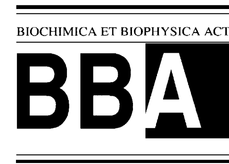
CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Elsevier - Publisher Connector



Available online at www.sciencedirect.com

Biochimica et Biophysica Acta 1746 (2005) 350 – 363

http://www.elsevier.com/locate/bba

Review

# Clathrin-independent endocytosis: New insights into caveolae and non-caveolar lipid raft carriers

Matthew Kirkham, Robert G. Parton*

*Institute for Molecular Bioscience, University of Queensland, Queensland 4072, Australia*
*Centre for Microscopy and Microanalysis, University of Queensland, Queensland 4072, Australia*

Received 24 March 2005; received in revised form 6 June 2005; accepted 6 June 2005

**Abstract**

A number of recent studies have provided new insights into the complexity of the endocytic pathways originating at the plasma membrane of mammalian cells. Many of the molecules involved in clathrin coated pit internalization are now well understood but other pathways are less well defined. Caveolae appear to represent a low capacity but highly regulated pathway in a restricted set of tissues in vivo. A third pathway, which is both clathrin- and caveolae-independent, may constitute a specialized high capacity endocytic pathway for lipids and fluid. The relationship of this pathway, if any, to macropinocytosis or to the endocytic pathways of lower eukaryotes remains an interesting open question. Our understanding of the regulatory mechanisms and molecular components involved in this pathway are at a relatively primitive stage. In this review, we will consider some of the characteristics of different endocytic pathways in high and lower eukaryotes and consider some of the common themes in endocytosis. One theme which becomes apparent from comparison of these pathways is that apparently different pathways can share common molecular machinery and that pathways considered to be distinct actually represent similar basic pathways to which additional levels of regulatory complexity have been added.
© 2005 Elsevier B.V. All rights reserved.

*Keywords:* Endocytosis; Lipid raft; Caveolae; Cholesterol; Clathrin

## 1. Introduction

It is now accepted that lipids and lipid–protein interactions play a crucial role in the functional compartmentalization of the plasma membrane into microdomains. The term 'lipid raft' has been used to define highly ordered lipid microdomains caused by the interaction of sterols and sphingolipids [1–4]. The existence of lipid rafts in vivo still remains an area of some debate [5] but the cholesterol-dependent clustering of a number of lipid-anchored proteins into submicroscopic microdomains in vivo has now been shown using a number of different approaches [6–8]. In addition, the lipid raft hypothesis provides an attractive explanation for findings in a number of different systems in which plasma membrane compartmentalization is important [2,9].

Of interest to the current review are a set of observations suggesting that the partitioning of certain macromolecules into lipid rafts facilitates their endocytosis via a pathway which is distinct from the classical well-studied clathrin coated pit pathway. This has perhaps been best characterized for internalization of glycosylphosphatidylinositol (GPI)-anchored proteins [6,10], bacterial toxins bound to surface glycolipids [11], and cytokine receptor subunits [12,13]. In these examples, lipid-based sorting as well as sensitivity to cholesterol perturbation have been shown to be important for the uptake process. In other cases, lipid raft endocytosis has often been defined operationally as an endocytic pathway that is sensitive to cholesterol perturbation. However, this crude definition covers a wide range of endocytic events, with a diverse set of receptors and ligands seemingly

DOI of original article: 10.1016/j.bbamcr.2005.06.002.
* Corresponding author. Institute for Molecular Bioscience, University of Queensland, Queensland 4072, Australia. Tel.: +61 7 33462032; fax: +61 7 33654422.

*E-mail address:* R.Parton@imb.uq.edu.au (R.G. Parton).

0167-4889/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2005.11.007

utilizing an array of different pathways to gain entry into the cell. Care must be taken in optimizing conditions to selectively inhibit specific pathways rather than perturbing general membrane properties as severe cholesterol depletion can even inhibit clathrin coated pit endocytosis [14,15]. Recent studies have revealed that caveolae budding, clathrin-mediated endocytosis, and clathrin-independent pathways can also use similar protein components, such as actin, dynamin, intersectin, cortactin, and epsin [13,16–18]. In fact, the reoccurrence of certain protein components through almost all of the lipid raft endocytic pathways raises the possibility that there might be basic common mechanisms that are regulated differently within each pathway or cell type. Another intriguing question raised by the discovery of multiple lipid raft endocytic mechanisms is how do these different pathways coordinate or interact with each other? It appears that the same ligand/receptor complexes can be internalized through distinct uptake mechanisms resulting in different signaling outcomes [19]. Before considering the complexity of the multiple endocytic pathways operating in mammalian cells, we will first summarize current knowledge of endocytosis in a lower eukaryotic system, the yeast *Saccharomyces cerevisiae*.

## 2. Endocytosis in yeast: evidence for lipid-based endocytosis

Lower eukaryotes have proven useful model systems to dissect fundamental aspects of membrane traffic. Many proteins first identified in yeast screens have orthologues that have been shown to be essential for endocytosis in higher eukaryotes. In *S. cerevisiae* receptor and fluid phase endocytosis is dependent on the yeast sterol, ergosterol (the major sterol of the yeast plasma membrane), suggesting similarities to cholesterol-dependent endocytosis in mammalian cells. The yeast plasma membrane contains a high fraction of sphingolipids (30%) relative to phospholipids [20] and ergosterol has been shown to have a higher propensity to form lipid rafts than cholesterol [21]. Thus, it is not surprising that a large number of yeast membrane receptors partition into detergent resistant domains consistent with the suggestion that a major part of the yeast plasma membrane is made up of lipid rafts [22,23].

Endocytosis in yeast has mainly been studied through the use of three markers, the membrane-impermeable fluid phase fluorescent dye lucifer yellow carbohydrazide (LY), the membrane dye FM4-64, and the internalization of mating-factor receptors. Haploid cells of the **a** mating type express the mating-factor receptor sterile2 (Ste2p), which binds α-factor peptide and activates signal transduction pathways that arrest cell division and are required for cell–cell fusion during mating [24]. The binding of α-factor to Ste2p causes a 5- to 10-fold increase of Ste2p internalization and degradation [25], demonstrating that Ste2p uptake can be regulated. Hyperphosphorylation of Ste2p is required for ubiquitination, which is in turn necessary for the internalization of the receptor [26,27]. One caveat is that developmental changes are occurring that may affect the internalization of the receptor over the time course of the mating signaling cascade.

The genetic tractability of yeast makes it a powerful tool for functional screens to identify mutants defective in specific cellular functions [28–30]. Screening for endocytic mutants revealed a large number of genes involved in regulation of the actin cytoskeleton. This included *End7/Act1* (actin) [31], *End4/Sla2* (huntingtin interacting protein) [32], and *End9/Arc35* (component of the Arp2/3 complex) [33,34]. Furthermore, actin organizing proteins such as Las17p/Bee1p and Vrp1p (Wasp and wasp interacting protein) have been shown to affect endocytosis [34,35]. Though the precise actin structures that are involved in endocytosis seem controversial [36–38], a fundamental role of actin in endocytosis has been established [39,40]. Evidence for a more direct role of actin patches in endocytosis has been provided by elegant studies of the dynamic association of actin and actin-associated proteins with putative endosomal structures [36,41]. The dynamic association of actin and associated proteins was shown to coincide with a change of motility and proposed fission and transport of early endocytic carriers to the interior of the cell. This dynamic recruitment of actin and role in the formation of early endocytic carriers from the plasma membrane within yeast has many similarities to that reported in mammal cells [16,42].

Other proteins identified in endocytic screens include orthologues of proteins identified in the clathrin coated pathway of mammalian cells including amphiphysin (End6p/ Rvs161p, Rvs167p) [29,43] and EH domain-containing proteins similar to Eps15 (End3p/Pan1p) [44, 45]. Yet there are apparently significant differences between endocytosis in yeast and the classical mammalian clathrin-coated pit pathway. Yeast has a single gene for clathrin heavy chain *CHC1* and clathrin light chain *CLC1*. Deletion of these genes resulted in receptor-mediated internalization still occurring but with slower kinetics [46–48]. Homologues of the adaptor complex subunits are not required for endocytosis, although they may not interact with clathrin [49,50]. Additionally, yeast has no classical dynamin homologue [51] but has three dynamin-like proteins. Two of these are involved in mitochondria morphology and inheritance [52,53] but Vps1p is involved in protein trafficking [54–57]. *Vps1*Δ yeast have a mild endocytic defect resulting from an abnormal actin cytoskeleton [57,58]. The absence of a fundamental involvement of these proteins in yeast endocytosis may indicate that there may be similarities between yeast endocytosis and clathrin-independent forms of endocytosis in mammalian cells.

If yeast endocytosis has similar characteristics to clathrin-independent endocytosis, could there be an involvement of lipid rafts in endocytosis of membrane receptors? Certainly, a number of observations provide support for the

hypothesis that lipids play an important role in endocytosis in yeast. Firstly, it has been shown that one endocytic mutant *end8* or *lcb-100* is defective in sphingolipid synthesis, [59]. Second, the transbilayer lipid arrangement of sphingolipids to phospholipids is important for endocytosis, as mutations in *Dnf1* and *Dnf2*, which code for aminophospholipid translocases, cause a defect in endocytic vesicle budding [60,61]. Third, specific mutations in enzymes involved in the biosynthesis of ergosterol disrupted the total amount of sterols in the plasma membrane and also the composition of the sterol species. This blocked both fluid-phase and receptor-mediated endocytosis although the membrane dye FM4-64 was still internalized but with delayed kinetics [62]. Interestingly, structural determinants shown to be important in endocytosis were also sterols that influence sphingolipid/cholesterol 'lipid raft' domain formation in artificial membranes [21,62,63]. Taken together, these studies suggest that lipids, and possibly lipid raft domains, play an important role in yeast endocytosis.

What role might lipid rafts play in the endocytic process? An intriguing possibility is that the specific lipid composition is important in the generation of budded endocytic vesicles but more indirect roles in endocytosis are perhaps more likely. For example, the kinases, Yck1p and Yck2p, are involved in the hyperphosphorylation of the membrane tail of Ste2p, which is required for ubiquitination and internalization of the receptor. Yck1p and Yck2p require palmitoylation for plasma membrane localization [64–66], and in mutants defective in ergosterol synthesis Ste2p is not phosphorylated. In addition, the reduced receptor endocytosis caused by defects in sphingolipid synthesis could be overcome by over-production of kinases Pkh1p, Pkh2p, or Yck2p [67]. Accordingly, the lipid raft domain may provide a platform to colocalize receptor and kinase to the same area of the plasma membrane.

Currently, it is unclear whether there are multiple endocytic mechanisms in yeast as in mammalian cells or one mechanism used to mediate all endocytosis. Uptake of FM4-64 in cells defective in the synthesis of ergosterol, where fluid phase and receptor-mediated endocytosis was inhibited, could in theory occur by a distinct pathway. Although to address the question of whether there are multiple endocytic mechanisms in yeast, new endocytic markers such as a GPI-anchored proteins, which undergo internalization, may need to be developed. In summary, studies of yeast have provided evidence for a clathrin- and dynamin-independent pathway, which is dependent on sterols. This raises the question of whether an equivalent pathway exists in higher eukaryotes.

## 3. New insights into clathrin-independent endocytic pathways

Evidence for a clathrin-independent pathway in mammalian cells has accumulated over many years from the study of a range of different endocytic markers but the endocytic vehicles involved have been a matter of some debate. The observation of uncoated flask-shaped invaginations covering the surface of many types of animal cells [68,69] suggested that such structures could represent a possible alternative endocytic carrier. The morphology of these structures, now termed caveolae, is certainly suggestive of 'vesicles' poised to bud. But caveolae are not essential for non-clathrin lipid raft endocytosis and few markers have been shown to be dependent on caveolae for uptake [11,70,71] (reviewed in [72]). It is well established that lipid rafts are present in cells without caveolae (some neuronal cells and lymphocytes, e.g. [2,73], and lipid raft endocytosis occurs in these cells. Moreover, recent evidence suggests that lipid raft caveolae-independent endocytosis also occurs in cells with abundant caveolae [70]. In this section, we will therefore consider this non-caveolar endocytic pathway, which we believe may represent a more universal primordial endocytic pathway, before considering the more specialized form of tissue-specific endocytosis mediated by caveolae in Section 4.

Some of the first studies to suggest a clathrin-independent endocytic pathway came from the pioneering studies of van Deurs and Sandvig. A series of elegant studies defined an endocytic pathway mediating the uptake of the plant toxin, ricin, which binds to glycolipids and glycoproteins. This pathway was shown to be insensitive to inhibitors of clathrin-mediated endocytosis and was also dynamin-independent [74]. The pathway originating at the apical surface of epithelial cells was particularly well studied. Caveolae are virtually absent at the apical surface of these cells [75] providing additional evidence that they were not responsible for the clathrin-independent uptake of ricin. A series of experiments showed that endocytosis was regulated by protein kinase C [76], protein kinase A [77], trimeric G proteins, and arachidonic acid [78]. Despite the apparently distinct uptake pathway, the clathrin-independent pathway converged with the clathrin pathway at the early endosome.

In other studies, evidence for a clathrin-independent non-caveolar pathway has come from analysis of cells expressing a temperature-sensitive mutant form of dynamin [79]. Interestingly, blocking clathrin- (and presumably caveolae-) mediated endocytosis caused an initial block in fluid phase internalization followed by a gradual compensatory increase. After 30 min fluid phase uptake returned to the same level as before the temperature shift while receptor-mediated endocytosis remained blocked [79]. Morphological analysis of the uptake pathway revealed vesicular, tubular and ring-shaped structures labeled after 10 min of uptake at the non-permissive temperature. Morphologically, similar structures were observed in studies of clathrin-independent endocytosis in the above studies of ricin internalization (e.g., [76]). Taken together, these studies suggested that clathrin and dynamin are not required for all types of endocytosis in mammalian cells.

Further insights into a clathrin-independent pathway have come from the study of the uptake of GPI-anchored proteins, classical markers of lipid raft domains. The GPI-anchor was shown to be required for internalization via an apparently novel clathrin- and dynamin-independent endocytic pathway, which was sensitive to cholesterol depletion [10]. Immediately after internalization GPI-anchored proteins were evident within labile tubular structures, termed GEECs (GPI-enriched endosomal compartments), which lacked markers of the clathrin pathway. Again, the GPI-anchored protein pathway and the transferrin clathrin-dependent pathway were then shown to converge in endosomes [10]. The Rho family GTPase cdc42, but not RhoA or Rac1, were shown to regulate this pathway that was again independent of caveolae. Other studies have supported the conclusion that GPI-anchored proteins are internalized via a lipid raft-dependent process into novel endosomes and implicated the GTP-binding protein, ARF6, as well as phosphatidylinositol (4,5) bisphosphate (PtdIns(4,5)$P_2$), in this process [80]. An endogenous GPI-anchored protein was shown to be internalized together with major histocompatibility protein class 1 (MHC1) in a cholesterol-dependent manner into ARF6-labeled endosomes before appearing in common endosomes containing a clathrin coated pit-internalized marker [80,81]. Further analysis of the role of ARF6 in this process should provide further insights into the mechanisms involved in this pathway. As ARF6 has also been implicated in macropinocytosis [82] and clathrin coated pit-mediated endocytosis [83], the specificity of endogenous ARF6 for this pathway remains to be established.

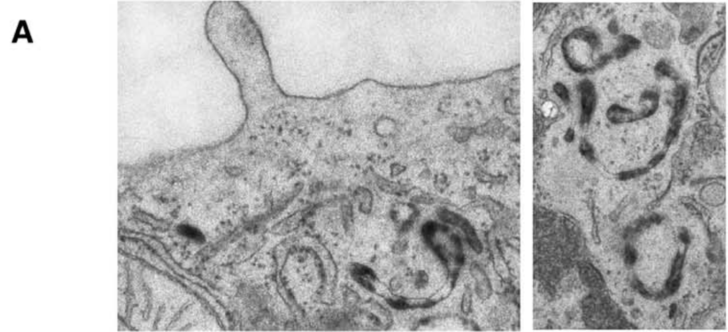
These and many other studies clearly indicate that clathrin-independent pathways exist in mammalian cells. Interestingly, in *Drosophila* hemocytes endocytosis of fluid and GPI-anchored proteins can also occur via a clathrin- and dynamin-independent pathway [84] suggesting that this is an evolutionarily conserved endocytic pathway. Since many of the clathrin-independent pathways are also dynamin-independent, or at least are not inhibited by dynamin mutants which block clathrin- (and caveolae-) mediated endocytosis, this raises a number of interesting questions about the molecular mechanisms operating in this pathway. What is the molecular machinery which generates these endocytic carriers, how is specific cargo concentrated in the carriers, and how are the carriers budded in the absence of clathrin and dynamin? As a starting point, a detailed analysis of the very first carriers involved in this pathway, the functional equivalent of the clathrin coated pit, is clearly required.

A recent study has started to address this question by using electron microscopy to examine the initial carriers involved in the uptake of the bacterial toxin, cholera toxin [70]. Cholera toxin (CT) binds via its binding (B) subunits to the lipid raft marker, the ganglioside GM1, and can be used as a general marker of internalization. While CT has been claimed to be a caveolar marker in some studies, postembedding (i.e., non-perturbing) EM labeling has shown that GM1 is present over the entire cell surface, including clathrin-coated pits, and is only slightly enriched in caveolae [85]. CTB can be internalized by clathrin-coated pits and by caveolae [86] but a significant fraction is internalized via a clathrin- and dynamin-independent pathway [70]. By using cells lacking caveolae, their contribution to CTB endocytosis could be ruled out and the initial vehicles mediating CTB entry were examined using a novel electron microscopic assay. After binding CTB to the cell surface and warming the cells for just 15 s, CTB could be detected in clathrin-coated pits, consistent with significant internalization by this pathway, but most interestingly, CTB was also observed in tubular and ring-shaped endocytic structures (see Fig. 1A). Labeling of these structures, but not clathrin-coated vesicles, was sensitive to cholesterol depletion suggesting that they were not related to the clathrin-coated pit pathway but constituted a novel internalization pathway. The short timescale of these experiments (to minimize interaction of budded structures with other organelles) suggested that the observed structures budded directly from the plasma membrane. No coat structures were evident to give clues as to their possible formation and they showed no detectable dynamin labeling consistent with their association with a dynamin-independent pathway [70].

Are these carriers (here termed CLICs for CLathrin-Independent Carriers see Fig. 1A) the morphological structures involved in clathrin-independent endocytosis in many different systems, such as those described above, or just one example of many different structures mediating clathrin-independent entry into the cell? Importantly, both fluid phase markers and GPI-anchored proteins were shown to co localize extensively with CTB under these conditions [70] linking these observations to previous studies of GEECs [10]. This suggests that CLICs may represent the first station in a major physiologically important internalization route. If so, it is somewhat surprising that their visualization has only now been reported. However, the morphology of CLICs is quite similar to endosomal structures as viewed by EM. They have also been reported to be highly labile and so could be prone to fixation artifacts [10]. The fusion of these structures with endosomal compartments, shortly after budding, may also have contributed to this problem. Many studies documenting a clathrin-independent uptake pathway have, in fact, reported morphologically similar structures but the very earliest carriers were not defined [76].

Clearly, the detailed characterization of these structures is a major goal for the future. Could lipids drive formation and sorting into these structures? The morphology of CLICs, which are presumably incredibly dynamic structures, gives few clues to their formation at present. Their high sensitivity to cholesterol depletion, enrichment in GPI-anchored proteins, and formation in a dynamin- and clathrin-independent manner all suggest similarities to the endocytic

A



B

| | Clathrin Coated pit | Macropinocytosis | Caveolae | CLIC-D | CLIC-DI |
|---|---|---|---|---|---|
| Examples of ligands | Anthrax-PA CTB | | CTB GP60 SV40 LacCer | IL-2 γc | SV40; GPI-AP Dextran CTB |
| Cholesterol depletion sensitive | Yes [14, 15, 135]. | Yes [121] | Yes [71, 94, 101, 102, 136] | Yes [12, 13] | Yes [70, 112] |
| Actin dependent | Yes [137] | Yes (ruffles) [121] | Yes [16] | Yes [13] | Possible [112] |
| Clathrin dependent | Yes [138, 139] | No | No | No [13] | No |
| Dynamin dependent | Yes [140] | No | Yes [95-97] | Yes [12, 13] | No [10, 70, 112] |
| Rho family GTPase | Rac1 [141] RhoA [141] cdc42 (indirect) [142, 143] | Rac1 [144] cdc42 [145, 146] | No direct evidence | RhoA [12] | cdc42 [10] |
| Tyrosine Kinase dependent | Yes: [147] Src family kinases [148, 149] | Src family kinases (induces formation) [150] | Src family kinases [16, 101, 102] | - | Yes (kinase unknown) [112] |
| Other Components: | Including: | PAK1 [151] | Gβγ Heterodimer [104] | | |
| | PKC [152] | PKC [121] | PKC [102] | | |
| | Cortactin [153, 154] | | | Cortactin [13] | |
| | Intersectin [155, 156] | | Intersectin [17] | | |
| | Arf6 [83, 157] | Arf6 [82] | | | Arf6 possible (post entry) [80, 81] |
| | Mono- ubiquitination Eps15/epsin [18, 158] | | | Mono- ubiquitination Eps15/epsin [18] | Mono- ubiquitination (Membrane-GFP) [119] |

pathway in yeast and could indicate that this represents a primordial pathway for endocytosis.

Other lipid raft-dependent pathways clearly exist in mammalian cells and this has been most clearly indicated by their dependence on dynamin. Caveolae budding is one such example, as will be discussed in the following section. However, dynamin-dependent lipid raft-mediated uptake has also been documented for non-caveolar carriers [12,13]. This has been best characterized for the uptake of the beta subunit of the interleukin (IL)-2 receptor and for the γc cytokine receptor that are both internalized through a dynamin-dependent process. Binding of IL-2 to the IL-2 receptor increases the association of the receptor with lipid rafts and this is coupled to receptor internalization [12]. Actin recruitment and the activity of RhoA were shown to be involved in γc internalization [13]. Interestingly, the dynamin-binding protein, cortactin, which is involved in clathrin-mediated endocytosis was required for clathrin-independent γc internalization suggesting that cortactin can link both clathrin-dependent and -independent pathways to the actin cytoskeleton [13]. This pathway is clearly distinct from the uptake of GPI-anchored proteins; as well as differing in dynamin-dependence, this pathway was inhibited by a dominant negative form of RhoA but not cdc42 and resulted in delivery of the internalized receptor to distinct early endosomal elements [10,12].

Taken together, these studies suggest that dynamin is required for some clathrin-independent pathways but not others. Lipid-based sorting, as judged by sensitivity to cholesterol-depleting agents and by association of internalized components with lipid rafts, appears to be an important feature of many of these pathways suggesting similarities to endocytic pathways of lower eukaryotes. These pathways may therefore represent a relatively primitive endocytic pathway. This makes an interesting comparison with more specialized endocytic pathways such as those involving caveolae (see Fig. 1B).

## 4. Caveolar endocytosis: a specialized regulated endocytic pathway

Caveolae are an abundant feature of many mammalian cells including adipocytes, endothelial cells, smooth muscle, and fibroblasts but are not detectable in all mammalian cells [73]. The formation of caveolae is dependent on the expression of caveolin-1 (Cav1) in non-muscle cells [87] and caveolin-3 in muscle cells [88]. Cells that do not express caveolins have no caveolae and transient expression of Cav1 causes the de novo formation of caveolae [89]. Caveolae have been considered to be a specialized form of lipid raft domain with a unique morphology generated by caveolin. Cav1 is an integral membrane protein with an unusual topology, a 33-amino acid intramembrane domain is believed to insert into the plasma membrane to form a hairpin loop with both N and C terminal regions of the protein cytoplasmic [90,91]. Cav1 directly interacts with cholesterol [92,93] and cholesterol depletion of the plasma membrane causes caveolae to flatten [94].

In endothelial cells, caveolae endocytosis has been well studied and appears to be a constitutive dynamin-dependent process [95–97]. In other cell types, caveolae endocytosis has been a controversial topic for some years but a consensus is now developing that budding of caveolae from the plasma membrane is a regulated process. However, unlike clathrin endocytosis, there is only a basic understanding of the machinery involved (summarized in Fig. 1B). Caveolae endocytosis is dependent on dynamin, which is localized to the neck of caveolae either constitutively in endothelial cells [96], or is recruited in response to specific signals [16]. In addition, a number of studies have implicated actin in caveolae endocytosis [16,98,99]. Studies of the uptake of the virus SV40, which binds to GM1 and enters cells at least partly through caveolae, have been particularly illuminating. Live cell imaging of SV40 uptake revealed the transient recruitment of dynamin, a burst of actin polymerization to form an actin tail, and the movement of caveolae (Cav1-GFP-labeled structures) into the cell [16]. This sequence of events is similar to that observed in clathrin endocytosis. Live cell imaging of actin at sites of clathrin endocytosis revealed that the inward movement of a vesicle occurred immediately after the transient requirement of actin assembly [42]. The transient recruitment of actin in endocytosis seems to be conserved within yeast [36], and thus it is feasible that this might be a universal mechanism in endocytosis.

Phosphorylation also plays an important part in caveolae budding. Inhibition of tyrosine kinase activity inhibits caveolae endocytosis, and okadaic acid a phosphatase inhibitor stimulates caveolae internalization [16,99]. The precise role of phosphorylation is presently unclear. Expression of both dominant negative Src and protein kinase Cα constructs, and the use of pharmacological

Fig. 1. The visualization of the first endocytic carriers involved in dynamin independent endocytosis, and a comparison of different modes of endocytosis. (A) Mouse embryo fibroblasts isolated from Cav1-null mice were allowed to internalize cholera toxin subunit B conjugated to HRP (CTB-HRP) for 15 s. Only internally localized CTB-HRP was visualized by using a combination of ascorbic acid and DAB histochemistry on live cells [70]. The majority of the internal structures visualized by EM were tubular and ring-like structures, proposed to be the earliest (plasma membrane-derived) carriers in a non-clathrin non-caveolar pathway. (B) A diagram illustrating features of the alternative endocytic pathways. Many components are common to a number of different pathways. Abbreviations: CTB, Cholera toxin subunit B; Anthrax-PA, Anthrax protective antigen; LacCer, lactosylceramide; IL-2, interleukin 2; GPI-AP, glycosylphosphatidylinositol anchored proteins; CLIC-D, dynamin-dependent clathrin-independent carriers; CLIC-DI, dynamin-independent clathrin-independent carriers [135–158].

inhibitors of these kinase families, inhibit caveolae-stimulated endocytosis [100–102]. However, the requirement for phosphorylation of Cav1 in endocytosis has only been addressed indirectly. Src family kinases can phosphorylate both Cav1 and dynamin [100,103] and the latter has been shown to be directly involved in caveolae internalization [16,95,104].

These studies suggest that caveolae internalization is a highly regulated process. Of particular interest in view of studies linking caveolae and caveolins to lipid regulation [105,106] is the recent finding that caveolae budding can be stimulated by specific lipids. Treatment of cells with lactosyl ceramide caused a dramatic stimulation of caveolae internalization [70,102,107]. The precise mechanism through which the lipid treatment stimulated caveolae internalization is unclear, although Src kinase activity was required [102]. A range of lipids could stimulate endocytosis, including cholesterol. Somewhat surprisingly, the sugar residue or the acyl chain length of the glycosphingolipids had no effect on the stimulatory properties of the lipids added but phosphatidylcholine, which does not contain a ceramide group, did not induce caveolae budding [108]. Whether these treatments also affect non-caveolar lipid raft endocytosis is not yet known.

As highlighted in Fig. 1B and in the text above, caveolae endocytosis shares many common features with clathrin-dependent pathways, including the same dynamic requirement of molecular machinery in the fission process. However, there are clearly striking differences between the dynamic association of clathrin with the plasma membrane and the stable association of caveolin. Unlike the transient association of clathrin with the plasma membrane [42], Cav1-GFP formed highly stable microdomains, with a slow rate of turnover similar to that of E-cadherin in junctional complexes [109,110]. However, the mobility and turnover of Cav1-GFP could be stimulated [109]. More recently, this finding have been confirmed through the use of a novel EM approach that distinguished internal caveolae structures from those connected to the plasma membrane [70]. Approximately 2% of surface caveolae appeared within the cell after warming for 1 min under control conditions but this could be increased using okadaic acid or lactosyl ceramide adding quantitative ultrastructural data to the light microscopic observations described above.

In summary, caveolae appear to be extremely uniform carriers poised to bud in response to a specific signal. An intriguing possibility is that specific subsets of caveolae or individual caveolae, in specific regions of the cell are triggered to bud in response to specific triggers. The physiological signals which regulate this budding and the role of caveolae endocytosis in vivo is yet to be determined. One system which has attracted recent attention is the endocytosis of transforming growth factor (TGF-beta)-receptors via two endocytic pathways, one involving caveolae and leading to degradation, and the other involving clathrin coated pits and leading to productive signal generation [19]. However, recent studies have questioned a role for caveolae in TGFbeta-receptor internalization [111]. In the absence of studies showing that specific markers are no longer endocytosed in cells lacking caveolae, the role of caveolae endocytosis in vivo remains an open question.

The fact that lipid raft internalization can occur through multiple pathways raises many questions. What factors determine entry into a specific pathway? What is the biological importance of one entry route over another? And how do these pathways interact with one another? In the next sections, links between these pathways will be described and possible alternative regulation discussed.

## 5. Links between caveolar and non-caveolar clathrin-independent pathways

Both CTB and the virus SV40 can be internalized by caveolae but in a cell lacking caveolae endocytosis of these markers still occurs (apparently via CLICs; [70,112]). In fact, comparing SV40 uptake in cells with and without caveolae, the rate of SV40 uptake is reduced in cells with caveolae [112]. A striking finding is that many of the characteristics of internalization are the same for uptake of SV40 via caveolae and via the non-caveolar pathway. For example, both pathways are sensitive to Src kinase inhibitors, and to cholesterol perturbation and lead to a novel endosomal compartment termed a caveosome [112]. This suggests that the two pathways share mechanistic similarities and some components of the same machinery. However, they do differ significantly in their dependence on dynamin; dynamin mutants inhibit caveolar internalization of SV40 but do not inhibit uptake in cells lacking caveolae [112]. This raises the possibility that the basic mechanisms involved in these two pathways are the same but caveolin and dynamin add an additional level of complexity, which allows further regulation of the pathway.

This idea can be expanded further by considering the effect of caveolin re-expression on endocytosis. Transient overexpression of Cav1, which induces caveolae formation, has a pronounced negative effect on lipid raft endocytosis in several different systems [70,101,113,114]. This somewhat paradoxical observation (at least from the viewpoint of caveolae as essential endocytic carriers) can be rationalized if one regards caveolin as a stabilizing component that restricts endocytosis from lipid raft domains (see [115]). One interesting possibility, which is consistent with this inhibitory effect of caveolin on endocytosis, is that caveolin acts as a ‘brake’ to inhibit internalization that can only be overcome by activation of dynamin. Caveolin and dynamin therefore act together to add a further level of regulation to an inherently endocytic structure. In this model, the formation of non-caveolar raft domains poised to bud could be due to an inherent property of the lipid raft domain [116–118]. These structures would normally undergo fission to produce endocytic carriers but in the presence of caveolin

these domains would be stabilized on the plasma membrane unless dynamin triggers their budding. Evidence for this comes from the observation that in transformed cells with few caveolae, expression of the dominant negative form of dynamin not only inhibits caveolae endocytosis but also causes an increase of caveolae-like vesicles at the plasma membrane [113,115]. Within some cell types, such as adipocytes, caveolae can make up to 30% of the total membrane. The presence of static caveolae could stabilize large areas of the membrane and act as a negative regulator of membrane dynamics.

## 6. New insights into sorting into clathrin-independent carriers

Lipid raft inclusion appears to be important for many surface components to be internalized via clathrin-independent carriers as outlined above (see Table 1). Recent studies suggest that ubiquitin might represent another sorting signal. Modification of plasma membrane proteins by monoubiquitination provides a signal for internalization of proteins and subsequent sorting to lysosomes for degradation. To test the role of ubiquitin in internalization, a chimeric model protein of GFP and ubiquitin was targeted to the plasma membrane through myristoylation and palmitoylation signals [119]. The hybrid protein was internalized through a clathrin-independent pathway. Internalization occurred independently of dynamin suggesting that it involves a non-caveolar pathway. Internalization required interaction of ubiquitin with epsin. This intriguing result suggests links between clathrin-dependent and independent internalization as epsin is well characterized as a clathrin binding protein. In fact, it appears that the interaction of epsin with ubiquitin is regulated by the interaction of epsin and clathrin. A recent study provided further insights into monoubiquitination of the EGF receptor as a signal influencing the mechanism of internalization [18]. High concentrations of EGF were shown to be endocytosed through a clathrin-independent lipid raft-dependent route. A hybrid containing EGF extracellular and transmembrane domains fused to ubiquitin was also internalized through a clathrin-independent but dynamin-dependent, apparently via caveolae. Espin and eps15 proteins were implicated in recognition of the ubiquitin signal. These two studies provide interesting new avenues of research that should provide further insights into the links between clathrin-dependent and independent pathways.

## 7. Macropinocytosis of lipid raft markers

The final endocytic pathway to be considered here is macropinocytosis, which involves the internalization of large areas of plasma membrane together with significant amounts of fluid. Macropinocytosis occurs when membrane protrusions fuse back with the plasma membrane to generate large (>1 μm) irregular vesicles called macropinosomes. Actin

Table 1
Examples of lipid raft involvement in the internalization of surface components

| Surface component/ligand | Lipid raft dependence determined by | Uptake sensitive to cholesterol depletion | Actin involved | Dynamin involved | Tyrosine kinase dependence | Other | Possible mechanism of uptake |
|---|---|---|---|---|---|---|---|
| Cannabinoid Anandamide | DRM (0.5% Triton X-100) [159] | Yes [159] | – | – | Yes [159] | Colocalized by LM with Cav1 [159] | Caveolae or CLIC |
| CD36/oxidized low density lipoprotein | DRM (1% Triton X-100) [160] | – | – | – | – | Colocalized by LM with GPI-AP [160] | CLIC |
| Glucagon-like peptide-2 receptor/ Glucagon-like peptide-2 | DRM (1% Triton X-100) [161] | Yes [161] | – | No [161] | No [161] | – | CLIC-DI |
| Proteoglycan syndecan-4/ Fibroblast growth factor 2 | – | Yes [162] | Indirectly: Rac1 cdc42 involved [162] | No [162] | – | Receptor clustering required [162] | CLIC-DI and macro-pinocytosis |
| A1 adenosine receptors | Caveolae enriched (detergent free isolation [163]) [164] | Yes [164] | – | – | – | Co-immunoprecipitation with Cav1 Colocalized by LM with Cav1 [164] | Caveolae |
| Norepinephrine transporters | DRM (1% Triton X-100) [165] | Yes [165] | – | No [165] | – | Induced internalization by PKC activation [165] | Macro-pinocytosis CLIC-DI |
| Bradykinin B2 receptor | Caveolae enriched [166] (detergent free isolation [167]) | – | – | No [168] | | Localized by EM to caveolae like structures [169] | CLIC-DI |

Abbreviations: DRM, detergent resistant membrane; CLIC, clathrin independent carrier; D, dynamin dependent; DI, dynamin independent; LM, Light microscopy; EM, electron microscopy.

ruffles caused by extensive actin rearrangement are a fundamental part of this process and factors that trigger membrane ruffling stimulate macropinocytosis. For example, stimulation of the Rho-family GTPase Rac1 by platelet derived growth factor or protein kinase C activation by phorbol esters stimulates membrane ruffles and micropinosome formation. Though it has not been demonstrated that macropinosomes differ in the composition to the membrane from which they are derived, membrane ruffles are apparently enriched in specific lipid rafts and phosphoinositides [120]. Lipid rafts have been implicated in macropinosome formation by the finding that cholesterol depletion inhibits both membrane ruffling and macropinocytosis [121]. Localization of Rac1 to the plasma membrane, but not activation, was inhibited by cholesterol depletion. Whether Rac1 requires lipid rafts to localize to the plasma membrane through direct interactions with the membrane or through additional protein–protein interactions is unclear. Cholesterol-dependent binding of Rac1 to artificial liposomes could not be observed in this study [121] but was observed in a subsequent investigation [122]. The small GTPase, ARF6, which has been implicated in localizing Rac to the plasma membrane, also showed reduced localization to the plasma membrane upon phorbol ester treatment of cholesterol-depleted cells [121]. The specificity of the cholesterol depletion is unclear in this system as the effect on other endocytic processes was not examined. These findings, and in general, any experiments using cholesterol depletion, should be considered in light of studies showing a global effect on the actin cytoskeleton through the redistribution of PtdIns(4,5)$P_2$ at the plasma membrane [123]. PtdIns(4,5)$P_2$ has a role in the organization of the actin cytoskeleton, stabilizing cortical actin and influencing the turnover of cytoplasmic stress fibers. Whether cholesterol disrupts specific PtdIns(4,5)$P_2$ microdomains or whether the effect on PtdIns(4,5)$P_2$ is a secondary consequence due to the loss of lipid rafts and thus protein–lipid organization of the plasma membrane is unclear. With the caveat mentioned above, these studies suggest that macropinocytosis can be added to the list of lipid raft-dependent endocytic processes.

## 8. Conclusions

It is now clear that multiple endocytic pathways exist in mammalian cells. These pathways vary in their dependence on clathrin, dynamin, and caveolin, but examination of the other machinery involved in these apparently distinct pathways suggests that other machinery can be shared. Cells with and without caveolae may appear to utilize fundamentally different endocytic machinery but in fact these pathways may represent slight modifications of a basic endocytic mechanism, perhaps analogous to the primordial endocytic pathway.

What is the evidence for a primordial mechanism? The endocytic mechanism within yeast has many similar features to the CLIC pathway, such as the requirement for sterols and independence of clathrin and dynamin function. The uptake of GPI-anchored proteins through a CLIC pathway has also been described in *Drosophila* [84]. In other metazoans, such as trypanosomes, GPI-anchored proteins make up a large percentage of the total surface protein [124,125]. In the bloodstream form of *Trypanosoma brucei*, there is a high level of endocytosis causing rapid GPI-anchored protein uptake [126]. There is now convincing evidence that this occurs through a pathway dependent on clathrin and actin but, interestingly, this extremely rapid high capacity pathway is independent of dynamin and the AP2 complex [127–130]. In the procyclic stage, the endocytic pathway is simplified and the rate of endocytosis is reduced [131]. At this stage, no coated endocytic structures are observed [131–133]. At the present time, it is therefore difficult to define the primordial endocytic pathway in terms of cargo and essential cellular components. A better understanding of the CLIC pathway and the molecular machinery utilized in this pathway are now required to compare lipid raft marker uptake in mammalian cells with other eukaryotes. Interestingly, a very recent study has provided new insights into dymanin-independent endocytosis [134]. This study implicated the CtBP-BARS protein in dynamin-independent trafficking pathways including fluid phase marker endocytosis [134].

Clathrin-independent pathways are now amenable to detailed analysis using the approaches that have proved so successful in studies of clathrin coated pits. The ability to characterize clathrin-independent carriers by electron microscopy, follow their dynamics and precise protein–protein interactions in real-time by light microscopy, and manipulate their molecular machinery through large-scale down-regulation of defined components should allow rapid insights to be gained into this poorly-understood process. Most importantly, we are now in a position in which we can start to examine the role of lipid–lipid and lipid–protein interactions in the generation of lipid raft-dependent carriers and to attempt to reconstitute such processes in vitro.

## Acknowledgments

Work in the authors' laboratory is supported by the NHMRC (Australia) and NIH (USA). The Institute for Molecular Bioscience is a Special Research Centre of the Australian research Council. We would like to thank Alan Munn for comments on the manuscript.

## References

[1] D.A. Brown, E. London, Functions of lipid rafts in biological membranes, Annu. Rev. Cell Dev. Biol. 14 (1998) 111–136.

[2] K. Simons, D. Toomre, Lipid rafts and signal transduction, Nat. Rev., Mol. Cell Biol. 1 (2000) 31–39.

[3] K. Simons, E. Ikonen, Functional rafts in cell membranes, Nature 387 (1997) 569–572.
[4] K. Simons, P. Dupree, K. Fiedler, L.A. Huber, T. Kobayashi, T. Kurzchalia, V. Olkkonen, S. Pimplikar, R. Parton, C. Dotti, Biogenesis of cell-surface polarity in epithelial cells and neurons, Cold Spring Harbor Symp. Quant. Biol. 57 (1992) 611–619.
[5] S. Munro, Lipid rafts. Elusive or illusive? Cell 115 (2003) 377–388.
[6] P. Sharma, R. Varma, R.C. Sarasij, Ira, K. Gousset, G. Krishnamoorthy, M. Rao, S. Mayor, Nanoscale organization of multiple GPI-anchored proteins in living cell membranes, Cell 116 (2004) 577–589.
[7] D.A. Zacharias, J.D. Violin, A.C. Newton, R.Y. Tsien, Partitioning of lipid-modified monomeric GFPs into membrane microdomains of live cells, Science 296 (2002) 913–916.
[8] I.A. Prior, C. Muncke, R.G. Parton, J.F. Hancock, Direct visualization of Ras proteins in spatially distinct cell surface microdomains, J. Cell Biol. 160 (2003) 165–170.
[9] R.G. Parton, J.F. Hancock, Lipid rafts and plasma membrane microorganization: insights from Ras, Trends Cell Biol. 14 (2004) 141–147.
[10] S. Sabharanjak, P. Sharma, R.G. Parton, S. Mayor, GPI-anchored proteins are delivered to recycling endosomes via a distinct cdc42-regulated, clathrin-independent pinocytic pathway, Dev. Cell 2 (2002) 411–423.
[11] A.A. Wolf, M.G. Jobling, S. Wimer-Mackin, M. Ferguson-Maltzman, J.L. Madara, R.K. Holmes, W.I. Lencer, Ganglioside structure dictates signal transduction by cholera toxin and association with caveolae-like membrane domains in polarized epithelia, J. Cell Biol. 141 (1998) 917–927.
[12] C. Lamaze, A. Dujeancourt, T. Baba, C.G. Lo, A. Benmerah, A. Dautry-Varsat, Interleukin 2 receptors and detergent-resistant membrane domains define a clathrin-independent endocytic pathway, Mol. Cell 7 (2001) 661–671.
[13] N. Sauvonnet, A. Dujeancourt, A. Dautry-Varsat, Cortactin and dynamin are required for the clathrin-independent endocytosis of gammac cytokine receptor, J. Cell Biol. 168 (2005) 155–163.
[14] A. Subtil, I. Gaidarov, K. Kobylarz, M.A. Lampson, J.H. Keen, T.E. McGraw, Acute cholesterol depletion inhibits clathrin-coated pit budding, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 6775–6780.
[15] S.K. Rodal, G. Skretting, O. Garred, F. Vilhardt, B. van Deurs, K. Sandvig, Extraction of cholesterol with methyl-beta-cyclodextrin perturbs formation of clathrin-coated endocytic vesicles, Mol. Biol. Cell 10 (1999) 961–974.
[16] L. Pelkmans, D. Puntener, A. Helenius, Local actin polymerization and dynamin recruitment in SV40-induced internalization of caveolae, Science 296 (2002) 535–539.
[17] S.A. Predescu, D.N. Predescu, B.K. Timblin, R.V. Stan, A.B. Malik, Intersectin regulates fission and internalization of caveolae in endothelial cells, Mol. Biol. Cell 14 (2003) 4997–5010.
[18] S. Sigismund, T. Woelk, C. Puri, E. Maspero, C. Tacchetti, P. Transidico, P.P. Di Fiore, S. Polo, Clathrin-independent endocytosis of ubiquitinated cargos, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 2760–2765.
[19] G.M. Di Guglielmo, C. Le Roy, A.F. Goodfellow, J.L. Wrana, Distinct endocytic pathways regulate TGF-beta receptor signalling and turnover, Nat. Cell Biol. 5 (2003) 410–421.
[20] J.L. Patton, R.L. Lester, The phosphoinositol sphingolipids of *Saccharomyces cerevisiae* are highly localized in the plasma membrane, J. Bacteriol. 173 (1991) 3101–3108.
[21] X. Xu, R. Bittman, G. Duportail, D. Heissler, C. Vilcheze, E. London, Effect of the structure of natural sterols and sphingolipids on the formation of ordered sphingolipid/sterol domains (rafts). Comparison of cholesterol to plant, fungal, and disease-associated sterols and comparison of sphingomyelin, cerebrosides, and ceramide, J. Biol. Chem. 276 (2001) 33540–33546.
[22] M. Bagnat, S. Keranen, A. Shevchenko, K. Simons, Lipid rafts function in biosynthetic delivery of proteins to the cell surface in yeast, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 3254–3259.
[23] S. Dupre, R. Haguenauer-Tsapis, Raft partitioning of the yeast uracil permease during trafficking along the endocytic pathway, Traffic 4 (2003) 83–96.
[24] L. Bardwell, J.G. Cook, C.J. Inouye, J. Thorner, Signal propagation and regulation in the mating pheromone response pathway of the yeast *Saccharomyces cerevisiae*, Dev. Biol. 166 (1994) 363–379.
[25] D.D. Jenness, P. Spatrick, Down regulation of the alpha-factor pheromone receptor in *S. cerevisiae*, Cell 46 (1986) 345–353.
[26] L. Hicke, B. Zanolari, H. Riezman, Cytoplasmic tail phosphorylation of the alpha-factor receptor is required for its ubiquitination and internalization, J. Cell Biol. 141 (1998) 349–358.
[27] L. Hicke, H. Riezman, Ubiquitination of a yeast plasma membrane receptor signals its ligand-stimulated endocytosis, Cell 84 (1996) 277–287.
[28] S. Raths, J. Rohrer, F. Crausaz, H. Riezman, end3 and end4: two mutants defective in receptor-mediated and fluid-phase endocytosis in *Saccharomyces cerevisiae*, J. Cell Biol. 120 (1993) 55–65.
[29] A.L. Munn, B.J. Stevenson, M.I. Geli, H. Riezman, end5, end6, and end7: mutations that cause actin delocalization and block the internalization step of endocytosis in *Saccharomyces cerevisiae*, Mol. Biol. Cell 6 (1995) 1721–1742.
[30] A.L. Munn, H. Riezman, Endocytosis is required for the growth of vacuolar H(+)-ATPase-defective yeast: identification of six new END genes, J. Cell Biol. 127 (1994) 373–386.
[31] H. Riezman, A. Munn, M.I. Geli, L. Hicke, Actin-, myosin- and ubiquitin-dependent endocytosis, Experientia 52 (1996) 1033–1041.
[32] A.E. Engqvist-Goldstein, M.M. Kessels, V.S. Chopra, M.R. Hayden, D.G. Drubin, 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 (1999) 1503–1518.
[33] C. Schaerer-Brodbeck, H. Riezman, Functional interactions between the p35 subunit of the Arp2/3 complex and calmodulin in yeast, Mol. Biol. Cell 11 (2000) 1113–1127.
[34] C.J. Merrifield, B. Qualmann, M.M. Kessels, W. Almers, Neural Wiskott Aldrich Syndrome Protein (N-WASP) and the Arp2/3 complex are recruited to sites of clathrin-mediated endocytosis in cultured fibroblasts, Eur. J. Cell Biol. 83 (2004) 13–18.
[35] S.N. Naqvi, R. Zahn, D.A. Mitchell, B.J. Stevenson, A.L. Munn, The WASp homologue Las17p functions with the WIP homologue End5p/verprolin and is essential for endocytosis in yeast, Curr. Biol. 8 (1998) 959–962.
[36] M. Kaksonen, Y. Sun, D.G. Drubin, A pathway for association of receptors, adaptors, and actin during endocytic internalization, Cell 115 (2003) 475–487.
[37] J. Mulholland, D. Preuss, A. Moon, A. Wong, D. Drubin, D. Botstein, Ultrastructure of the yeast actin cytoskeleton and its association with the plasma membrane, J. Cell Biol. 125 (1994) 381–391.
[38] J. Mulholland, J. Konopka, B. Singer-Kruger, M. Zerial, D. Botstein, Visualization of receptor-mediated endocytosis in yeast, Mol. Biol. Cell 10 (1999) 799–817.
[39] A.L. Munn, Molecular requirements for the internalisation step of endocytosis: insights from yeast, Biochim. Biophys. Acta 1535 (2001) 236–257.
[40] A.E. Engqvist-Goldstein, D.G. Drubin, Actin assembly and endocytosis: from yeast to mammals, Annu. Rev. Cell Dev. Biol. 19 (2003) 287–332.
[41] T.M. Huckaba, A.C. Gay, L.F. Pantalena, H.C. Yang, L.A. Pon, Live cell imaging of the assembly, disassembly, and actin cable-dependent movement of endosomes and actin patches in the budding yeast, *Saccharomyces cerevisiae*, J. Cell Biol. 167 (2004) 519–530.
[42] C.J. Merrifield, M.E. Feldman, L. Wan, W. Almers, Imaging actin and dynamin recruitment during invagination of single clathrin-coated pits, Nat. Cell Biol. 4 (2002) 691–698.
[43] R. Lombardi, H. Riezman, Rvs161p and Rvs167p, the two yeast amphiphysin homologs, function together in vivo, J. Biol. Chem. 276 (2001) 6016–6022.
[44] H. Benedetti, S. Raths, F. Crausaz, H. Riezman, The END3 gene

encodes a protein that is required for the internalization step of endocytosis and for actin cytoskeleton organization in yeast, Mol. Biol. Cell 5 (1994) 1023–1037.
[45] B. Wendland, S.D. Emr, Pan1p, yeast eps15, functions as a multivalent adaptor that coordinates protein–protein interactions essential for endocytosis, J. Cell Biol. 141 (1998) 71–84.
[46] G.S. Payne, D. Baker, E. van Tuinen, R. Schekman, Protein transport to the vacuole and receptor-mediated endocytosis by clathrin heavy chain-deficient yeast, J. Cell Biol. 106 (1988) 1453–1461.
[47] D.S. Chu, B. Pishvaee, G.S. Payne, The light chain subunit is required for clathrin function in *Saccharomyces cerevisiae*, J. Biol. Chem. 271 (1996) 33123–33130.
[48] B.G. Yeung, G.S. Payne, Clathrin interactions with C-terminal regions of the yeast AP-1 beta and gamma subunits are important for AP-1 association with clathrin coats, Traffic 2 (2001) 565–576.
[49] K.M. Huang, K. D'Hondt, H. Riezman, S.K. Lemmon, Clathrin functions in the absence of heterotetrameric adaptors and AP180-related proteins in yeast, EMBO J. 18 (1999) 3897–3908.
[50] B.G. Yeung, H.L. Phan, G.S. Payne, Adaptor complex-independent clathrin function in yeast, Mol. Biol. Cell 10 (1999) 3643–3659.
[51] G.J. Praefcke, H.T. McMahon, The dynamin superfamily: universal membrane tubulation and fission molecules? Nat. Rev., Mol. Cell Biol. 5 (2004) 133–147.
[52] D. Otsuga, B.R. Keegan, E. Brisch, J.W. Thatcher, G.J. Hermann, W. Bleazard, J.M. Shaw, The dynamin-related GTPase, Dnm1p, controls mitochondrial morphology in yeast, J. Cell Biol. 143 (1998) 333–349.
[53] K.A. Shepard, M.P. Yaffe, The yeast dynamin-like protein, Mgm1p, functions on the mitochondrial outer membrane to mediate mitochondrial inheritance, J. Cell Biol. 144 (1999) 711–720.
[54] J.H. Rothman, C.K. Raymond, T. Gilbert, P.J. O'Hara, T.H. Stevens, A putative GTP binding protein homologous to interferon-inducible Mx proteins performs an essential function in yeast protein sorting, Cell 61 (1990) 1063–1074.
[55] C.A. Vater, C.K. Raymond, K. Ekena, I. Howald-Stevenson, T.H. Stevens, The VPS1 protein, a homolog of dynamin required for vacuolar protein sorting in *Saccharomyces cerevisiae*, is a GTPase with two functionally separable domains, J. Cell Biol. 119 (1992) 773–786.
[56] S. Gurunathan, D. David, J.E. Gerst, Dynamin and clathrin are required for the biogenesis of a distinct class of secretory vesicles in yeast, EMBO J. 21 (2002) 602–614.
[57] X. Yu, M. Cai, The yeast dynamin-related GTPase Vps1p functions in the organization of the actin cytoskeleton via interaction with Sla1p, J. Cell Sci. 117 (2004) 3839–3853.
[58] S.F. Nothwehr, E. Conibear, T.H. Stevens, Golgi and vacuolar membrane proteins reach the vacuole in vps1 mutant yeast cells via the plasma membrane, J. Cell Biol. 129 (1995) 35–46.
[59] B. Zanolari, S. Friant, K. Funato, C. Sutterlin, B.J. Stevenson, H. Riezman, Sphingoid base synthesis requirement for endocytosis in *Saccharomyces cerevisiae*, EMBO J. 19 (2000) 2824–2833.
[60] Z. Hua, P. Fatheddin, T.R. Graham, An essential subfamily of Drs2p-related P-type ATPases is required for protein trafficking between Golgi complex and endosomal/vacuolar system, Mol. Biol. Cell 13 (2002) 3162–3177.
[61] T. Pomorski, R. Lombardi, H. Riezman, P.F. Devaux, G. van Meer, J.C. Holthuis, Drs2p-related P-type ATPases Dnf1p and Dnf2p are required for phospholipid translocation across the yeast plasma membrane and serve a role in endocytosis, Mol. Biol. Cell 14 (2003) 1240–1254.
[62] A. Heese-Peck, H. Pichler, B. Zanolari, R. Watanabe, G. Daum, H. Riezman, Multiple functions of sterols in yeast endocytosis, Mol. Biol. Cell 13 (2002) 2664–2680.
[63] A.L. Munn, A. Heese-Peck, B.J. Stevenson, H. Pichler, H. Riezman, Specific sterols required for the internalization step of endocytosis in yeast, Mol. Biol. Cell 10 (1999) 3943–3957.
[64] Y. Feng, N.G. Davis, Akr1p and the type I casein kinases act prior to the ubiquitination step of yeast endocytosis: Akr1p is required for kinase localization to the plasma membrane, Mol. Cell. Biol. 20 (2000) 5350–5359.
[65] A.F. Roth, Y. Feng, L. Chen, N.G. Davis, The yeast DHHC cysteine-rich domain protein Akr1p is a palmitoyl transferase, J. Cell Biol. 159 (2002) 23–28.
[66] P. Babu, J.D. Bryan, H.R. Panek, S.L. Jordan, B.M. Forbrich, S.C. Kelley, R.T. Colvin, L.C. Robinson, Plasma membrane localization of the Yck2p yeast casein kinase 1 isoform requires the C-terminal extension and secretory pathway function, J. Cell Sci. 115 (2002) 4957–4968.
[67] S. Friant, R. Lombardi, T. Schmelzle, M.N. Hall, H. Riezman, Sphingoid base signaling via Pkh kinases is required for endocytosis in yeast, EMBO J. 20 (2001) 6783–6792.
[68] G.E. Palade, Fine structure of blood capillaries, J. Appl. Phys. 24 (1953) 1424.
[69] E. Yamada, The fine structures of the gall bladder epithelium of the mouse, J. Biophys. Biochem. Cytol. 1 (1955) 445–458.
[70] M. Kirkham, A. Fujita, R. Chadda, S.J. Nixon, T.V. Kurzchalia, D.K. Sharma, R.E. Pagano, J.F. Hancock, S. Mayor, R.G. Parton, Ultrastructural identification of uncoated caveolin-independent early endocytic vehicles, J. Cell Biol. 168 (2005) 465–476.
[71] P.A. Orlandi, P.H. Fishman, Filipin-dependent inhibition of cholera toxin: evidence for toxin internalization and activation through caveolae-like domains, J. Cell Biol. 141 (1998) 905–915.
[72] R.G. Parton, A.A. Richards, Lipid rafts and caveolae as portals for endocytosis: new insights and common mechanisms, Traffic 4 (2003) 724–738.
[73] A.M. Fra, E. Williamson, K. Simons, R.G. Parton, Detergent-insoluble glycolipid microdomains in lymphocytes in the absence of caveolae, J. Biol. Chem. 269 (1994) 30745–30748.
[74] A. Llorente, A. Rapak, S.L. Schmid, B. van Deurs, K. Sandvig, Expression of mutant dynamin inhibits toxicity and transport of endocytosed ricin to the Golgi apparatus, J. Cell Biol. 140 (1998) 553–563.
[75] P. Verkade, T. Harder, F. Lafont, K. Simons, Induction of caveolae in the apical plasma membrane of Madin–Darby canine kidney cells, J. Cell Biol. 148 (2000) 727–739.
[76] P.K. Holm, P. Eker, K. Sandvig, B. van Deurs, Phorbol myristate acetate selectively stimulates apical endocytosis via protein kinase C in polarized MDCK cells, Exp. Cell Res. 217 (1995) 157–168.
[77] P. Eker, P.K. Holm, B. van Deurs, K. Sandvig, Selective regulation of apical endocytosis in polarized Madin–Darby canine kidney cells by mastoparan and cAMP, J. Biol. Chem. 269 (1994) 18607–18615.
[78] A. Llorente, B. van Deurs, O. Garred, P. Eker, K. Sandvig, Apical endocytosis of ricin in MDCK cells is regulated by the cyclooxygenase pathway, J. Cell Sci. 113 (Pt. 7) (2000) 1213–1221.
[79] H. Damke, T. Baba, A.M. van der Bliek, S.L. Schmid, Clathrin-independent pinocytosis is induced in cells overexpressing a temperature-sensitive mutant of dynamin, J. Cell Biol. 131 (1995) 69–80.
[80] N. Naslavsky, R. Weigert, J.G. Donaldson, Characterization of a nonclathrin endocytic pathway: membrane cargo and lipid requirements, Mol. Biol. Cell 15 (2004) 3542–3552.
[81] N. Naslavsky, R. Weigert, J.G. Donaldson, Convergence of non-clathrin- and clathrin-derived endosomes involves Arf6 inactivation and changes in phosphoinositides, Mol. Biol. Cell 14 (2003) 417–431.
[82] H. Radhakrishna, R.D. Klausner, J.G. Donaldson, Aluminum fluoride stimulates surface protrusions in cells overexpressing the ARF6 GTPase, J. Cell Biol. 134 (1996) 935–947.
[83] M. Krauss, M. Kinuta, M.R. Wenk, P. De Camilli, K. Takei, V. Haucke, ARF6 stimulates clathrin/AP-2 recruitment to synaptic

membranes by activating phosphatidylinositol phosphate kinase type Igamma, J. Cell Biol. 162 (2003) 113–124.

[84] A. Guha, V. Sriram, K.S. Krishnan, S. Mayor, Shibire mutations reveal distinct dynamin-independent and -dependent endocytic pathways in primary cultures of *Drosophila* hemocytes, J. Cell Sci. 116 (2003) 3373–3386.

[85] R.G. Parton, Ultrastructural localization of gangliosides; GM1 is concentrated in caveolae, J. Histochem. Cytochem. 42 (1994) 155–166.

[86] M.L. Torgersen, G. Skretting, B. van Deurs, K. Sandvig, Internalization of cholera toxin by different endocytic mechanisms, J. Cell Sci. 114 (2001) 3737–3747.

[87] M. Drab, P. Verkade, M. Elger, M. Kasper, M. Lohn, B. Lauterbach, J. Menne, C. Lindschau, F. Mende, F.C. Luft, A. Schedl, H. Haller, T.V. Kurzchalia, Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice, Science 9 (2001) 9.

[88] F. Galbiati, J.A. Engelman, D. Volonte, X.L. Zhang, C. Minetti, M. Li, H. Hou Jr., B. Kneitz, W. Edelmann, M.P. Lisanti, Caveolin-3 null mice show a loss of caveolae, changes in the microdomain distribution of the dystrophin–glycoprotein complex, and t-tubule abnormalities, J. Biol. Chem. 276 (2001) 21425–21433.

[89] A.M. Fra, E. Williamson, K. Simons, R.G. Parton, De novo formation of caveolae in lymphocytes by expression of VIP21-caveolin, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 8655–8659.

[90] P. Dupree, R.G. Parton, G. Raposo, T.V. Kurzchalia, K. Simons, Caveolae and sorting in the trans-Golgi network of epithelial cells, EMBO J. 12 (1993) 1597–1605.

[91] S. Monier, R.G. Parton, F. Vogel, J. Behlke, A. Henske, T.V. Kurzchalia, VIP21-caveolin, a membrane protein constituent of the caveolar coat, oligomerizes in vivo and in vitro, Mol. Biol. Cell 6 (1995) 911–927.

[92] C. Thiele, M.J. Hannah, F. Fahrenholz, W.B. Huttner, Cholesterol binds to synaptophysin and is required for biogenesis of synaptic vesicles, Nat. Cell Biol. 2 (2000) 42–49.

[93] M. Murata, J. Peranen, R. Schreiner, F. Wieland, T.V. Kurzchalia, K. Simons, VIP21/caveolin is a cholesterol-binding protein, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 10339–10343.

[94] K.G. Rothberg, Y.S. Ying, B.A. Kamen, R.G. Anderson, Cholesterol controls the clustering of the glycophospholipid-anchored membrane receptor for 5-methyltetrahydrofolate, J. Cell Biol. 111 (1990) 2931–2938.

[95] J.R. Henley, E.W. Krueger, B.J. Oswald, M.A. McNiven, Dynamin-mediated internalization of caveolae, J. Cell Biol. 141 (1998) 85–99.

[96] P. Oh, D.P. McIntosh, J.E. Schnitzer, Dynamin at the neck of caveolae mediates their budding to form transport vesicles by GTP-driven fission from the plasma membrane of endothelium, J. Cell Biol. 141 (1998) 101–114.

[97] J.E. Schnitzer, Caveolae: from basic trafficking mechanisms to targeting transcytosis for tissue-specific drug and gene delivery in vivo, Adv. Drug Deliv. Rev. 49 (2001) 265–280.

[98] L. Pelkmans, J. Kartenbeck, A. Helenius, Caveolar endocytosis of simian virus 40 reveals a new two-step vesicular-transport pathway to the ER, Nat. Cell Biol. 3 (2001) 473–483.

[99] R.G. Parton, B. Joggerst, K. Simons, Regulated internalization of caveolae, J. Cell Biol. 127 (1994) 1199–1215.

[100] A.N. Shajahan, B.K. Timblin, R. Sandoval, C. Tiruppathi, A.B. Malik, R.D. Minshall, Role of Src-induced dynamin-2 phosphorylation in caveolae-mediated endocytosis in endothelial cells, J. Biol. Chem. 279 (2004) 20392–20400.

[101] R.D. Minshall, C. Tiruppathi, S.M. Vogel, W.D. Niles, A. Gilchrist, H.E. Hamm, A.B. Malik, Endothelial cell-surface gp60 activates vesicle formation and trafficking via G(i)-coupled Src kinase signaling pathway, J. Cell Biol. 150 (2000) 1057–1070.

[102] D.K. Sharma, J.C. Brown, A. Choudhury, T.E. Peterson, E. Holicky, D.L. Marks, R. Simari, R.G. Parton, R.E. Pagano, Selective stimulation of caveolar endocytosis by glycosphingolipids and cholesterol, Mol. Biol. Cell (2004).

[103] R. Nomura, T. Fujimoto, Tyrosine-phosphorylated caveolin-1: immunolocalization and molecular characterization, Mol. Biol. Cell 10 (1999) 975–986.

[104] A.N. Shajahan, C. Tiruppathi, A.V. Smrcka, A.B. Malik, R.D. Minshall, Gbetagamma activation of Src induces caveolae-mediated endocytosis in endothelial cells, J. Biol. Chem. 279 (2004) 48055–48062.

[105] E. Ikonen, R.G. Parton, Caveolins and cellular cholesterol balance, Traffic 1 (2000) 212–217.

[106] A. Pol, R. Luetterforst, M. Lindsay, S. Heino, E. Ikonen, R.G. Parton, A caveolin dominant negative mutant associates with lipid bodies and induces intracellular cholesterol imbalance, J. Cell Biol. 152 (2001) 1057–1070.

[107] V. Puri, R. Watanabe, R.D. Singh, M. Dominguez, J.C. Brown, C.L. Wheatley, D.L. Marks, R.E. Pagano, Clathrin-dependent and -independent internalization of plasma membrane sphingolipids initiates two Golgi targeting pathways, J. Cell Biol. 154 (2001) 535–547.

[108] R.D. Singh, V. Puri, J.T. Valiyaveettil, D.L. Marks, R. Bittman, R.E. Pagano, Selective caveolin-1-dependent endocytosis of glycosphingolipids, Mol. Biol. Cell 14 (2003) 3254–3265.

[109] P. Thomsen, K. Roepstorff, M. Stahlhut, B. van Deurs, Caveolae are highly immobile plasma membrane microdomains, which are not involved in constitutive endocytic trafficking, Mol. Biol. Cell 13 (2002) 238–250.

[110] B.J. Nichols, A distinct class of endosome mediates clathrin-independent endocytosis to the Golgi complex, Nat. Cell Biol. 4 (2002) 374–378.

[111] H. Mitchell, A. Choudhury, R.E. Pagano, E.B. Leof, Ligand-dependent and -independent transforming growth factor-beta receptor recycling regulated by clathrin-mediated endocytosis and Rab11, Mol. Biol. Cell 15 (2004) 4166–4178.

[112] E.M. Damm, L. Pelkmans, J. Kartenbeck, A. Mezzacasa, T. Kurzchalia, A. Helenius, Clathrin- and caveolin-1-independent endocytosis: entry of simian virus 40 into cells devoid of caveolae, J. Cell Biol. 168 (2005) 477–488.

[113] P.U. Le, G. Guay, Y. Altschuler, I.R. Nabi, Caveolin-1 is a negative regulator of caveolae-mediated endocytosis to the endoplasmic reticulum, J. Biol. Chem. 277 (2002) 3371–3379.

[114] P.U. Le, I.R. Nabi, Distinct caveolae-mediated endocytic pathways target the Golgi apparatus and the endoplasmic reticulum, J. Cell Sci. 116 (2003) 1059–1071.

[115] I.R. Nabi, P.U. Le, Caveolae/raft-dependent endocytosis, J. Cell Biol. 161 (2003) 673–677.

[116] T. Baumgart, S.T. Hess, W.W. Webb, Imaging coexisting fluid domains in biomembrane models coupling curvature and line tension, Nature 425 (2003) 821–824.

[117] F. Julicher, R. Lipowsky, Domain-induced budding of vesicles, Phys. Rev. Lett. 70 (1993) 2964–2967.

[118] K. Bacia, P. Schwille, T. Kurzchalia, Sterol structure determines the separation of phases and the curvature of the liquid-ordered phase in model membranes, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 3272–3277.

[119] H. Chen, P. De Camilli, The association of epsin with ubiquitinated cargo along the endocytic pathway is negatively regulated by its interaction with clathrin, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 2766–2771.

[120] S. Manes, R. Ana Lacalle, C. Gomez-Mouton, A.C. Martinez, From rafts to crafts: membrane asymmetry in moving cells, Trends Immunol. 24 (2003) 320–326.

[121] S. Grimmer, B. van Deurs, K. Sandvig, Membrane ruffling and macropinocytosis in A431 cells require cholesterol, J. Cell Sci. 115 (2002) 2953–2962.

[122] M.A. del Pozo, N.B. Alderson, W.B. Kiosses, H.H. Chiang, R.G. Anderson, M.A. Schwartz, Integrins regulate Rac targeting

by internalization of membrane domains, Science 303 (2004) 839–842.

[123] J. Kwik, S. Boyle, D. Fooksman, L. Margolis, M.P. Sheetz, M. Edidin, Membrane cholesterol, lateral mobility, and the phosphatidylinositol 4,5-bisphosphate-dependent organization of cell actin, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 13964–13969.

[124] M.J. McConville, K.A. Mullin, S.C. Ilgoutz, R.D. Teasdale, Secretory pathway of trypanosomatid parasites, Microbiol. Mol. Biol. Rev. 66 (2002) 122–154.

[125] P. Overath, M. Engstler, Endocytosis, membrane recycling and sorting of GPI-anchored proteins: trypanosoma brucei as a model system, Mol. Microbiol. 53 (2004) 735–744.

[126] M. Engstler, L. Thilo, F. Weise, C.G. Grunfelder, H. Schwarz, M. Boshart, P. Overath, Kinetics of endocytosis and recycling of the GPI-anchored variant surface glycoprotein in *Trypanosoma brucei*, J. Cell Sci. 117 (2004) 1105–1115.

[127] M.C. Field, M. Carrington, Intracellular membrane transport systems in *Trypanosoma brucei*, Traffic 5 (2004) 905–913.

[128] C.L. Allen, D. Goulding, M.C. Field, Clathrin-mediated endocytosis is essential in *Trypanosoma brucei*, EMBO J. 22 (2003) 4991–5002.

[129] J.A. Garcia-Salcedo, D. Perez-Morga, P. Gijon, V. Dilbeck, E. Pays, D.P. Nolan, A differential role for actin during the life cycle of *Trypanosoma brucei*, EMBO J. 23 (2004) 780–789.

[130] G.W. Morgan, D. Goulding, M.C. Field, The single dynamin-like protein of *Trypanosoma brucei* regulates mitochondrial division and is not required for endocytosis, J. Biol. Chem. 279 (2004) 10692–10701.

[131] G.W. Morgan, C.L. Allen, T.R. Jeffries, M. Hollinshead, M.C. Field, Developmental and morphological regulation of clathrin-mediated endocytosis in *Trypanosoma brucei*, J. Cell Sci. 114 (2001) 2605–2615.

[132] S.G. Langreth, A.E. Balber, Protein uptake and digestion in bloodstream and culture forms of *Trypanosoma brucei*, J. Protozool. 22 (1975) 40–53.

[133] P. Webster, Endocytosis by African trypanosomes: I. Three-dimensional structure of the endocytic organelles in *Trypanosoma brucei* and *T. congolense*, Eur. J. Cell Biol. 49 (1989) 295–302.

[134] M. Bonazzi, S. Spano, G. Turacchio, C. Cericola, C. Valente, A. Colanzi, H.S. Kweon, V.W. Hsu, E.V. Polishchuck, R.S. Polishchuck, M. Sallese, T. Pulvirenti, D. Corda, A. Luini, CtBP3/BARS drives membrane fission in dynamin-independent transport pathways, Nat. Cell Biol. 7 (2005) 570–580.

[135] L. Abrami, S. Liu, P. Cosson, S.H. Leppla, F.G. van der Goot, Anthrax toxin triggers endocytosis of its receptor via a lipid raft-mediated clathrin-dependent process, J. Cell Biol. 160 (2003) 321–328.

[136] H.A. Anderson, Y. Chen, L.C. Norkin, Bound simian virus 40 translocates to caveolin-enriched membrane domains, and its entry is inhibited by drugs that selectively disrupt caveolae, Mol. Biol. Cell 7 (1996) 1825–1834.

[137] D. Yarar, C.M. Waterman-Storer, S.L. Schmid, A dynamic actin cytoskeleton functions at multiple stages of clathrin-mediated endocytosis, Mol. Biol. Cell 16 (2005) 964–975.

[138] B.M. Pearse, Clathrin: a unique protein associated with intracellular transfer of membrane by coated vesicles, Proc. Natl. Acad. Sci. U. S. A. 73 (1976) 1255–1259.

[139] R.K. Draper, Y. Goda, F.M. Brodsky, S.R. Pfeffer, Antibodies to clathrin inhibit endocytosis but not recycling to the trans Golgi network in vitro, Science 248 (1990) 1539–1541.

[140] A.M. van der Bliek, T.E. Redelmeier, H. Damke, E.J. Tisdale, E.M. Meyerowitz, S.L. Schmid, Mutations in human dynamin block an intermediate stage in coated vesicle formation, J. Cell Biol. 122 (1993) 553–563.

[141] C. Lamaze, T.H. Chuang, L.J. Terlecky, G.M. Bokoch, S.L. Schmid, Regulation of receptor-mediated endocytosis by Rho and Rac, Nature 382 (1996) 177–179.

[142] N.K. Hussain, S. Jenna, M. Glogauer, C.C. Quinn, S. Wasiak, M. Guipponi, S.E. Antonarakis, B.K. Kay, T.P. Stossel, N. Lamarche-Vane, P.S. McPherson, Endocytic protein intersectin-l regulates actin assembly via Cdc42 and N-WASP, Nat. Cell Biol. 3 (2001) 927–932.

[143] W. Yang, C.G. Lo, T. Dispenza, R.A. Cerione, The Cdc42 target ACK2 directly interacts with clathrin and influences clathrin assembly, J. Biol. Chem. 276 (2001) 17468–17473.

[144] A.J. Ridley, H.F. Paterson, C.L. Johnston, D. Diekmann, A. Hall, The small GTP-binding protein rac regulates growth factor-induced membrane ruffling, Cell 70 (1992) 401–410.

[145] M. Symons, N. Rusk, Control of vesicular trafficking by Rho GTPases, Curr. Biol. 13 (2003) R409–R418.

[146] W.S. Garrett, L.M. Chen, R. Kroschewski, M. Ebersold, S. Turley, S. Trombetta, J.E. Galan, I. Mellman, Developmental control of endocytosis in dendritic cells by Cdc42, Cell 102 (2000) 325–334.

[147] V. Korolchuk, G. Banting, Kinases in clathrin-mediated endocytosis, Biochem. Soc. Trans. 31 (2003) 857–860.

[148] A. Wilde, E.C. Beattie, L. Lem, D.A. Riethof, S.H. Liu, W.C. Mobley, P. Soriano, F.M. Brodsky, EGF receptor signaling stimulates SRC kinase phosphorylation of clathrin, influencing clathrin redistribution and EGF uptake, Cell 96 (1999) 677–687.

[149] S. Ahn, S. Maudsley, L.M. Luttrell, R.J. Lefkowitz, Y. Daaka, Src-mediated tyrosine phosphorylation of dynamin is required for beta2-adrenergic receptor internalization and mitogen-activated protein kinase signaling, J. Biol. Chem. 274 (1999) 1185–1188.

[150] A. Veithen, P. Cupers, P. Baudhuin, P.J. Courtoy, v-Src induces constitutive macropinocytosis in rat fibroblasts, J. Cell Sci. 109 (Pt. 8) (1996) 2005–2012.

[151] S. Dharmawardhane, A. Schurmann, M.A. Sells, J. Chernoff, S.L. Schmid, G.M. Bokoch, Regulation of macropinocytosis by p21-activated kinase-1, Mol. Biol. Cell 11 (2000) 3341–3352.

[152] P.J. Robinson, J.M. Sontag, J.P. Liu, E.M. Fykse, C. Slaughter, H. McMahon, T.C. Sudhof, Dynamin GTPase regulated by protein kinase C phosphorylation in nerve terminals, Nature 365 (1993) 163–166.

[153] J. Zhu, K. Zhou, J.J. Hao, J. Liu, N. Smith, X. Zhan, Regulation of cortactin/dynamin interaction by actin polymerization during the fission of clathrin-coated pits, J. Cell Sci. 118 (2005) 807–817.

[154] H. Cao, J.D. Orth, J. Chen, S.G. Weller, J.E. Heuser, M.A. McNiven, Cortactin is a component of clathrin-coated pits and participates in receptor-mediated endocytosis, Mol. Cell. Biol. 23 (2003) 2162–2170.

[155] M. Yamabhai, N.G. Hoffman, N.L. Hardison, P.S. McPherson, L. Castagnoli, G. Cesareni, B.K. Kay, Intersectin, a novel adaptor protein with two Eps15 homology and five Src homology 3 domains, J. Biol. Chem. 273 (1998) 31401–31407.

[156] F. Simpson, N.K. Hussain, B. Qualmann, R.B. Kelly, B.K. Kay, P.S. McPherson, S.L. Schmid, SH3-domain-containing proteins function at distinct steps in clathrin-coated vesicle formation, Nat. Cell Biol. 1 (1999) 119–124.

[157] K. Tanabe, T. Torii, W. Natsume, S. Braesch-Andersen, T. Watanabe, M. Satake, A novel GTPase-activating protein for ARF6 directly interacts with clathrin and regulates clathrin-dependent endocytosis, Mol. Biol. Cell 16 (2005) 1617–1628.

[158] P.P. Di Fiore, S. Polo, K. Hofmann, When ubiquitin meets ubiquitin receptors: a signalling connection, Nat. Rev., Mol. Cell Biol. 4 (2003) 491–497.

[159] M.J. McFarland, A.C. Porter, F.R. Rakhshan, D.S. Rawat, R.A. Gibbs, E.L. Barker, A role for caveolae/lipid rafts in the uptake and recycling of the endogenous cannabinoid anandamide, J. Biol. Chem. 279 (2004) 41991–41997.

[160] Y. Zeng, N. Tao, K.N. Chung, J.E. Heuser, D.M. Lublin, Endocytosis of oxidized low density lipoprotein through scavenger receptor CD36 utilizes a lipid raft pathway that does not require caveolin-1, J. Biol. Chem. 278 (2003) 45931–45936.

[161] J.L. Estall, B. Yusta, D.J. Drucker, Lipid raft-dependent glucagon-like peptide-2 receptor trafficking occurs independently of agonist-induced desensitization, Mol. Biol. Cell 15 (2004) 3673–3687.
[162] E. Tkachenko, E. Lutgens, R.V. Stan, M. Simons, Fibroblast growth factor 2 endocytosis in endothelial cells proceed via syndecan-4-dependent activation of Rac1 and a Cdc42-dependent macropinocytic pathway, J. Cell Sci. 117 (2004) 3189–3199.
[163] K.S. Song, S. Li, T. Okamoto, L.A. Quilliam, M. Sargiacomo, M.P. Lisanti, Co-purification and direct interaction of Ras with caveolin, an integral membrane protein of caveolae microdomains. Detergent-free purification of caveolae microdomains, J. Biol. Chem. 271 (1996) 9690–9697.
[164] S. Gines, F. Ciruela, J. Burgueno, V. Casado, E.I. Canela, J. Mallol, C. Lluis, R. Franco, Involvement of caveolin in ligand-induced recruitment and internalization of A(1) adenosine receptor and adenosine deaminase in an epithelial cell line, Mol. Pharmacol. 59 (2001) 1314–1323.
[165] L.D. Jayanthi, D.J. Samuvel, S. Ramamoorthy, Regulated internalization and phosphorylation of the native norepinephrine transporter in response to phorbol esters. Evidence for localization in lipid rafts and lipid raft-mediated internalization, J. Biol. Chem. 279 (2004) 19315–19326.
[166] M.E. Lamb, C. Zhang, T. Shea, D.J. Kyle, L.M. Leeb-Lundberg, Human B1 and B2 bradykinin receptors and their agonists target caveolae-related lipid rafts to different degrees in HEK293 cells, Biochemistry 41 (2002) 14340–14347.
[167] E.J. Smart, Y.S. Ying, C. Mineo, R.G. Anderson, A detergent-free method for purifying caveolae membrane from tissue culture cells, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 10104–10108.
[168] M.E. Lamb, W.F. De Weerd, L.M. Leeb-Lundberg, Agonist-promoted trafficking of human bradykinin receptors: arrestin- and dynamin-independent sequestration of the B2 receptor and bradykinin in HEK293 cells, Biochem. J. 355 (2001) 741–750.
[169] M. Haasemann, J. Cartaud, W. Muller-Esterl, I. Dunia, Agonist-induced redistribution of bradykinin B2 receptor in caveolae, J. Cell Sci. 111 (Pt. 7) (1998) 917–928.