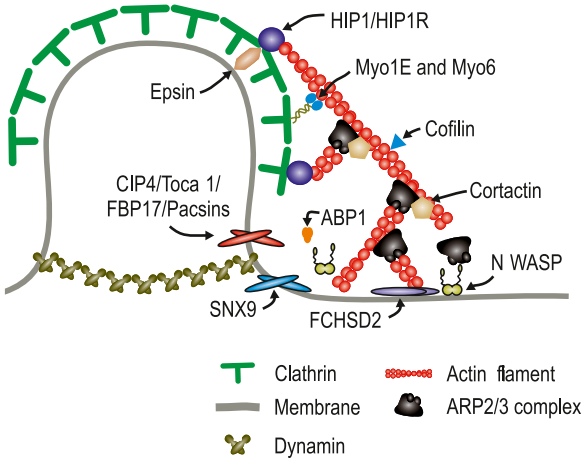

Fig. 1. CCP localisation of various endocytic cytoskeleton proteins. The illustration depicts localisation of molecules based on either imaging methods, protein-protein interactions, or predicted function. For some (i.e. Cofilin and myosins), the localisations may not be accurate as there is scarce data available or conflict within the literature. Most actin regulators in CME described so far were shown to be recruited just before scission, when actin polymerisation peaks. For this reason, we show protein localisation at a single CCP stage. It is important to note that many actin regulators (CIP4, Toca-1, FBP17, Pacsins, SNX9 and ABP1) are recruited by interactions with dynamin at the neck of CCPs.

presence of N-WASP, cortactin may enhance and stabilise the branching of actin filaments (Helgeson et al., 2014; Helgeson and Nolen, 2013; Weaver et al., 2001). Conversely, *in vitro* models show that, when in complex with dynamin and cortactin, actin filaments become loosely attached and more vulnerable to severing by cofilin upon dynamin activation (Mooren et al., 2009). Furthermore, cortactin can bind and inhibit cofilin in invadopodia, and this inhibition is released upon cortactin phosphorylation (Oser et al., 2009), but whether this also happens in CME remains to be seen. All in all, it is evident that N-WASP is the main NPF required for the initial formation of the branched actin network. Once a sufficient number of filaments and branches have formed, it is plausible that cortactin plays an important role in synergistically supporting actin polymerisation or remodelling the cytoskeleton to facilitate CME progression, or both.

Finally, the activation of N-WASP at CCPs is regulated by other proteins, including FCHSD2 (Almeida-Souza et al., 2018), Abp-1 (Kessels et al., 2001; Pinyol et al., 2007), sorting nexin 9 (SNX9) (Lundmark and Carlsson, 2002; Yarar et al., 2007), Syndapin/Pacsin 1, 2, and 3 (Dharmalingam et al., 2009; Qualmann et al., 1999; Qualmann and Kelly, 2000), and CIP4/Toca-1/formin-binding protein 17 (FBP17) (Feng et al., 2010; Fricke et al., 2009; Kamioka et al., 2004; Shimada et al., 2007).

Of all the N-WASP activators present at CCPs, our recent work showed that FCHSD2 is the most significant contributor (Almeida-Souza et al., 2018). By comparing the reduction in transferrin uptake in wild-type and FCHSD2 KO cells, combined or not with ARP3 knockdown, we found that FCHSD2 is responsible for around half of all ARP2/3 endocytic contribution. We hypothesise that this major role is a result of the optimal localisation of FCHSD2 at the surrounding regions of CCPs (Almeida-Souza et al., 2018). A study published the same year further reinforced the role of FCHSD2 in CME (Xiao et al., 2018). However, the authors report a role for FCHSD2 in CME initiation (Xiao et al., 2018), while we reported a later recruitment of FCHSD2, mediated by its interactor intersectin (Almeida-Souza et al., 2018). Whether this discrepancy is based in differing experimental setups or simply reflects variation between different cell types will require further studies. Recently, FCHSD2 was also shown to control vesicular trafficking of receptor tyrosine kinases (RTKs) in a Rab7-dependent manner (Xiao and Schmid, 2020). Interestingly, these results are in line with the vesicular recycling function shown for the *Drosophila* FCHSD2 homologue, Nervous Wreck (nwk) (Rodal et al., 2011). Whether the FCHSD2 functions in vesicle formation and endocytic recycling are independent or part of a wider cellular system that connects actin polymerisation at CME sites to vesicle fate is currently not known.

It is worth noting that the insights obtained on FCHSD2 were only possible due to a series of high-quality studies on *Drosophila* Nervous Wreck (nwk). Nwk was initially described as the gene that, when mutated, caused a paralysing phenotype in flies accompanied by excessive neuronal branching and synaptic boutons (Coyle et al., 2004). Further studies established nwk as an endocytic regulator of BMP receptor signalling (O'Connor-Giles et al., 2008) and discovered its molecular connection to the endocytic and actin machinery (Kelley et al., 2015; Rodal et al., 2011, 2008). Despite many commonalities, a few differences still exist between these homologues. For example, their F-BAR domains differ in their overall shape, with FCHSD2 F-BAR being flat (Almeida-Souza et al., 2018) and the nwk F-BAR being curved (Becalska et al., 2013; Stanishneva-Konovalova et al., 2016). Moreover, nwk is found in a primed state at the synaptic periactive zone, ensuring fast and productive actin bursts when activated (Del Signore et al., 2021), while FCHSD2 is recruited to endocytic sites after initiation (Almeida-Souza et al., 2018). We still do not fully understand the reason for these differences, but they may be an example of the versatility of the endocytic cytoskeleton, which needs to adapt to various force and kinetic requirements found in different cells *in vivo*.

Huntingtin-interacting protein 1/Huntingtin-interacting protein 1-related (HIP1/HIP1R)—the mammalian homologues of the yeast protein Sla2p—strongly co-localise with clathrin and other markers of CME (Engqvist-Goldstein et al., 1999). HIP1/HIP1R form rod-shaped dimers with membrane-binding and actin-binding domains on opposite ends, separated by central clathrin-binding domains. HIP1R promotes the formation of clathrin cages *in vitro* and links actin filaments to clathrin-coated vesicles (Clarke and Royle, 2018; Engqvist-Goldstein et al., 2001). Knocking down HIP1R results in a reduction in transferrin uptake in a dose-dependent manner as well as the formation of enlarged, membrane-bound F-actin structures that also contain the endocytic machinery (Engqvist-Goldstein et al., 2004). Cryo-ET showed that HIP1R localises to the necks and tips of CCPs, providing a site of attachment for actin filaments and allowing them to generate force efficiently (Serwas et al., 2021).

Similar to their function in yeast (Garcia-Alai et al., 2018; Skruzny et al., 2012), mammalian epsins also play an important role in CME (Chen et al., 1998). Epsin binds several CCP components (Chen et al., 1998; Rosenthal et al., 1999) and super-resolution correlative light EM revealed that both epsins 1 and 2 localise to CCPs in a pattern similar to HIP1R (Sochacki et al., 2017). Epsin interacts with both HIP1R and actin filaments, and epsin triple KO cells show accumulation of F-actin and a lack of HIP1R recruitment at CCPs (Messa et al., 2014). Thus, it is plausible that epsin contributes to the endocytic cytoskeleton by recruiting HIP1R to CCPs and facilitating its interaction with F-actin.

Myosin motors also play a role in mammalian CME (Buss et al., 2001; Krendel et al., 2007; Spudich et al., 2007). In yeast, type I myosins act as anchors and promote actin polymerisation and membrane bending during CME (Manenschijn et al., 2019; Pedersen and Drubin, 2019). In mammalian cells, both myosin IE (Myo1E) and myosin VI (Myo6) localise to CME sites, showing peak localisation around the same time as dynamin, as seen by live-cell imaging (Taylor et al., 2011). Myo6 is recruited to CCPs through binding to Disabled-2 (Dab2) (Morris et al., 2002; Spudich et al., 2007), and inhibiting Myo6 function results in a reduction in transferrin uptake (Aschenbrenner et al., 2003; Buss et al., 2001). Importantly, when localised to CCPs, Myo6 predominantly exists as a dimer capable of processive motor function (Altman et al., 2007; Spudich et al., 2007), consistent with its apparent role in transporting uncoated endocytic vesicles away from the cell periphery (Aschenbrenner et al., 2004, 2003). Consistent with these findings, Biancospino et al. (2019) reported that the long isoform of Myo6 (myosin VI_{long}, exclusively expressed in highly polarised tissue) is recruited to CCPs through direct interaction with clathrin light chain, rather than through binding to Dab2. Importantly, this interaction is mutually exclusive with HIP1R, suggesting these two proteins may function sequentially.

Like Myo6, Myo1E localises to CME sites in mammalian cells through binding to dynamin and synaptojanin-1, and inhibiting Myo1E function results in a reduction in transferrin uptake (Krendel et al., 2007). In addition to class I myosins, a more recent study revealed a critical role for non-muscle myosin II during mammalian CME. Inhibiting myosin II function results in prominent CME defects exemplified by an inhibition of transferrin uptake, an increase in the proportion of shallow pits on the membrane, and asymmetric and malformed CCPs (Chandrasekar et al., 2014). Thus, it is clear that myosins are an intrinsic part of mammalian CME, and future work will shed light on their contribution in time and space.

Cofilin is required for endocytosis in yeast (Chen and Pollard, 2013; Idrissi et al., 2002; Lappalainen and Drubin, 1997). In mammalian cells, cofilin gradually accumulates at CME sites and its concentration peaks shortly after dynamin (Taylor et al., 2011), suggesting a role in network disassembly. The precise role of cofilin in the regulation of the mammalian endocytic cytoskeleton is, however, not clear. Nonetheless, the inhibition of cofilin function leads to a potent inhibition of CME in mammalian cells (Bertling et al., 2004; Hryciw et al., 2003).

Box 1
The contributions of *C. elegans* and *Drosophila* studies to the understanding of the endocytic cytoskeleton

Clathrin-mediated endocytosis is fundamental to all eukaryotes. A lot of our knowledge on mammalian CME took advantage of insights using yeast. Similarly, other model systems such as the nematode *C. elegans* and the fruit fly also made, and continue to make, significant contributions to our understanding of CME. Below we highlight some of the key studies on the endocytic cytoskeleton in *C. elegans* and fruit flies.

By monitoring the uptake of GFP-tagged yolk protein, Grant and Hirsh (1999) showed that *C. elegans* share the same basic components of CME with vertebrates. Consistently, Fares and Greenwald (2001) identified several components of endocytosis in *C. elegans* that were also conserved in mammals (reviewed in Fares and Grant, 2002). Unsurprisingly, the actin cytoskeleton plays an important role in *C. elegans* CME (Shi et al., 2019).

The *C. elegans* F-BAR containing TOCA family of proteins bend the membrane and regulate actin polymerisation through their SH3 domains, and perturbing their function reduces the efficiency of CME in oocytes (Giuliani et al., 2009). Interestingly, a similar reduction in CME efficiency is observed upon perturbing the function of WVE-1 (nematode homologue of mammalian nucleation promoting factor WAVE/SCAR) or—to a lesser extent—WSP-1 (nematode homologue of mammalian N-WASP) (Giuliani et al., 2009). Indeed, both mammalian and *C. elegans* TOCA proteins associate with WVE-1 (through ABI-1 (Innocenti et al., 2004)) and WSP-1, and TOCA mutants that cannot bind WVE-1 cause a significant reduction in CME (Giuliani et al., 2009). In line with this, perturbing WVE-1—but not WSP-1—function leads to a significant reduction in transferrin uptake, indicating deficiencies in CME (Patel and Soto, 2013). Importantly, the interaction between DBN-1 (nematode homologue of Abp1) and F-actin at CME sites in *C. elegans* is required for recruiting dynamin (Shi et al., 2019; Shivas and Skop, 2012), a finding that represents a rare insight into the interaction between dynamin recruitment and actin polymerisation.

The discovery of the role of dynamin in CME—pivotal to our current understanding of the process—can be traced back to seminal work on the *shibire* mutation in *Drosophila* (Chen et al., 1991; Grigliatti et al., 1973; Poodry and Edgar, 1979; van der Bliek and Meyerowitz, 1991). Flies carrying the *shibire* mutation experienced reversible temperature-sensitive paralysis as a result of the depletion of synaptic vesicles. In fact, in characterising the effects of the *shibire* mutation, Poodry et al. (1973) concluded that it 'affects a fundamental cell process common to many cell types'.

Studies in flies also helped to stress the importance of the endocytic cytoskeleton. Disruption of the actin cytoskeleton (with latrunculin A or cytochalasin D) led to significant endocytic defects *in vivo* and on dissociated cells (Kochubey et al., 2006; Kuromi and Kidokoro, 1998). Similar to *C. elegans,* perturbing the function of Cip4—the only member of the CIP4/FBP17/Toca1 family in *Drosophila*—leads to defective E-cadherin endocytosis in epithelial cells (Leibfried et al., 2008) and abnormal wing hair development (a process dependent on endocytosis) (Fricke et al., 2009). Importantly, these phenotypes resemble that of blocking dynamin function (Fricke et al., 2009; Leibfried et al., 2008), and the defects in E-cadherin endocytosis also result from loss of ARP2/3 or WASP function (Leibfried et al., 2008). As discussed in the main text, seminal studies with the fly homologue of FCHSD2, Nervous Wreck (Coyle et al., 2004; O'Connor-Giles et al., 2008; Rodal et al., 2011, 2008; Stanishneva-Konovalova et al., 2016), paved the way for the discovery of this important actin activator in mammalian endocytosis (Almeida-Souza et al., 2018; Xiao and Schmid, 2020; Xiao et al., 2018).

Studies in these organisms clearly demonstrate that the basic mechanisms governing the endocytic cytoskeleton share considerable similarity across metazoan cells and play important roles *in vivo.* Decades of work on *C. elegans* and the fruit fly make them well-characterised multicellular model systems offering several advantages over mammalian models, most notably simpler genetics (Harris et al., 2001) and relative ease of conducting experiments and imaging *in vivo (*Jha and Traub, 2014; Wang and Audhya, 2014). The latter may indeed be crucial to cement and develop our understanding of the endocytic cytoskeleton—and CME in general—as the dynamics of CME may significantly differ in intact tissue *in vivo* (Masedunskas et al., 2012; Weigert, 2014).

6. The participation of the endocytic cytoskeleton in human disease

Owing to its central role in metazoan physiology, defects in CME manifest in several disease conditions, ranging from metabolic malfunction to neurodegenerative disorders (Azarnia Tehran et al., 2019). Knockout animals of the core CME components are not viable (Bazinet et al., 1993; Ferguson et al., 2009; Mitsunari et al., 2005). Unsurprisingly, mutations in core and accessory CME proteins are implicated in a sizeable list of disease conditions (reviewed in Yarwood et al., 2020).

Several *bona fide* regulators of the endocytic cytoskeleton are implicated in disease. As its name suggests, HIP1 interacts with Huntingtin, a protein mutated in Huntington's disease (Kalchman et al., 1997; Wanker et al., 1997). HIP1 is expressed primarily in the brain (Kalchman et al., 1997) and its role in Huntington's disease is proposed to be related to its apoptotic function (Bhattacharyya et al., 2008). The connection between HIP1's endocytic and apoptotic functions and if they contribute independently to Huntington's pathology is not fully understood. The ubiquitously expressed HIP1 paralog, HIP1R, is linked to colon cancer (Scanlan et al., 2002) and chronic lymphocytic leukaemia (Porpaczy et al., 2009). Similarly, cortactin overexpression is frequently present in breast cancer and squamous cell carcinoma (Schuuring et al., 1992).

FCHSD2 expression levels inversely correlate with chemotherapy response in acute myeloid leukaemia patients (El Dahshan et al., 2014; Han et al., 2012), while high levels of FCHSD2 expression are associated with better survival in non-small cell lung cancers (Xiao et al., 2018). Importantly, the latter is caused by an FCHSD2-dependent modulation of the endocytic fate of epidermal growth factor receptor (EGFR), which regulates proliferative and migratory signalling (Xiao and Schmid, 2020; Xiao et al., 2018). Furthermore, individuals with mutations in the STARD10 locus (an enhancer cluster controlling FCHSD2 expression (Hu et al., 2021)) have a higher risk of type 2 diabetes (Nielsen et al., 2011; Voight et al., 2010), and FCHSD2-KO pancreatic beta cells exhibit reduced glucose-induced insulin secretion (Hu et al., 2021).

Another aspect of CME's role in disease lies in its utility as an entry point for pathogens. The uptake of viral particles often requires increasing the size of a CCP or altering its geometry, or both. CME is the most commonly used endocytic pathway for viral entry (Marsh and Helenius, 2006), and some viruses can promote the *de novo* initiation of CCPs (Ehrlich et al., 2004; Rust et al., 2004). Notably, the virus responsible for COVID-19, SARS-CoV-2, also uses CME for its internalisation (Bayati et al., 2021). Viruses utilise a variety of strategies for infection, and a single virus can often enter the cell through multiple pathways (Barrow et al., 2013; Marsh and Helenius, 2006). The first stage of the entry of influenza virus into BSC-1 cells, characterised by

association with the membrane and CME markers, is actin-dependent (Rust et al., 2004). Similarly, adenovirus endocytosis is actin-dependent and is blocked upon treatment with the actin-disrupting drug cytoD (Li et al., 1998). More strikingly, the rod-shaped vesicular stomatitis virus, with a length of ~ 200 nm, enters cells through CME where only the tip of the endocytic vesicle is coated with clathrin and the base coated with actin (Cureton et al., 2009). Interestingly, a shorter (~ 75 nm) variant of the same virus also enters cells through CME, but the vesicles are completely coated with clathrin and viral entry is not affected by latrunculin B treatment (Cureton et al., 2010). The authors also reported an increase in the recruitment of cortactin to pits containing the full-size version of the virus compared to the smaller particle (Cureton et al., 2010). In summary, viral entry through CME is an important pathway, but one that involves multiple mechanisms. Thus, the role of the endocytic cytoskeleton in this pathway may be potentially equally variable and requires further study.

The various examples we describe above, linking the endocytic cytoskeleton and its regulators to different disease processes, clearly demonstrate the physiological importance of actin polymerisation during CME. Therefore, a deep knowledge of the endocytic cytoskeleton is essential to translate these disease links into novel mechanistic insights that may help treat patients and understand human biology.

7. Unanswered questions about the endocytic cytoskeleton biology

The involvement of the actin cytoskeleton in mammalian CME is now firmly established. However, while recent work has led to important revelations about the nature and significance of the role of actin and its regulation in mammalian CME, several key questions remain unanswered.

To start, many studies point to the fact that the contribution of the endocytic cytoskeleton to CME progression is not fixed nor predetermined (Boulant et al., 2011; Kaplan et al., 2021; Kaur et al., 2014; Fujimoto et al., 2000; Yarar et al., 2005), but, rather, a variable parameter controlled by a series of intrinsic and extrinsic cellular factors. Parameters such as membrane tension, cargo size, receptor identity, cellular localisation, and cell cycle stage are just a few of the possible parameters controlling endocytic cytoskeleton participation. In addition, each of these parameters is likely to have different weights depending on cell type. Indeed, individual cells can show persistent variations in CME dynamics between different regions (Willy et al., 2017). Ultimately, *in vivo* , changes in membrane tension (Willy et al., 2021) and even mechanical constraints (Pouille et al., 2009) are among a plethora of other physiological factors that may also serve as endocytic cytoskeleton regulating factors. Thus, studies in intact tissue and using organoids or other 3-dimensional experimental models will undoubtedly provide valuable insight. Keeping in mind these possibilities while studying the endocytic cytoskeleton is an essential step to understand the logic of this structure.

Alongside the questions regarding the role of the endocytic cytoskeleton in CME progression, many details of the molecular composition and architecture of this structure remain to be addressed. A few of these unanswered questions are illustrated in Fig. 2 and discussed below:

(1) What is the role of the cortical actin cytoskeleton in CME initiation? Do cells actively remodel the cortical actin at sites of CME to allow for the assembly of the endocytic machinery? Are there sites where the cortical actin is 'more permissive' for CME initiation? How is this regulated, and what are the key players involved?
(2) When does actin polymerisation start, and what is the origin of the 'mother filaments' required for this to happen (Collins et al., 2011; Serwas et al., 2021)? EM studies show an endocytic cytoskeleton at an early stage in CME (Collins et al., 2011); whether these early networks have a functional role or represent a "preparation-phase" for later stages is not known.
(3) How does the actin network change in architecture and composition as the CCP bends from a flat to a deeply curved membrane? Given the radical changes in membrane curvature as CME progresses, it is likely that the actin cytoskeleton may need to adapt by changing its biophysical properties. It is tempting to hypothesise that myosins may play a significant role in this process.
(4) What is the significance of the presence of multiple N-WASP activators at CME sites? Are they redundant? What is their relative contribution? Different N-WASP activators have distinct capacities in promoting actin polymerisation (Almeida-Souza et al., 2018). Does the spatiotemporal separation of these activators at CCPs allow fine control of actin polymerisation rates? In addition, what are the factors responsible for nucleating unbranched actin

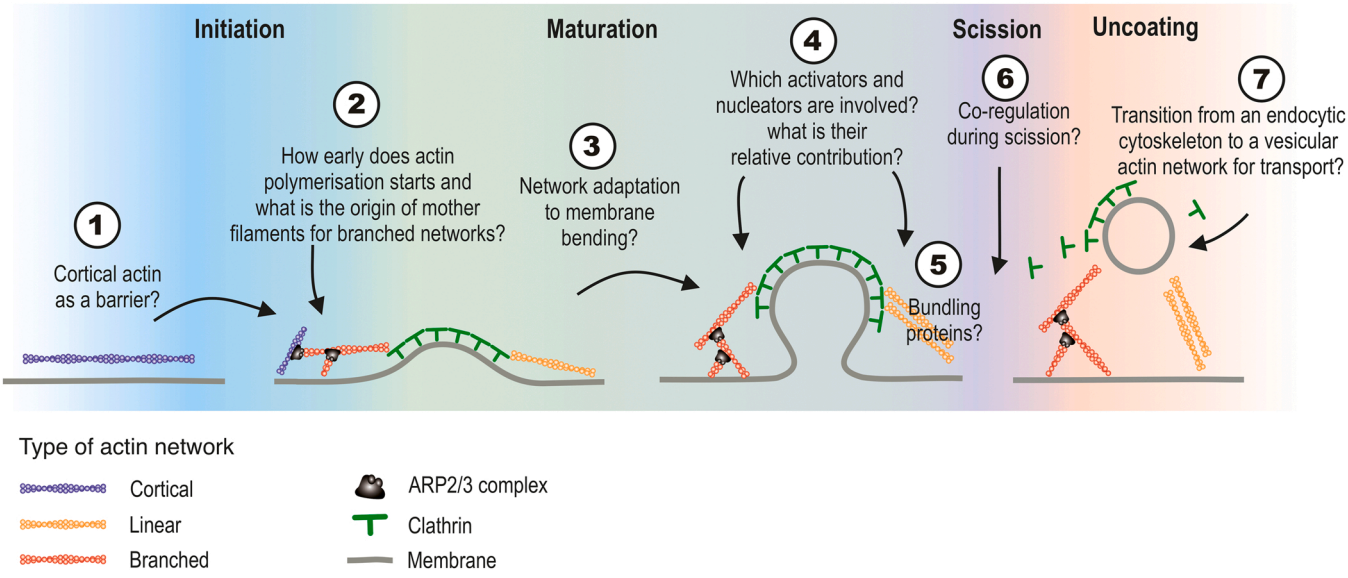

Fig. 2. Illustration showing endocytic progression and mechanistic details missing on the regulation of the endocytic cytoskeleton. Read text for discussion on each point.

filaments (Serwas et al., 2021), and what is their function in CME progression?

(5) What is the significance of actin filament bundling in mammalian CME? The actin crosslinking and bundling protein fimbrin is important for CME in yeast (Skau et al., 2011). The relevance of actin bundling in mammalian CME—and the players involved—, however, remain to be elucidated.

(6) Late CME is characterised by an acceleration of dynamin recruitment and a concurrent surge in actin polymerisation (Taylor et al., 2011)? Are these two processes inter-dependent and/or regulated by the same factors?

(7) Post-scission, what happens to the endocytic cytoskeleton after internalisation, and how does the cell mediate the transition from the plasma membrane-associated endocytic cytoskeleton to the molecular machinery responsible for establishing actin networks around internalised vesicles? At the membrane, is the endocytic cytoskeleton completely disassembled or does a vestigial structure remain that may favour the formation of new endocytic events and explain observed CME hotspots (Henne et al., 2010; Nunez et al., 2011)?

We believe that the answers to these questions will represent important milestones in our understanding of actin regulation during CME. Moreover, considering that the endocytic cytoskeleton formation and disassembly is confined in time and space, it can be used as a powerful model to understand actin regulation in various other cellular contexts. Finally, the multitude of connections between the endocytic cytoskeleton and various human diseases further highlight the importance of understanding the minutiae of this important cellular structure.

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References

Aghamohammadzadeh, S., Ayscough, K.R., 2009. Differential requirements for actin during yeast and mammalian endocytosis. Nat. Cell Biol. 11, 1039–1042. https://doi.org/10.1038/ncb1918.

Akamatsu, M., Vasan, R., Serwas, D., Ferrin, M., Rangamani, P., Drubin, D.G., 2020. Principles of self-organization and load adaptation by the actin cytoskeleton during clathrin-mediated endocytosis. Elife 9. https://doi.org/10.7554/eLife.49840.

Almeida-Souza, L., Frank, R.A.W., García-Nafría, J., Colussi, A., Gunawardana, N., Johnson, C.M., Yu, M., Howard, G., Andrews, B., Vallis, Y., McMahon, H.T., 2018. A flat BAR protein promotes actin polymerization at the base of clathrin-coated pits. Cell 174, 325–337. https://doi.org/10.1016/j.cell.2018.05.020 (.e14).

Altman, D., Goswami, D., Hasson, T., Spudich, J.A., Mayor, S., 2007. Precise positioning of myosin VI on endocytic vesicles in vivo. PLoS Biol. 5, 1712–1722. https://doi.org/10.1371/JOURNAL.PBIO.0050210.

Aschenbrenner, L., Lee, T.T., Hasson, T., 2003. Myo6 facilitates the translocation of endocytic vesicles from cell peripheries. Mol. Biol. Cell 14, 2728–2743. https://doi.org/10.1091/MBC.E02-11-0767.

Aschenbrenner, L., Naccache, S.N., Hasson, T., 2004. Uncoated endocytic vesicles require the unconventional myosin, Myo6, for rapid transport through actin barriers. Mol. Biol. Cell 15, 2253–2263. https://doi.org/10.1091/mbc.E04-01-0002.

Azarnia Tehran, D., López-Hernández, T., Maritzen, T., 2019. Endocytic adaptor proteins in health and disease: lessons from model organisms and human mutations. Cells 8. https://doi.org/10.3390/CELLS8111345.

Barrow, E., Nicola, A.V., Liu, J., 2013. Multiscale perspectives of virus entry via endocytosis. Virol. J. 10 https://doi.org/10.1186/1743-422X-10-177.

Bayati, A., Kumar, R., Francis, V., McPherson, P.S., 2021. SARS-CoV-2 infects cells after viral entry via clathrin-mediated endocytosis. J. Biol. Chem. 296. https://doi.org/10.1016/J.JBC.2021.100306.

Bazinet, C., Katzen, A.L., Morgan, M., Mahowald, A.P., Lemmon, S.K., 1993. The Drosophila clathrin heavy chain gene: clathrin function is essential in a multicellular organism. Genetics 134, 1119–1134. https://doi.org/10.1093/GENETICS/134.4.1119.

Becalska, A.N., Kelley, C.F., Berciu, C., Stanishneva-Konovalova, T.B., Fu, X., Wang, S.Y., Sokolova, O.S., Nicastro, D., Rodal, A.A., 2013. Formation of membrane ridges and scallops by the F-BAR protein nervous wreck. Mol. Biol. Cell 24, 2406–2418. https://doi.org/10.1091/mbc.E13-05-0271.

Benesch, S., Polo, S., Lai, F.P., Anderson, K.I., Stradal, T.E.B., Wehland, J., Rottner, K., 2005. N-WASP deficiency impairs EGF internalization and actin assembly at clathrin-coated pits. J. Cell Sci. 118, 3103–3115. https://doi.org/10.1242/jcs.02444.

Bertling, E., Hotulainen, P., Mattila, P.K., Matilainen, T., Salminen, M., Lappalainen, P., 2004. Cyclase-associated protein 1 (CAP1) promotes cofilin-induced actin dynamics in mammalian nonmuscle cells. Mol. Biol. Cell 15, 2324–2334. https://doi.org/10.1091/MBC.E04-01-0048.

Bhattacharyya, N.P., Banerjee, M., Majumder, P., 2008. Huntington's disease: roles of huntingtin-interacting protein 1 (HIP-1) and its molecular partner HIPPI in the regulation of apoptosis and transcription. FEBS J. 275, 4271–4279. https://doi.org/10.1111/J.1742-4658.2008.06563.X.

Bhave, M., Mino, R.E., Wang, X., Lee, J., Grossman, H.M., Lakoduk, A.M., Danuser, G., Schmid, S.L., Mettlen, M., 2020. Functional characterization of 67 endocytic accessory proteins using multiparametric quantitative analysis of CCP dynamics. Proc. Natl. Acad. Sci. USA 117, 31591–31602. https://doi.org/10.1073/pnas.2020346117.

Biancospino, M., Buel, G.R., Niño, C.A., Maspero, E., di Perrotolo, R.S., Raimondi, A., Redlingshöfer, L., Weber, J., Brodsky, F.M., Walters, K.J., Polo, S., 2019. Clathrin light chain A drives selective myosin VI recruitment to clathrin-coated pits under membrane tension. Nat. Commun. 10, 4974. https://doi.org/10.1038/s41467-019-12855-6.

Bitsikas, V., Corrêa, I.R., Nichols, B.J., 2014. Clathrin-independent pathways do not contribute significantly to endocytic flux. Elife 3, e03970. https://doi.org/10.7554/eLife.03970.

Blanchoin, L., Boujemaa-Paterski, R., Sykes, C., Plastino, J., 2014. Actin dynamics, architecture, and mechanics in cell motility. Physiol. Rev. 94, 235–263. https://doi.org/10.1152/physrev.00018.2013.

Boucrot, E., Saffarian, S., Massol, R., Kirchhausen, T., Ehrlich, M., 2006. Role of lipids and actin in the formation of clathrin-coated pits. Exp. Cell Res. 312, 4036–4048. https://doi.org/10.1016/j.yexcr.2006.09.025.

Boulant, S., Kural, C., Zeeh, J.-C., Ubelmann, F., Kirchhausen, T., 2011. Actin dynamics counteract membrane tension during clathrin-mediated endocytosis. Nat. Cell Biol. 13, 1124–1131. https://doi.org/10.1038/ncb2307.

Buss, F., Luzio, J.P., Kendrick-Jones, J., 2001. Myosin VI, a new force in clathrin mediated endocytosis. FEBS Lett. 508, 295–299. https://doi.org/10.1016/S0014-5793(01)03065-4.

Cao, H., Orth, J.D., Chen, J., Weller, S.G., Heuser, J.E., McNiven, M.A., 2003. Cortactin is a component of clathrin-coated pits and participates in receptor-mediated endocytosis. Mol. Cell. Biol. 23, 2162–2170. https://doi.org/10.1128/MCB.23.6.2162-2170.2003.

Chandrasekar, I., Goeckeler, Z.M., Turney, S.G., Wang, P., Wysolmerski, R.B., Adelstein, R.S., Bridgman, P.C., 2014. Nonmuscle myosin II is a critical regulator of clathrin-mediated endocytosis. Traffic 15, 418–432. https://doi.org/10.1111/TRA.12152.

Chen, H., Fre, S., Slepnev, V.I., Capua, M.R., Takei, K., Butler, M.H., Di Fiore, P.P., De Camilli, P., 1998. Epsin is an EH-domain-binding protein implicated in clathrin-mediated endocytosis. Nature 394, 793–797. https://doi.org/10.1038/29555.

Chen, M.S., Obar, R.A., Schroeder, C.C., Austin, T.W., Poodry, C.A., Wadsworth, S.C., Vallee, R.B., 1991. Multiple forms of dynamin are encoded by shibire, a Drosophila gene involved in endocytosis. Nature 351, 583–586. https://doi.org/10.1038/351583a0.

Chen, Q., Pollard, T.D., 2013. Actin filament severing by cofilin dismantles actin patches and produces mother filaments for new patches. Curr. Biol. 23, 1154–1162. https://doi.org/10.1016/J.CUB.2013.05.005.

Clarke, N.I., Royle, S.J., 2018. FerriTag is a new genetically-encoded inducible tag for correlative light-electron microscopy. Nat. Commun. 9. https://doi.org/10.1038/S41467-018-04993-0.

Collins, A., Warrington, A., Taylor, K.A., Svitkina, T., 2011. Structural organization of the actin cytoskeleton at sites of clathrin-mediated endocytosis. Curr. Biol. 21, 1167–1175. https://doi.org/10.1016/j.cub.2011.05.048.

Coyle, I.P., Koh, Y.H., Lee, W.C.M., Slind, J., Fergestad, T., Littleton, J.T., Ganetzky, B., 2004. Nervous wreck, an SH3 adaptor protein that interacts with Wsp, regulates synaptic growth in Drosophila. Neuron 41, 521–534. https://doi.org/10.1016/S0896-6273(04)00016-9.

Cureton, D.K., Massol, R.H., Saffarian, S., Kirchhausen, T.L., Whelan, S.P.J., 2009. Vesicular stomatitis virus enters cells through vesicles incompletely coated with clathrin that depend upon actin for internalization. PLoS Pathog. 5 https://doi.org/10.1371/JOURNAL.PPAT.1000394.

Cureton, D.K., Massol, R.H., Whelan, S.P.J., Kirchhausen, T., 2010. The length of vesicular stomatitis virus particles dictates a need for actin assembly during clathrin-dependent endocytosis. PLoS Pathog. 6 https://doi.org/10.1371/JOURNAL.PPAT.1001127.

Del Signore, S.J., Kelley, C.F., Messelaar, E.M., Lemos, T., Marchan, M.F., Ermanoska, B., Mund, M., Fai, T.G., Kaksonen, M., Rodal, A.A., 2021. An autoinhibitory clamp of actin assembly constrains and directs synaptic endocytosis. Elife 10. https://doi.org/10.7554/ELIFE.69597.

Dharmalingam, E., Haeckel, A., Pinyol, R., Schwintzer, L., Koch, D., Kessels, M.M., Qualmann, B., 2009. F-BAR proteins of the syndapin family shape the plasma

membrane and are crucial for neuromorphogenesis. J. Neurosci. 29, 13315–13327. https://doi.org/10.1523/JNEUROSCI.3973-09.2009.
Doherty, G.J., McMahon, H.T., 2009. Mechanisms of endocytosis. Annu. Rev. Biochem. 78, 857–902. https://doi.org/10.1146/annurev.biochem.78.081307.110540.
Ehrlich, M., Boll, W., Van Oijen, A., Hariharan, R., Chandran, K., Nibert, M.L., Kirchhausen, T., 2004. Endocytosis by random initiation and stabilization of clathrin-coated pits. Cell 118, 591–605. https://doi.org/10.1016/J.CELL.2004.08.017.
El Dahshan, D., Metwaly, H., Sheir, R., Aly, T., 2014. High expression of FCHSD2 is associated with chemoresistance in adult acute myeloid leukemia. Comp. Clin. Pathol. 23, 1441–1446. https://doi.org/10.1007/s00580-013-1802-9.
Engqvist-Goldstein, Å.E.Y., Kessels, M.M., Chopra, V.S., Hayden, M.R., Drubin, D.G., 1999. An actin-binding protein of the Sla2/Huntingtin interacting protein 1 family is a novel component of clathrin-coated pits and vesicles. J. Cell Biol. 147, 1503–1518. https://doi.org/10.1083/JCB.147.7.1503.
Engqvist-Goldstein, Å.E.Y., Warren, R.A., Kessels, M.M., Keen, J.H., Heuser, J., Drubin, D.G., 2001. The actin-binding protein Hip1R associates with clathrin during early stages of endocytosis and promotes clathrin assembly in vitro. J. Cell Biol. 154, 1209–1223. https://doi.org/10.1083/JCB.200106089.
Engqvist-Goldstein, Å.E.Y., Zhang, C.X., Carreno, S., Barroso, C., Heuser, J.E., Drubin, D. G., 2004. RNAi-mediated Hip1R silencing results in stable association between the endocytic machinery and the actin assembly machinery. Mol. Biol. Cell 15, 1666–1679. https://doi.org/10.1091/MBC.E03-09-0639.
Fares, H., Grant, B., 2002. Deciphering endocytosis in Caenorhabditis elegans. Traffic 3, 11–19. https://doi.org/10.1034/j.1600-0854.2002.30103.x.
Fares, H., Greenwald, I., 2001. Genetic analysis of endocytosis in caenorhabditis elegans: coelomocyte uptake defective mutants. Genetics 159, 133–145. https://doi.org/10.1093/genetics/159.1.133.
Feng, Y., Hartig, S.M., Bechill, J.E., Blanchard, E.G., Caudell, E., Corey, S.J., 2010. The Cdc42-interacting protein-4 (CIP4) gene knock-out mouse reveals delayed and decreased endocytosis. J. Biol. Chem. 285, 4348–4354. https://doi.org/10.1074/JBC.M109.041038.
Ferguson, J.P., Huber, S.D., Willy, N.M., Aygün, E., Goker, S., Atabey, T., Kural, C., 2017. Mechanoregulation of clathrin-mediated endocytosis. J. Cell Sci. 130, 3631–3636. https://doi.org/10.1242/jcs.205930.
Ferguson, S., Raimondi, A., Paradise, S., Shen, H., Mesaki, K., Ferguson, A., Destaing, O., Ko, G., Takasaki, J., Cremona, O., O' Toole, E., De Camilli, P., 2009. Coordinated actions of actin and BAR proteins upstream of dynamin at endocytic clathrin-coated pits. Dev. Cell 17, 811–822. https://doi.org/10.1016/j.devcel.2009.11.005.
Franck, A., Lainé, J., Moulay, G., Lemerle, E., Trichet, M., Gentil, C., Benkhelifa-Ziyyat, S., Lacène, E., Bui, M.T., Brochier, G., Guicheney, P., Romero, N., Bitoun, M., Vassilopoulos, S., 2019. Clathrin plaques and associated actin anchor intermediate filaments in skeletal muscle. Mol. Biol. Cell 30, 579–590. https://doi.org/10.1091/mbc.E18-11-0718.
Fricke, R., Gohl, C., Dharmalingam, E., Grevelhörster, A., Zahedi, B., Harden, N., Kessels, M., Qualmann, B., Bogdan, S., 2009. Drosophila Cip4/Toca-1 integrates membrane trafficking and actin dynamics through WASP and SCAR/WAVE. Curr. Biol. 19, 1429–1437. https://doi.org/10.1016/j.cub.2009.07.058.
Fujimoto, L.M., Roth, R., Heuser, J.E., Schmid, S.L., 2000. Actin assembly plays a variable, but not obligatory role in receptor-mediated endocytosis in mammalian cells. Traffic 1, 161–171. https://doi.org/10.1034/j.1600-0854.2000.010208.x.
Fujiwara, T., Ritchie, K., Murakoshi, H., Jacobson, K., Kusumi, A., 2002. Phospholipids undergo hop diffusion in compartmentalized cell membrane. J. Cell Biol. 157, 1071–1081. https://doi.org/10.1083/JCB.200202050.
Garcia-Alai, M.M., Heidemann, J., Skruzny, M., Gieras, A., Mertens, H.D.T., Svergun, D. I., Kaksonen, M., Uetrecht, C., Meijers, R., 2018. Epsin and Sla2 form assemblies through phospholipid interfaces. Nat. Commun. 9, 328. https://doi.org/10.1038/s41467-017-02443-x.
Giuliani, C., Troglio, F., Bai, Z., Patel, F.B., Zucconi, A., Malabarba, M.G., Disanza, A., Stradal, T.B., Cassata, G., Confalonieri, S., Hardin, J.D., Soto, M.C., Grant, B.D., Scita, G., 2009. Requirements for F-BAR proteins TOCA-1 and TOCA-2 in actin dynamics and membrane trafficking during Caenorhabditis elegans oocyte growth and embryonic epidermal morphogenesis. PLoS Genet. 5, e1000675 https://doi.org/10.1371/journal.pgen.1000675.
Goddette, D.W., Frieden, C., 1986. Actin polymerization. The mechanism of action of cytochalasin D. J. Biol. Chem. 261, 15974–15980. https://doi.org/10.1016/S0021-9258(18)66662-1.
Goley, E.D., Welch, M.D., 2006. The ARP2/3 complex: an actin nucleator comes of age. Nat. Rev. Mol. Cell Biol. 7, 713–726. https://doi.org/10.1038/NRM2026.
Goode, B.L., Eskin, J.A., Wendland, B., 2015. Actin and endocytosis in budding yeast. Genetics 199, 315–358. https://doi.org/10.1534/genetics.112.145540.
Gottlieb, T.A., Ivanov, I.E., Adesnik, M., Sabatini, D.D., 1993. Actin microfilaments play a critical role in endocytosis at the apical but not the basolateral surface of polarized epithelial cells. J. Cell Biol. 120, 695–710. https://doi.org/10.1083/JCB.120.3.695.
Grant, B., Hirsh, D., 1999. Receptor-mediated endocytosis in the Caenorhabditis elegans oocyte. Mol. Biol. Cell 10, 4311–4326. https://doi.org/10.1091/MBC.10.12.4311/ASSET/IMAGES/LARGE/MK1291061009.JPEG.
Grassart, A., Cheng, A.T., Hong, S.H., Zhang, F., Zenzer, N., Feng, Y., Briner, D.M., Davis, G.D., Malkov, D., Drubin, D.G., 2014. Actin and dynamin2 dynamics and interplay during clathrin-mediated endocytosis. J. Cell Biol. 205, 721–735. https://doi.org/10.1083/jcb.201403041.
Grigliatti, T.A., Hall, L., Rosenbluth, R., Suzuki, D.T., 1973. Temperature-sensitive mutations in Drosophila melanogaster. Mol. Gen. Genet. MGG 120, 107–114. https://doi.org/10.1007/BF00267238.
Han, Y., Cui, J., Lu, Y., Sue, S., Arpaia, E., Mak, T.W., Minden, M.D., 2012. FCHSD2 predicts response to chemotherapy in acute myeloid leukemia patients. Leuk. Res. 36, 1339–1346. https://doi.org/10.1016/j.leukres.2012.06.011.
Harris, T.W., Schuske, K., Jorgensen, E.M., 2001. Studies of synaptic vesicle endocytosis in the Nematode C. elegans. Traffic 2, 597–605. https://doi.org/10.1034/J.1600-0854.2001.002009597.X.
Hassinger, J.E., Oster, G., Drubin, D.G., Rangamani, P., 2017. Design principles for robust vesiculation in clathrin-mediated endocytosis. Proc. Natl. Acad. Sci. USA 114, E1118–E1127. https://doi.org/10.1073/pnas.1617705114.
Helgeson, L.A., Nolen, B.J., 2013. Mechanism of synergistic activation of Arp2/3 complex by cortactin and N-WASP. Elife 2, e00884. https://doi.org/10.7554/eLife.00884.
Helgeson, L.A., Prendergast, J.G., Wagner, A.R., Rodnick-Smith, M., Nolen, B.J., 2014. Interactions with actin monomers, actin filaments, and Arp2/3 complex define the roles of WASP family proteins and cortactin in coordinately regulating branched actin networks. J. Biol. Chem. 289, 28856–28869. https://doi.org/10.1074/jbc.M114.587527.
Henne, W.M., Boucrot, E., Meinecke, M., Evergren, E., Vallis, Y., Mittal, R., McMahon, H. T., 2010. FCHo proteins are nucleators of clathrin-mediated endocytosis. Science (80-) 328, 1281–1284. https://doi.org/10.1126/science.1188462.
Heuser, J., Evans, L., 1980. Three-dimensional visualization of coated vesicle formation in fibroblasts. J. Cell Biol. 84, 560–583. https://doi.org/10.1083/JCB.84.3.560.
Hryciw, D.H., Wang, Y., Devuyst, O., Pollock, C.A., Poronnik, P., Guggino, W.B., 2003. Cofilin interacts with ClC-5 and regulates albumin uptake in proximal tubule cell lines. J. Biol. Chem. 278, 40169–40176. https://doi.org/10.1074/JBC.M307890200.
Hu, M., Cebola, I., Carrat, G., Jiang, S., Nawaz, S., Khamis, A., Canouil, M., Froguel, P., Schulte, A., Solimena, M., Ibberson, M., Marchetti, P., Cardenas-Diaz, F.L., Gadue, P. J., Hastoy, B., Almeida-Souza, L., McMahon, H., Rutter, G.A., 2021. Chromatin 3D interaction analysis of the STARD10 locus unveils FCHSD2 as a regulator of insulin secretion. Cell Rep. 34, 108703 https://doi.org/10.1016/j.celrep.2021.108703.
Idrissi, F.Z., Blasco, A., Espinal, A., Geli, M.I., 2012. Ultrastructural dynamics of proteins involved in endocytic budding. Proc. Natl. Acad. Sci. USA 109, E2587–E2594. https://doi.org/10.1073/pnas.1202789109.
Idrissi, F.Z., Wolf, B.L., Geli, M.I., 2002. Cofilin, but not profilin, is required for myosin-I-induced actin polymerization and the endocytic uptake in yeast. Mol. Biol. Cell 13, 4074–4087. https://doi.org/10.1091/MBC.02-04-0052.
Innocenti, M., Zucconi, A., Disanza, A., Frittoli, E., Areces, L.B., Steffen, A., Stradal, T.E. B., Di Fiore, P.P., Carlier, M.F., Scita, G., 2004. Abi1 is essential for the formation and activation of a WAVE2 signalling complex. Nat. Cell Biol. 6, 319–327. https://doi.org/10.1038/ncb1105.
Jackman, M.R., Shurety, W., Ellis, J.A., Luzio, J.P., 1994. Inhibition of apical but not basolateral endocytosis of ricin and folate in Caco-2 cells by cytochalasin D. J. Cell Sci. 107 (Pt 9), 2547–2556. https://doi.org/10.1242/jcs.107.9.2547.
Jha, A., Traub, L.M., 2014. Visualization of clathrin-mediated endocytosis in live Drosophila egg chambers. Methods Mol. Biol. 1174, 349–360. https://doi.org/10.1007/978-1-4939-0944-5_24.
Jin, M., Shirazinejad, C., Wang, B., Yan, A., Schöneberg, J., Upadhyayula, S., Xu, K., Drubin, D.G., 2021. Rescue of stalled clathrin-mediated endocytosis by asymmetric Arp2/3-mediated actin assembly. bioRxiv, 2021.07.16.452693. ⟨https://doi.org/10.1101/2021.07.16.452693⟩.
Kaksonen, M., Roux, A., 2018. Mechanisms of clathrin-mediated endocytosis. Nat. Rev. Mol. Cell Biol. 19, 313–326. https://doi.org/10.1038/nrm.2017.132.
Kaksonen, M., Sun, Y., Drubin, D.G., 2003. A pathway for association of receptors, adaptors, and actin during endocytic internalization. Cell 115, 475–487. https://doi.org/10.1016/S0092-8674(03)00883-3.
Kalchman, M.A., Koide, H.B., McCutcheon, K., Graham, R.K., Nichol, K., Nishiyama, K., Kazemi-Esfarjani, P., Lynn, F.C., Wellington, C., Metzler, M., Goldberg, Y.P., Kanazawa, I., Gietz, R.D., Hayden, M.R., 1997. HIP1, a human homologue of S. cerevisiae Sla2p, interacts with membrane-associated huntingtin in the brain. Nat. Genet. 16, 44–53. https://doi.org/10.1038/NG0597-44.
Kamioka, Y., Fukuhara, S., Sawa, H., Nagashima, K., Masuda, M., Matsuda, M., Mochizuki, N., 2004. A novel dynamin-associating molecule, formin-binding protein 17, induces tubular membrane invaginations and participates in endocytosis. J. Biol. Chem. 279, 40091–40099. https://doi.org/10.1074/JBC.M404899200.
Kaplan, C., Kenny, S.J., Chen, X., Schöneberg, J., Sitarska, E., Diz-Muñoz, A., Akamatsu, M., Xu, K., Drubin, D.G., 2021. Load adaptation of endocytic actin networks. bioRxiv, 2020.04.05.026559. ⟨https://doi.org/10.1101/2020.04.05.026559⟩.
Kaur, S., Fielding, A.B., Gassner, G., Carter, N.J., Royle, S.J., 2014. An unmet actin requirement explains the mitotic inhibition of clathrin-mediated endocytosis. Elife 3, e00829. https://doi.org/10.7554/elife.00829.
Kelley, C.F., Messelaar, E.M., Eskin, T.L., Wang, S., Song, K., Vishnia, K., Becalska, A.N., Shupliakov, O., Hagan, M.F., Danino, D., Sokolova, O.S., Nicastro, D., Rodal, A.A., 2015. Membrane charge directs the outcome of F-BAR domain lipid binding and autoregulation. Cell Rep. 13, 2597–2609. https://doi.org/10.1016/j.celrep.2015.11.044.
Kessels, M.M., Engqvist-Goldstein, Å.E.Y., Drubin, D.G., Qualmann, B., 2001. Mammalian Abp1, a signal-responsive F-actin-binding protein, links the actin cytoskeleton to endocytosis via the GTPase dynamin. J. Cell Biol. 153, 351–366. https://doi.org/10.1083/JCB.153.2.351.
Kessels, M.M., Qualmann, B., 2002. Syndapins integrate N-WASP in receptor-mediated endocytosis. EMBO J. 21, 6083–6094. https://doi.org/10.1093/emboj/cdf604.
Kochubey, O., Majumdar, A., Klingauf, J., 2006. Imaging clathrin dynamics in Drosophila melanogaster hemocytes reveals a role for actin in vesicle fission. Traffic 7, 1614–1627. https://doi.org/10.1111/j.1600-0854.2006.00492.x.

Krendel, M., Osterweil, E.K., Mooseker, M.S., 2007. Myosin 1E interacts with synaptojanin-1 and dynamin and is involved in endocytosis. FEBS Lett. 581, 644–650. https://doi.org/10.1016/J.FEBSLET.2007.01.021.
Kukulski, W., Schorb, M., Kaksonen, M., Briggs, J.A.G., 2012. Plasma membrane reshaping during endocytosis is revealed by time-resolved electron tomography. Cell 150, 508–520. https://doi.org/10.1016/j.cell.2012.05.046.
Kuromi, H., Kidokoro, Y., 1998. Two distinct pools of synaptic vesicles in single presynaptic boutons in a temperature-sensitive Drosophila mutant, shibire. Neuron 20, 917–925. https://doi.org/10.1016/S0896-6273(00)80473-0.
Lamaze, C., Fujimoto, L.M., Yin, H.L., Schmid, S.L., 1997. The actin cytoskeleton is required for receptor-mediated endocytosis in mammalian cells. J. Biol. Chem. 272, 20332–20335. https://doi.org/10.1074/jbc.272.33.20332.
Lappalainen, P., Drubin, D.G., 1997. Cofilin promotes rapid actin filament turnover in vivo. Nature 388, 78–82. https://doi.org/10.1038/40418.
Le Clainche, C., Pauly, B.S., Zhang, C.X., Engqvist-Goldstein, Å.E.Y., Cunningham, K., Drubin, D.G., 2007. A Hip1R-cortactin complex negatively regulates actin assembly associated with endocytosis. EMBO J. 26, 1199–1210. https://doi.org/10.1038/sj.emboj.7601576.
Leibfried, A., Fricke, R., Morgan, M.J., Bogdan, S., Bellaiche, Y., 2008. Drosophila Cip4 and WASp define a branch of the Cdc42-Par6-aPKC pathway regulating E-cadherin endocytosis. Curr. Biol. 18, 1639–1648. https://doi.org/10.1016/j.cub.2008.09.063.
Li, E., Stupack, D., Bokoch, G.M., Nemerow, G.R., 1998. Adenovirus endocytosis requires actin cytoskeleton reorganization mediated by Rho family GTPases. J. Virol. 72, 8806–8812. https://doi.org/10.1128/JVI.72.11.8806-8812.1998.
Lundmark, R., Carlsson, S.R., 2002. The beta-appendages of the four adaptor-protein (AP) complexes: structure and binding properties, and identification of sorting nexin 9 as an accessory protein to AP-2. Biochem. J. 362, 597–607. https://doi.org/10.1042/0264-6021:3620597.
Manenschijn, H.E., Picco, A., Mund, M., Rivier-Cordey, A.S., Ries, J., Kaksonen, M., 2019. Type-I myosins promote actin polymerization to drive membrane bending in endocytosis. Elife 8. https://doi.org/10.7554/ELIFE.44215.
Maples, C.J., Ruiz, W.G., Apodaca, G., 1997. Both microtubules and actin filaments are required for efficient postendocytotic traffic of the polymeric immunoglobulin receptor in polarized Madin-Darby canine kidney cells. J. Biol. Chem. 272, 6741–6751. https://doi.org/10.1074/jbc.272.10.6741.
Marsh, M., Helenius, A., 2006. Virus entry: open sesame. Cell 124, 729–740. https://doi.org/10.1016/J.CELL.2006.02.007.
Masedunskas, A., Porat-Shliom, N., Rechache, K., Aye, M.P., Weigert, R., 2012. Intravital microscopy reveals differences in the kinetics of endocytic pathways between cell cultures and live animals. Cells 1, 1121–1132. https://doi.org/10.3390/CELLS1041121.
Mayor, S., Pagano, R.E., 2007. Pathways of clathrin-independent endocytosis. Nat. Rev. Mol. Cell Biol. 8, 603–612. https://doi.org/10.1038/nrm2216.
McMahon, H.T., Boucrot, E., 2011. Molecular mechanism and physiological functions of clathrin-mediated endocytosis. Nat. Rev. Mol. Cell Biol. 12, 517–533. https://doi.org/10.1038/nrm3151.
McNiven, M.A., Kim, L., Krueger, E.W., Orth, J.D., Cao, H., Wong, T.W., 2000. Regulated interactions between dynamin and the actin-binding protein cortactin modulate cell shape. J. Cell Biol. 151, 187–198. https://doi.org/10.1083/jcb.151.1.187.
Merrifield, C.J., Feldman, M.E., Wan, L., Almers, W., 2002. Imaging actin and dynamin recruitment during invagination of single clathrin-coated pits. Nat. Cell Biol. 4, 691–698. https://doi.org/10.1038/ncb837.
Merrifield, C.J., Perrais, D., Zenisek, D., 2005. Coupling between clathrin-coated-pit invagination, cortactin recruitment, and membrane scission observed in live cells. Cell 121, 593–606. https://doi.org/10.1016/j.cell.2005.03.015.
Merrifield, C.J., Qualmann, B., Kessels, M.M., Almers, W., 2004. Neural Wiskott Aldrich Syndrome Protein (N-WASP) and the Arp 2/3 complex are recruited to sites of clathrin-mediated endocytosis in cultured fibroblasts. Eur. J. Cell Biol. 83, 13–18. https://doi.org/10.1078/0171-9335-00356.
Messa, M., Fernández-Busnadiego, R., Sun, E.W., Chen, H., Czapla, H., Wrasman, K., Wu, Y., Ko, G., Ross, T., Wendland, B., De Camilli, P., 2014. Epsin deficiency impairs endocytosis by stalling the actin-dependent invagination of endocytic clathrin-coated pits. Elife 3, 1–25. https://doi.org/10.7554/eLife.03311.
Mitsunari, T., Nakatsu, F., Shioda, N., Love, P.E., Grinberg, A., Bonifacino, J.S., Ohno, H., 2005. Clathrin adaptor AP-2 is essential for early embryonal development. Mol. Cell. Biol. 25, 9318–9323. https://doi.org/10.1128/MCB.25.21.9318-9323.2005.
Montagnac, G., Meas-Yedid, V., Irondelle, M., Castro-Castro, A., Franco, M., Shida, T., Nachury, M.V., Benmerah, A., Olivo-Marin, J.C., Chavrier, P., 2013. αtAT1 catalyses microtubule acetylation at clathrin-coated pits. Nature 502, 567–570. https://doi.org/10.1038/nature12571.
Mooren, O.L., Kotova, T.I., Moore, A.J., Schafer, D.A., 2009. Dynamin2 GTPase and cortactin remodel actin filaments. J. Biol. Chem. 284, 23995–24005. https://doi.org/10.1074/jbc.M109.024398.
Morris, S.M., Arden, S.D., Roberts, R.C., Kendrick-Jones, J., Cooper, J.A., Luzio, J.P., Buss, F., 2002. Myosin VI binds to and localises with Dab2, potentially linking receptor-mediated endocytosis and the actin cytoskeleton. Traffic 3, 331–341. https://doi.org/10.1034/J.1600-0854.2002.30503.X.
Moskowitz, H.S., Yokoyama, C.T., Ryan, T.A., 2005. Highly cooperative control of endocytosis by clathrin. Mol. Biol. Cell 16, 1769–1776. https://doi.org/10.1091/mbc.e04-08-0739.
Nielsen, T., Sparsø, T., Grarup, N., Jørgensen, T., Pisinger, C., Witte, D.R., Hansen, T., Pedersen, O., 2011. Type 2 diabetes risk allele near CENTD2 is associated with decreased glucose-stimulated insulin release. Diabetologia 54, 1052–1056. https://doi.org/10.1007/S00125-011-2054-3/TABLES/1.
Nunez, D., Antonescu, C., Mettlen, M., Liu, A., Schmid, S.L., Loerke, D., Danuser, G., 2011. Hotspots organize clathrin-mediated endocytosis by efficient recruitment and retention of nucleating resources. Traffic 12, 1868–1878. https://doi.org/10.1111/j.1600-0854.2011.01273.x.
O'Connor-Giles, K.M., Ho, L.L., Ganetzky, B., 2008. Nervous wreck interacts with thickveins and the endocytic machinery to attenuate retrograde BMP signaling during synaptic growth. Neuron 58, 507–518. https://doi.org/10.1016/J.NEURON.2008.03.007.
Oser, M., Yamaguchi, H., Mader, C.C., Bravo-Cordero, J.J., Arias, M., Chen, X., Desmarais, V., van Rheenen, J., Koleske, A.J., Condeelis, J., 2009. Cortactin regulates cofilin and N-WASp activities to control the stages of invadopodium assembly and maturation. J. Cell Biol. 186, 571–587. https://doi.org/10.1083/jcb.200812176.
Patel, F.B., Soto, M.C., 2013. WAVE/SCAR promotes endocytosis and early endosome morphology in polarized C. elegans epithelia. Dev. Biol. 377, 319–332. https://doi.org/10.1016/j.ydbio.2013.03.012.
Pedersen, R.T.A., Drubin, D.G., 2019. Type i myosins anchor actin assembly to the plasma membrane during clathrin-mediated endocytosis. J. Cell Biol. 218, 1138–1147. https://doi.org/10.1083/jcb.201810005.
Picco, A., Kukulski, W., Manenschijn, H.E., Specht, T., Briggs, J.A.G., Kaksonen, M., 2018. The contributions of the actin machinery to endocytic membrane bending and vesicle formation. Mol. Biol. Cell 29, 1346–1358. https://doi.org/10.1091/mbc.E17-11-0688.
Pinyol, R., Haeckel, A., Ritter, A., Qualmann, B., Kessels, M.M., 2007. Regulation of N-WASP and the Arp2/3 complex by Abp1 controls neuronal morphology. PLoS One 2. https://doi.org/10.1371/JOURNAL.PONE.0000400.
Poodry, C.A., Edgar, L., 1979. Reversible alteration in the neuromuscular junctions of Drosophila melanogaster bearing a temperature-sensitive mutation, shibire. J. Cell Biol. 81, 520–527. https://doi.org/10.1083/jcb.81.3.520.
Poodry, C.A., Hall, L., Suzuki, D.T., 1973. Developmental properties of shibirets1: a pleiotropic mutation affecting larval and adult locomotion and development. Dev. Biol. 32, 373–386. https://doi.org/10.1016/0012-1606(73)90248-0.
Porpaczy, E., Bilban, M., Heinze, G., Gruber, M., Vanura, K., Schwarzinger, I., Stilgenbauer, S., Streubel, B., Fonatsch, C., Jaeger, U., 2009. Gene expression signature of chronic lymphocytic leukaemia with Trisomy 12. Eur. J. Clin. Invest. 39, 568–575. https://doi.org/10.1111/J.1365-2362.2009.02146.X.
Pouille, P.-A., Ahmadi, P., Brunet, A.-C., Farge, E., 2009. Mechanical signals trigger Myosin II redistribution and mesoderm invagination in Drosophila embryos. Sci. Signal. 2, ra16. https://doi.org/10.1126/scisignal.2000098.
Qualmann, B., Kelly, R.B., 2000. Syndapin isoforms participate in receptor-mediated endocytosis and actin organization. J. Cell Biol. 148, 1047–1062. https://doi.org/10.1083/jcb.148.5.1047.
Qualmann, B., Roos, J., DiGregorio, P.J., Kelly, R.B., 1999. Syndapin I, a synaptic dynamin-binding protein that associates with the neural Wiskott-Aldrich syndrome protein. Mol. Biol. Cell 10, 501–513. https://doi.org/10.1091/mbc.10.2.501.
Rodal, A.A., Blunk, A.D., Akbergenova, Y., Jorquera, R.A., Buhl, L.K., Littleton, J.T., 2011. A presynaptic endosomal trafficking pathway controls synaptic growth signaling. J. Cell Biol. 193, 201–217. https://doi.org/10.1083/JCB.201009052.
Rodal, A.A., Motola-Barnes, R.N., Littleton, J.T., 2008. Nervous wreck and Cdc42 cooperate to regulate endocytic actin assembly during synaptic growth. J. Neurosci. 28, 8316–8325. https://doi.org/10.1523/JNEUROSCI.2304-08.2008.
Rosenthal, J.A., Chen, H., Slepnev, V.I., Pellegrini, L., Salcini, A.E., Di Fiore, P.P., De Camilli, P., 1999. The epsins define a family of proteins that interact with components of the clathrin coat and contain a new protein module. J. Biol. Chem. 274, 33959–33965. https://doi.org/10.1074/jbc.274.48.33959.
Rust, M.J., Lakadamyali, M., Zhang, F., Zhuang, X., 2004. Assembly of endocytic machinery around individual influenza viruses during viral entry. Nat. Struct. Mol. Biol. 11, 567–573. https://doi.org/10.1038/NSMB769.
Saffarian, S., Cocucci, E., Kirchhausen, T., 2009. Distinct dynamics of endocytic clathrin-coated pits and coated plaques. PLoS Biol. 7, e1000191 https://doi.org/10.1371/JOURNAL.PBIO.1000191.
Sandvig, K., Van Deurs, B., 1990. Selective modulation of the endocytic uptake of ricin and fluid phase markers without alteration in transferrin endocytosis. J. Biol. Chem. 265, 6382–6388. https://doi.org/10.1016/S0021-9258(19)39337-8.
Santini, F., Gaidarov, I., Keen, J.H., 2002. G protein-coupled receptor/arrestin3 modulation of the endocytic machinery. J. Cell Biol. 156, 665–676. https://doi.org/10.1083/JCB.200110132.
Scanlan, M.J., Welt, S., Gordon, C.M., Chen, Y.-T., Gure, A.O., Stockert, E., Jungbluth, A. A., Ritter, G., Jäger, D., Jäger, E., Knuth, A., Old, L.J., 2002. Cancer-related serological recognition of human colon cancer. Cancer Res. 62, 4041–4047.
Schliwa, M., 1982. Action of cytochalasin D on cytoskeletal networks. J. Cell Biol. 92, 79–91. https://doi.org/10.1083/JCB.92.1.79.
Schmid, E.M., McMahon, H.T., 2007. Integrating molecular and network biology to decode endocytosis. Nature 448, 883–888. https://doi.org/10.1038/nature06031.
Schmid, S.L., Sorkin, A., Zerial, M., 2014. Endocytosis: past, present, and future. Cold Spring Harb. Perspect. Biol. 6, a022509 https://doi.org/10.1101/CSHPERSPECT.A022509.
Schuuring, E., Verhoeven, E., Mooi, W.J., Michalides, R.J., 1992. Identification and cloning of two overexpressed genes, U21B31/PRAD1 and EMS1, within the amplified chromosome 11q13 region in human carcinomas. Oncogene 7, 355–361.
Seetharaman, S., Etienne-Manneville, S., 2020. Cytoskeletal crosstalk in cell migration. Trends Cell Biol. 30, 720–735. https://doi.org/10.1016/j.tcb.2020.06.004.
Serwas, D., Akamatsu, M., Moayed, A., Vegesna, K., Vasan, R., Hill, J.M., Schoeneberg, J., Davies, K.M., Rangamani, P., Drubin, D.G., 2021. Actin force generation in vesicle formation: mechanistic insights from cryo-electron tomography. bioRxiv. 2021.06.28.450262. ⟨https://doi.org/10.1101/2021.06.28.450262⟩.

Shi, X., Duan, F., Lin, L., Xu, Q., Xu, T., Zhang, R., 2019. WIP-1 and DBN-1 promote scission of endocytic vesicles by bridging actin and Dynamin-1 in the C. elegans intestine. J. Cell Sci. 132. https://doi.org/10.1242/jcs.228023.

Shimada, A., Niwa, H., Tsujita, K., Suetsugu, S., Nitta, K., Hanawa-Suetsugu, K., Akasaka, R., Nishino, Y., Toyama, M., Chen, L., Liu, Z.J., Wang, B.C., Yamamoto, M., Terada, T., Miyazawa, A., Tanaka, A., Sugano, S., Shirouzu, M., Nagayama, K., Takenawa, T., Yokoyama, S., 2007. Curved EFC/F-BAR-domain dimers are joined end to end into a filament for membrane invagination in endocytosis. Cell 129, 761–772. https://doi.org/10.1016/J.CELL.2007.03.040.

Shivas, J.M., Skop, A.R., 2012. Arp2/3 mediates early endosome dynamics necessary for the maintenance of PAR asymmetry in Caenorhabditis elegans. Mol. Biol. Cell 23, 1917–1927. https://doi.org/10.1091/mbc.E12-01-0006.

Skau, C.T., Courson, D.S., Bestul, A.J., Winkelman, J.D., Rock, R.S., Sirotkin, V., Kovar, D.R., 2011. Actin filament bundling by fimbrin is important for endocytosis, cytokinesis, and polarization in fission yeast. J. Biol. Chem. 286, 26964–26977. https://doi.org/10.1074/jbc.M111.239004.

Skruzny, M., Brach, T., Ciuffa, R., Rybina, S., Wachsmuth, M., Kaksonen, M., 2012. Molecular basis for coupling the plasma membrane to the actin cytoskeleton during clathrin-mediated endocytosis. Proc. Natl. Acad. Sci. USA 109, E2533–E2542. https://doi.org/10.1073/pnas.1207011109.

Sochacki, K.A., Dickey, A.M., Strub, M.-P., Taraska, J.W., 2017. Endocytic proteins are partitioned at the edge of the clathrin lattice in mammalian cells. Nat. Cell Biol. 19, 352–361. https://doi.org/10.1038/ncb3498.

Spudich, G., Chibalina, M.V., Au, J.S.Y., Arden, S.D., Buss, F., Kendrick-Jones, J., 2007. Myosin VI targeting to clathrin-coated structures and dimerization is mediated by binding to disabled-2 and PtdIns(4,5)P2. Nat. Cell Biol. 9, 176–183. https://doi.org/10.1038/NCB1531.

Stanishneva-Konovalova, T.B., Kelley, C.F., Eskin, T.L., Messelaar, E.M., Wasserman, S. A., Sokolova, O.S., Rodal, A.A., 2016. Coordinated autoinhibition of F-BAR domain membrane binding and WASp activation by Nervous Wreck. Proc. Natl. Acad. Sci. USA 113, E5552–E5561. https://doi.org/10.1073/PNAS.1524412113.

Sun, Y., Leong, N.T., Wong, T., Drubin, D.G., 2015. A Pan1/End3/Sla1 complex links Arp2/3-mediated actin assembly to sites of clathrinmediated endocytosis. Mol. Biol. Cell 26, 3841–3856. https://doi.org/10.1091/mbc.E15-04-0252.

Taylor, M.J., Lampe, M., Merrifield, C.J., 2012. A feedback loop between dynamin and actin recruitment during clathrin-mediated endocytosis. PLoS Biol. 10, e1001302 https://doi.org/10.1371/journal.pbio.1001302.

Taylor, Marcus J., Perrais, D., Merrifield, C.J., 2011. A high precision survey of the molecular dynamics of mammalian clathrin-mediated endocytosis. PLoS Biol. 9, e1000604 https://doi.org/10.1371/journal.pbio.1000604.

Uruno, T., Liu, J., Zhang, P., Fan, Y., Egile, C., Li, R., Mueller, S.C., Zhan, X., 2001. Activation of Arp2/3 complex-mediated actin polymerization by cortactin. Nat. Cell Biol. 3, 259–266. https://doi.org/10.1038/35060051.

van der Bliek, A.M., Meyerowitz, E.M., 1991. Dynamin-like protein encoded by the Drosophila shibire gene associated with vesicular traffic. Nature 351, 411–414. https://doi.org/10.1038/351411a0.

Voight, B.F., Scott, L.J., Steinthorsdottir, V., Morris, A.P., Dina, C., Welch, R.P., Zeggini, E., Huth, C., Aulchenko, Y.S., Thorleifsson, G., McCulloch, L.J., Ferreira, T., Grallert, H., Amin, N., Wu, G., Willer, C.J., Raychaudhuri, S., McCarroll, S.A., Langenberg, C., Hofmann, O.M., Dupuis, J., Qi, L., Segrè, A.V., Van Hoek, M., Navarro, P., Ardlie, K., Balkau, B., Benediktsson, R., Bennett, A.J., Blagieva, R., Boerwinkle, E., Bonnycastle, L.L., Boström, K.B., Bravenboer, B., Bumpstead, S., Burtt, N.P., Charpentier, G., Chines, P.S., Cornelis, M., Couper, D.J., Crawford, G., Doney, A.S.F., Elliott, K.S., Elliott, A.L., Erdos, M.R., Fox, C.S., Franklin, C.S., Ganser, M., Gieger, C., Grarup, N., Green, T., Griffin, S., Groves, C.J., Guiducci, C., Hadjadj, S., Hassanali, N., Herder, C., Isomaa, B., Jackson, A.U., Johnson, P.R.V., Jørgensen, T., Kao, W.H.L., Klopp, N., Kong, A., Kraft, P., Kuusisto, J., Lauritzen, T., Li, M., Lieverse, A., Lindgren, C.M., Lyssenko, V., Marre, M., Meitinger, T., Midthjell, K., Morken, M.A., Narisu, N., Nilsson, P., Owen, K.R., Payne, F., Perry, J.R. B., Petersen, A.K., Platou, C., Proença, C., Prokopenko, I., Rathmann, W., Rayner, N. W., Robertson, N.R., Rocheleau, G., Roden, M., Sampson, M.J., Saxena, R., Shields, B.M., Shrader, P., Sigurdsson, G., Sparsø, T., Strassburger, K., Stringham, H. M., Sun, Q., Swift, A.J., Thorand, B., Tichet, J., Tuomi, T., Van Dam, R.M., Van Haeften, T.W., Van Herpt, T., Van Vliet-Ostaptchouk, J.V., Walters, G.B., Weedon, M. N., Wijmenga, C., Witteman, J., Bergman, R.N., Cauchi, S., Collins, F.S., Gloyn, A.L., Gyllensten, U., Hansen, T., Hide, W.A., Hitman, G.A., Hofman, A., Hunter, D.J., Hveem, K., Laakso, M., Mohlke, K.L., Morris, A.D., Palmer, C.N.A., Pramstaller, P.P., Rudan, I., Sijbrands, E., Stein, L.D., Tuomilehto, J., Uitterlinden, A., Walker, M., Wareham, N.J., Watanabe, R.M., Abecasis, G.R., Boehm, B.O., Campbell, H., Daly, M.J., Hattersley, A.T., Hu, F.B., Meigs, J.B., Pankow, J.S., Pedersen, O., Wichmann, H.E., Barroso, I., Florez, J.C., Frayling, T.M., Groop, L., Sladek, R., Thorsteinsdottir, U., Wilson, J.F., Illig, T., Froguel, P., Van Duijn, C.M., Stefansson, K., Altshuler, D., Boehnke, M., McCarthy, M.I., 2010. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nat. Genet. 42, 579–589. https://doi.org/10.1038/ng.609.

Wang, L., Audhya, A., 2014. In vivo imaging of C. elegans endocytosis. Methods 68, 518–528. https://doi.org/10.1016/j.ymeth.2014.03.028.

Wanker, E.E., Rovira, C., Scherzinger, E., Hasenbank, R., Wälter, S., Tait, D., Colicelli, J., Lehrach, H., 1997. HIP-I: a huntingtin interacting protein isolated by the yeast two-hybrid system. Hum. Mol. Genet. 6, 487–495. https://doi.org/10.1093/HMG/6.3.487.

Weaver, A.M., Karginov, A.V., Kinley, A.W., Weed, S.A., Li, Y., Parsons, J.T., Cooper, J. A., 2001. Cortactin promotes and stabilizes Arp2/3-induced actin filament network formation. Curr. Biol. 11, 370–374. https://doi.org/10.1016/s0960-9822(01)00098-7.

Weigert, R., 2014. Imaging the dynamics of endocytosis in live mammalian tissues. Cold Spring Harb. Perspect. Biol. 6. https://doi.org/10.1101/CSHPERSPECT.A017012.

Willy, N.M., Colombo, F., Huber, S., Smith, A.C., Norton, E.G., Kural, C., Cocucci, E., 2021. CALM supports clathrin-coated vesicle completion upon membrane tension increase. Proc. Natl. Acad. Sci. USA, vol. 118. ⟨https://doi.org/10.1073/pnas.2010438118⟩.

Willy, N.M., Ferguson, J.P., Huber, S.D., Heidotting, S.P., Aygün, E., Wurm, S.A., Johnston-Halperin, E., Poirier, M.G., Kural, C., 2017. Membrane mechanics govern spatiotemporal heterogeneity of endocytic clathrin coat dynamics. Mol. Biol. Cell 28, 3480–3488. https://doi.org/10.1091/mbc.E17-05-0282.

Xiao, G.-Y., Schmid, S.L., 2020. FCHSD2 controls oncogenic ERK1/2 signaling outcome by regulating endocytic trafficking. PLoS Biol. 18, e3000778 https://doi.org/10.1371/journal.pbio.3000778.

Xiao, G.Y., Mohanakrishnan, A., Schmid, S.L., 2018. Role for ERK1/2-dependent activation of FCHSD2 in cancer cell-selective regulation of clathrinmediated endocytosis. Proc. Natl. Acad. Sci. USA 115, E9570–E9579. https://doi.org/10.1073/pnas.1810209115.

Yamada, H., Ohashi, E., Abe, T., Kusumi, N., Li, S.A., Yoshida, Y., Watanabe, M., Tomizawa, K., Kashiwakura, Y., Kumon, H., Matsui, H., Takei, K., 2007. Amphiphysin 1 is important for actin polymerization during phagocytosis. Mol. Biol. Cell 18, 4669–4680. https://doi.org/10.1091/mbc.E07-04-0296.

Yarar, D., Waterman-Storer, C.M., Schmid, S.L., 2007. SNX9 couples actin assembly to phosphoinositide signals and is required for membrane remodeling during endocytosis. Dev. Cell 13, 43–56. https://doi.org/10.1016/J.DEVCEL.2007.04.014.

Yarar, D., Waterman-Storer, C.M., Schmid, S.L., 2005. A dynamic actin cytoskeleton functions at multiple stages of clathrin-mediated endocytosis. Mol. Biol. Cell 16, 964–975. https://doi.org/10.1091/mbc.E04-09-0774.

Yarwood, R., Hellicar, J., Woodman, P.G., Lowe, M., 2020. Membrane trafficking in health and disease. Dis. Model. Mech. 13. https://doi.org/10.1242/DMM.043448.

Yoshida, A., Sakai, N., Uekusa, Y., Imaoka, Y., Itagaki, Y., Suzuki, Y., Yoshimura, S.H., 2018. Morphological changes of plasma membrane and protein assembly during clathrin-mediated endocytosis. PLoS Biol. 16, e2004786 https://doi.org/10.1371/journal.pbio.2004786.

Zhu, J., Zhou, K., Hao, J.-J., Liu, J., Smith, N., Zhan, X., 2005. Regulation of cortactin/dynamin interaction by actin polymerization during the fission of clathrin-coated pits. J. Cell Sci. 118, 807–817. https://doi.org/10.1242/jcs.01668.