Actin regulation in endocytosis

Elizabeth Smythe[1] and Kathryn R. Ayscough[2,*]

Departments of [1]Biomedical Science and [2]Molecular Biology and Biotechnology, Firth Court, Western Bank, University of Sheffield, Sheffield, S10 2TN, UK

*Author for correspondence (e-mail: k.ayscough@sheffield.ac.uk)

Accepted 6 September 2006
Journal of Cell Science 119, 4589-4598 Published by The Company of Biologists 2006
doi:10.1242/jcs.03247

Summary

Increasing evidence from a variety of cell types has highlighted the importance of the actin cytoskeleton during endocytosis. No longer is actin viewed as a passive barrier that must be removed to allow endocytosis to proceed. Rather, actin structures are dynamically organised to assist the remodelling of the cell surface to allow inward movement of vesicles. The majority of our mechanistic insight into the role of actin in endocytosis has come from studies in budding yeast. Although endocytosis in mammalian cells is clearly more complex and subject to a greater array of regulatory signals, recent advances have revealed actin, and actin-regulatory proteins, to be present at endocytic sites. Furthermore, live cell imaging indicates that spatiotemporal aspects of actin recruitment and vesicle formation are likely to be conserved across eukaryotic evolution.

Key words: Clathrin, Budding yeast, Vesicle trafficking

Introduction

Endocytosis is the process through which the plasma membrane invaginates into the cell, resulting in the production of a vesicle that is then able to fuse with endosomes and enter the endo-lysosomal membrane system. Endocytosis is required for recycling of plasma membrane lipids and trafficking proteins, and for uptake or downregulation of cell-surface receptors. The endocytic process can also be exploited by pathogens and toxins to allow their entry into cells. Uptake of material by endocytosis of clathrin-coated vesicles has been visualised by electron microscopy for over 40 years (Roth and Porter, 1964). Clathrin-coated pits assemble at the plasma membrane, select cargo, invaginate and pinch off. Clathrin-coated vesicles then move into the cell, lose their coat and the uncoated endocytic vesicles fuse with the early endosome to deliver their content. In addition to clathrin-coated endocytosis, some eukaryotic cells are able to internalise external material by an engulfment process called phagocytosis, and more recently non-clathrin-coated-vesicle internalisation processes have been identified.

Actin is an ubiquitous eukaryotic protein that is able to form dynamic polar filamentous structures (F-actin), which constitute a major part of the cell cytoskeleton. F-actin associates with a plethora of binding proteins that regulate the rate of filament assembly and disassembly, as well as organising the filaments into higher-order structures, such as bundles or crosslinked meshworks (Winder and Ayscough, 2005). Many studies have observed that actin localises to regions underlying the plasma membrane of cells. However, it is from studies of budding yeast that the clearest data supporting a role for actin in endocytosis have emerged. Indeed, until recently this role for actin was generally assumed to be a rather specialised function for yeast. Only since the advent of high-resolution, dual-labelling and real-time fluorescence microscopy has the clear participation of actin in mammalian cell endocytosis been observed. This has led the way for experiments that now indicate that the role of actin in endocytosis is likely to have been evolutionarily conserved.

In this Commentary, we limit our discussion to the role of actin in clathrin-mediated endocytosis. This is the major uptake pathway of many mammalian cells. However, the significance of other endocytic pathways is becoming increasingly recognised (Conner and Schmid, 2003). In all cases, actin has been implicated in the mechanics of uptake, although to varying degrees. In the case of phagocytosis, the importance of actin in pseudopod extension and engulfment has been known for many years. However, our knowledge of the mechanistic basis for actin involvement in macropinocytosis and non-clathrin-mediated uptake is currently limited.

Below, we outline the current understanding of the function of actin during endocytosis and highlight how actin is regulated to allow the formation and movement of endocytic vesicles from the plasma membrane. Initially we focus on the data from yeast, which form the basis for most of our current understanding of the role of actin during the process. We then consider endocytosis in mammalian cells, and discuss recent advances that demonstrate the importance of the actin cytoskeleton in this context.

Actin in yeast and the mechanism of endocytosis

Actively growing budding yeast contains three distinct actin structures that are visible by fluorescence microscopy of Rhodamine-phalloidin-stained cells: cortical actin patches, which are now thought to represent sites of endocytosis; actin cables, which are crucial for polarity and for movement of exocytic vesicles; and a contractile actin ring, which plays a role in cytokinesis (Adams and Pringle, 1984).

The first evidence linking actin to the endocytic process was published 13 years ago. A genetic screen for yeast mutants exhibiting defective uptake of a fluid-phase marker revealed

that, as well as displaying defective endocytosis, these mutants also contain aberrant actin (Kübler and Riezman, 1993; Raths et al., 1993). Over subsequent years, links between proteins of cortical actin patches and the ability to effectively endocytose became increasingly apparent. However, although >30 proteins were identified and characterised as components of the cortical actin patches, how the actin cytoskeleton is involved in the process remained unclear (reviewed in Engqvist-Goldstein and Drubin, 2003).

Key advances have come from studies of the behaviour of actin patch proteins in real-time. Several studies have assessed the behaviour and lifetime of these patches (Doyle and Botstein, 1996; Waddle et al., 1996; Warren et al., 2002). Such studies show that a patch is formed at the plasma membrane and after a few seconds it moves rapidly into the cell (Fig. 1). However, other proteins that regulate actin appear to have distinct behaviours, often remaining relatively non-motile at the membrane or showing just small inward movement (Warren et al., 2002). How this behaviour relates to the process of endocytosis was explained in a very elegant study in 2003, when Kaksonen and colleagues used dual-colour, real-time fluorescence microscopy to study kinetic aspects of the endocytic process (Kaksonen et al., 2003). This study led them to propose a model linking the recruitment of actin to the endocytic process. Essentially, endocytic vesicle coat proteins are thought to be recruited to sites of the plasma membrane, where they act to deform the membrane, causing invagination. Actin is then recruited to assist the later stages of vesicle formation and the rapid movement of the vesicle away from the membrane.

The laboratories of David Drubin and others have subsequently added to this core data to propose a clear mechanism through which organisation and regulation of actin is integral to the endocytic process (Jonsdottir and Li, 2004; Kaksonen et al., 2003; Kaksonen et al., 2005; Newpher et al., 2005; Soulard et al., 2005; Warren et al., 2002). As depicted in Fig. 2, the process involves distinct stages of non-motile, slow-moving and fast-moving patches that can be defined by the presence of discrete protein modules. The four main modules that assemble at endocytic sites in a coordinated and sequential manner are: the endocytic coat complex, the actin network growth machinery, the actin dynamic regulation module and the vesicle scission module.

Actin regulation during endocytosis in budding yeast

The endocytic coat and actin nucleation

Prior to actin recruitment, the site of vesicle formation is determined and the initial stages of coat formation are completed. The selection and establishment of an endocytic site is achieved by a group of proteins referred to as the ‘endocytic coat complex’. This group contains many proteins, which perform a range of functions. Clathrin is able to form a basket-like network around a forming vesicle that is thought to assist in maintaining curvature. Other proteins such as Ent1p and End3p contain ENTH domains that interact with specific lipids, such as phosphoinositol (4,5)-bisphosphate [(PtdIns(4,5)P_2], and are proposed to drive initial stages of membrane curvature. A third group of proteins including Sla1p, Sla2p and Pan1p contain multiple interaction domains. Sla1p binds endocytic cargo and other coat proteins and negatively regulates the Arp2/3 activator Las17p; Sla2p contains a membrane-interaction domain, a central domain that binds other coat components and a C-terminal actin-binding domain; Pan1p binds coat proteins End3p and Yap1801/2, as well as functioning as an activator of Arp2/3 (see Table 1 for known mammalian orthologues of these proteins). These proteins associate early and are visualised as a non-motile patch that later moves a small distance into the cell. This movement is proposed to occur during the invagination and scission steps, after which these proteins disassemble from the vesicle.

What then is the role of actin in the process, and how is it regulated? The importance of F-actin in endocytosis is clearly illustrated by the effect of addition of the actin-monomer-sequestering drug latrunculin A. Within 5 minutes of its addition, actin patches are no longer visible and endocytosis is completely abrogated (Ayscough et al., 1997; Morton et al., 2000). The stage in the endocytic process at which actin is observed is concomitant with onset of slow vesicle movement. This is the point at which the yeast WASP orthologue Las17p and the type I myosins (Myo3p and Myo5p) begin to nucleate actin filaments through activation of the Arp2/3 complex (Sun et al., 2006). This group of proteins has been called the ‘actin network growth machinery’.

Las17p is present on the membrane for a prolonged period, whereas type I myosins and several regulatory proteins (Vrp1p,

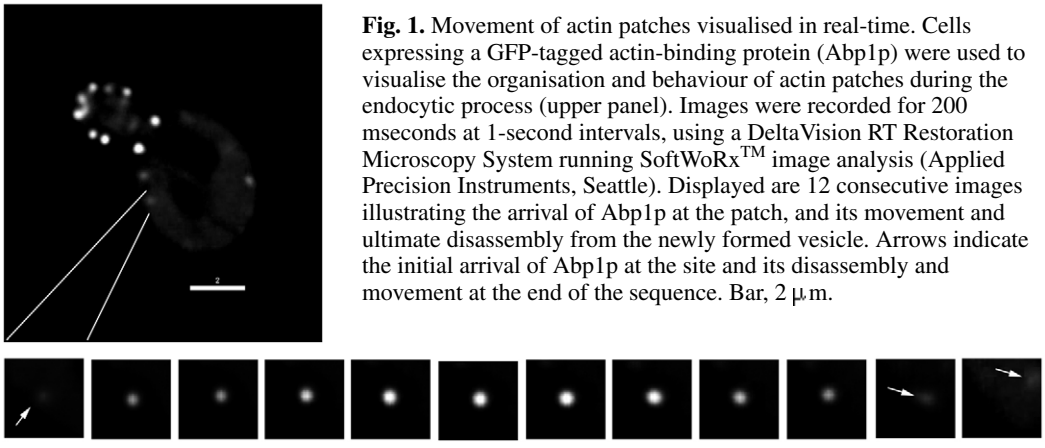

Fig. 1. Movement of actin patches visualised in real-time. Cells expressing a GFP-tagged actin-binding protein (Abp1p) were used to visualise the organisation and behaviour of actin patches during the endocytic process (upper panel). Images were recorded for 200 mseconds at 1-second intervals, using a DeltaVision RT Restoration Microscopy System running SoftWoRx^{TM} image analysis (Applied Precision Instruments, Seattle). Displayed are 12 consecutive images illustrating the arrival of Abp1p at the patch, and its movement and ultimate disassembly from the newly formed vesicle. Arrows indicate the initial arrival of Abp1p at the site and its disassembly and movement at the end of the sequence. Bar, 2 μm.

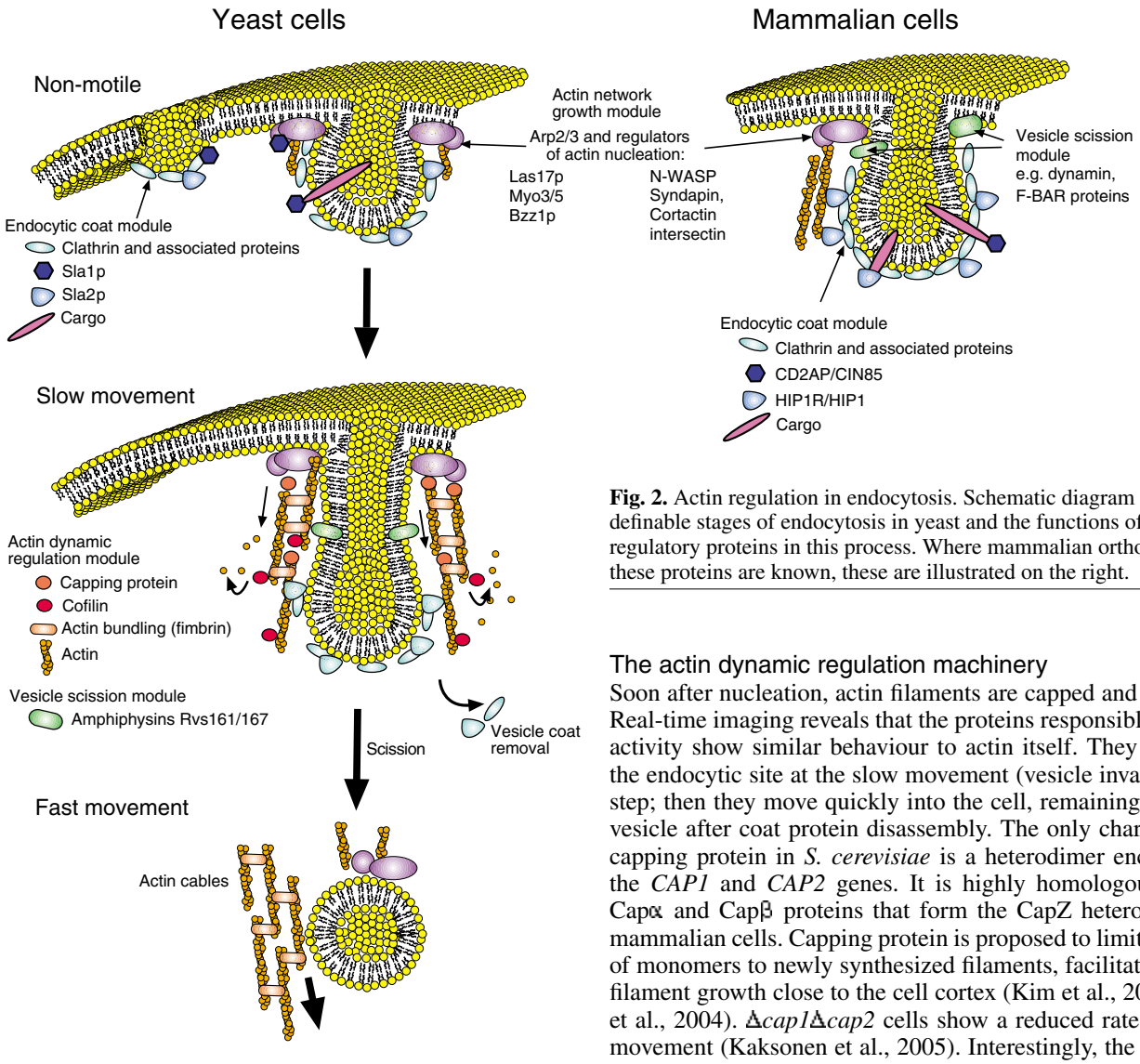

Fig. 2. Actin regulation in endocytosis. Schematic diagram depicting definable stages of endocytosis in yeast and the functions of actin-regulatory proteins in this process. Where mammalian orthologues of these proteins are known, these are illustrated on the right.

Bzz1p and Bbc1p) arrive just prior to actin (see Table 1 for the mammalian orthologues). Live cell imaging reveals that these proteins remain in a non-motile complex at the plasma membrane throughout the invagination and scission process. The current idea is that Las17p (WASP) interacts with coat components including Sla1p, which maintain it in an inactive state. Las17p subsequently recruits Vrp1p (the WIP orthologue) and Bzz1p (the syndapin orthologue) (Sirotkin et al., 2005; Soulard et al., 2002). Bzz1p relieves the inhibition of Las17p by Sla1p to allow initiation of actin nucleation at the endocytic site. Vrp1p and F-actin recruit Myo3p and Myo5p to the site and the actin-nucleation-promotion activity of Myo5p is then activated by Vrp1p (Sun et al., 2006; Jonsdottir and Li, 2004; Soulard et al., 2005; Soulard et al., 2002). Fine-tuning of the level of actin nucleation required is proposed to be the function of Bbc1p, Abp1p and coronin (Humphries et al., 2002; Rodal et al., 2003; Rodal et al., 2005). As illustrated in Fig. 2, filament formation then drives membrane invagination and 'pushes' the forming vesicle away from the plasma membrane. After scission, these proteins are observed to disassemble from the endocytic site.

The actin dynamic regulation machinery

Soon after nucleation, actin filaments are capped and bundled. Real-time imaging reveals that the proteins responsible for this activity show similar behaviour to actin itself. They arrive at the endocytic site at the slow movement (vesicle invagination) step; then they move quickly into the cell, remaining with the vesicle after coat protein disassembly. The only characterised capping protein in *S. cerevisiae* is a heterodimer encoded by the *CAP1* and *CAP2* genes. It is highly homologous to the Capα and Capβ proteins that form the CapZ heterodimer in mammalian cells. Capping protein is proposed to limit addition of monomers to newly synthesized filaments, facilitating rapid filament growth close to the cell cortex (Kim et al., 2006; Kim et al., 2004). Δ*cap1*Δ*cap2* cells show a reduced rate of patch movement (Kaksonen et al., 2005). Interestingly, the effect on endocytosis is not highly penetrant, which indicates that either capping is a relatively minor participant in the process or that other capping proteins exist in yeast.

Following actin polymerisation, the filaments are bound together by the activity of Sac6p/fimbrin a highly conserved actin-bundling protein. In the absence of Sac6p, actin polymerises at endocytic sites, but there is no internalisation (Kaksonen et al., 2005; Kübler and Riezman, 1993). Actin bundles are stronger than single filaments and are thought to provide the strength required to allow generation of sufficient force to allow inward movement of the forming vesicle. Other proteins, including Scp1p, associate with actin at this stage and are likely to provide further stability to the actin network (Winder et al., 2003). However, the functions of many of these proteins await further analysis of their effects on actin dynamics and the endocytic process.

The filaments must then be depolymerised to release monomers to allow further rounds of endocytosis. The best characterised depolymerisation factor in yeast is cofilin, which, in common with many of its orthologues in other eukaryotes, is essential (Iida et al., 1993; Lappalainen and Drubin, 1997; Moon et al., 1993). Mutant alleles of cofilin that cause actin

Table 1. Actin regulatory functions for specific yeast proteins

Actin regulatory activity	Yeast protein	Endocytic phenotype of deletion	Mammalian orthologue	Endocytic phenotype	References
Regulation of Arp2/3 nucleated F-actin assembly	Las17p	+	N-WASP	+	(Benesch et al., 2005; Innocenti et al., 2005; Merrifield, 2004; Naqvi et al., 1998)
	Pan1p	+[m]	Eps15	+[na]	
	Abp1p	+/–	mAbp1p	+[na]	(Kaksonen et al., 2003; Kessels et al., 2001)
	Myo3/5p	+	Myosin 1	n.d	(Geli and Riezman, 1996)
	Bzz1p	–	Syndapin	+	(Kessels and Qualmann, 2002; Soulard et al., 2002)
			Cortactin	+	(Merrifield et al., 2005; Sauvonnet et al., 2005; Zhu et al., 2005)
			Intersectin	+	(McGavin et al., 2001)
Regulators of WASP	Sla1p	+/–	CD2AP	+	(Badour et al., 2003; Warren et al., 2002) (Mochida et al., 2002)
	Bbc1p	–	?		
	Vrp1	+	WIP/WIRE	+	(Aspenstrom, 2004; Naqvi et al., 1998)
	coronin	–	coronin	n.d.[ph]	(de Hostos, 1999; Goode et al., 1999)
F-actin capping	cap1/cap2	+/–	CapZ	n.d	(Kaksonen et al., 2005)
Bundling and stabilising	Sac6p	+	Fimbrin	n.d.	(Kübler and Riezman, 1993)
	Scp1p	–	Transgelin	n.d.	(Winder et al., 2003)
F-actin depolymerising	Cof1p	+[m]	Cofilin	+	(Lappalainen and Drubin, 1997; Nishimura et al., 2006)
Scaffold between F-actin and vesicle	Sla2p	+	HIP1R	+	(Engqvist-Goldstein et al., 2004; Gourlay et al., 2003)
	Abp1p	–	mAbp1	+	(Connert et al., 2006; Han et al., 2005; Kessels et al., 2001; Wesp et al., 1997)
Scaffold between actin regulatory protein and vesicle	Sla1p	+/–[c]	CD2AP/CIN85	+/–[c]	(Badour et al., 2003; Bruck et al., 2006; Gourlay et al., 2003)
	Bzz1p	–	Syndapin	+	(Kessels and Qualmann, 2002; Otsuki et al., 2003; Soulard et al., 2002) (Innocenti et al., 2005)
			Abi1	+	(Buss et al., 2002)
			Myosin VI	+	(Kovacs et al., 2006; Salazar et al., 2003)
			Tuba	+	
Phosphoregulation of actin structures	Ark1/Prk1p	+[ΔΔ]	AAK1/GAK	+[na]	(Conner and Schmid, 2002; Cope et al., 1999; Jackson et al., 2003; Olusanya et al., 2001; Ricotta et al., 2002; Zeng et al., 2001; Zhang et al., 2005)

Known orthologues of these proteins are given and reported effects in endocytosis noted. Proteins are mentioned more than once if multiple functions have been ascribed to them.

+, marked effect on endocytosis; +/–, weak or subtle effect on endocytosis; –, no detected effect on endocytosis; n.d., effect on endocytosis not determined; [m], essential genes, but mutants have strong endocytic phenotypes; [ph], protein is involved in phagocytosis; [c], cargo-specific effects; [ΔΔ], strong effect when deleted in combination; [na], effect not directly linked to actin or not directly tested.

patches to grow larger have been generated and there is an associated defect in endocytosis (Lappalainen and Drubin, 1997). The importance of a dynamic actin cytoskeleton rather than the presence of F-actin alone is also illustrated by experiments involving addition of the drug jasplakinolide, which stabilises F-actin in cells; this completely abrogates endocytosis in yeast (Ayscough, 2000)

Mutations in at least 20 proteins are known to affect the function of actin during this process, and the presence of a recognised level of redundancy among the proteins involved means that this number is likely to be higher. In addition to proteins that directly bind to and affect actin dynamics, there is a further level of control exerted over the actin-binding proteins themselves. One of the best characterised of these mechanisms is phosphoregulation. The key kinases that have been studied are Ark1p and Prk1p, which are related to the mammalian kinases AAK1 and GAK (Smythe and Ayscough, 2003). In yeast, these phosphorylate several components of the endocytic coat, including Pan1p, Ent1p and Sla1p (Ayscough, 2005; Huang et al., 2003; Watson et al., 2001; Zeng et al., 2001). Phosphorylation of Pan1p has been shown to inhibit its ability to activate Arp2/3-dependent actin polymerisation (Duncan et al., 2001; Toshima et al., 2005), whereas phosphorylation of Sla1p is required for its release from the coat (Zeng et al., 2001). If Sla1p is not removed from the coat, actin turnover becomes reduced and endocytosis is inhibited. The kinases are recruited to the sites of endocytosis by another actin-binding protein, Abp1p (Fazi et al., 2002).

Actin and vesicle scission

Invagination is followed by vesicle scission, and then movement of the vesicle away from the membrane. The role of actin in these stages is less well characterised. The vesicle scission module contains the two yeast amphiphysin proteins Rvs161p and Rvs167p. These proteins contain BAR (Bin-Amphiphysin-Rvs) domains that bind to and tubulate

membranes in vitro (Peter et al., 2004). Tubulation is then suggested to facilitate the scission process. The yeast amphiphysins have been shown to bind to a number of known endocytic proteins, including Las17p, Sla1p and actin itself (Lombardi and Riezman, 2001; Friesen et al., 2003; Madania et al., 1999; Stamenova et al., 2004). The proteins arrive after actin, remain immobile for a few seconds, then show transient movement into the cell. In *rvs161* or *rvs167* deletion mutants, cells show frequent retraction of the forming vesicle after an initial internalisation movement, which may suggest failed endocytosis.

It is not currently clear whether actin plays a direct role in scission. However, actin and associated proteins recruit amphiphysins and thus mark the site at which membrane tubulation should occur. It should also be noted that endocytosis can still occur in the absence of the amphiphysins, which suggests that other mechanisms for scission exist. One possibility is that other proteins, such as dynamin, are involved. Dynamin is a GTPase that plays a key role in the endocytic scission process in mammalian cells (Song and Schmid, 2003). Although dynamin relatives are present in yeast, there is no experimental support for a role for this family of proteins in endocytosis (Engqvist-Goldstein and Drubin, 2003). Alternatively, in the absence of amphiphysins, continued inward movement of the endocytic pit mediated by interactions between the coat and actin bundles could lead to neck elongation and eventually to pinching off. Very elongated vesicle necks are seen in a number of strains that possess mutations in genes encoding actin regulatory proteins (Jonsdottir and Li, 2004; Wendland et al., 1996), indicating the possible importance of actin at this scission step.

Actin and vesicle movement

Following scission, the vesicle is uncoated and moves away from the membrane until it fuses with an endosome. There are two possible roles for actin at this final stage: vesicles could move along actin cables; alternatively actin could be nucleated at the vesicle surface to facilitate their movement within the cell. Evidence for the former idea includes the demonstration that endocytic vesicles containing the lipophilic dye FM4-64 undergo linear movement along actin cables (Huckaba et al., 2004). Furthermore, a recent study of receptor-mediated uptake and trafficking of fluorescently labelled alpha-factor derivatives revealed that endocytic vesicles and endosomes move towards each other along the actin cables (Toshima et al., 2006). Genetic studies revealing links between proteins involved in formation of actin cables (e.g. formins) and endocytic proteins such as Sla2p lend further support to the idea that actin cables act as tracks to facilitate the endocytic process (Yoshiuchi et al., 2006). Clearly, we must now identify the proteins that link vesicles to these actin cables.

Currently, there is no clear evidence that actin nucleation drives vesicle movement. Such a process would be analagous to that used by *Listeria* where actin is polymerised at the bacterial surface and can be visualised as ‘comet-tails’ within cells as the *Listeria* move. The presence of Abp1p and Pan1p on the newly formed vesicles, which are both activators of Arp2/3-dependent actin polymerisation (Duncan et al., 2001; Goode et al., 2001) might, however, mean that although the majority of the F-actin polymerisation drives the initial invagination process, a lower level of filament formation facilitates movement of vesicles away from the membrane.

Actin and endocytosis in mammalian cells

Until recently, the role(s) of actin in clathrin-mediated endocytosis in mammalian cells was difficult to define, although there were intriguing observations connecting actin and its binding partners to components of the endocytic machinery (Qualmann and Kessels, 2002). For example, in neuronal cells, actin filaments were visualised in close proximity to sites of endocytosis at the synapse (Shupliakov et al., 2002). However, treatment of cells with drugs that interfere with the actin cytoskeleton resulted in variable effects on endocytosis, which suggested at the very least that actin does not have a mandatory role in clathrin-mediated endocytosis and simply facilitates the process, depending on the context (Fujimoto et al., 2000). Recent studies, largely based on live-cell imaging, now provide more compelling evidence for a role for actin in endocytosis and progress has been made to elucidate the mechanistic basis of its involvement.

Association of actin with mammalian clathrin-coated vesicles

A major step forward definitively linking actin polymerisation to clathrin-coated vesicle formation came from the live-cell imaging studies of Merrifield and colleagues (Merrifield et al., 2002; Merrifield et al., 2005). These studies combined epifluorescence with total internal reflection microscopy (TIRF) to follow the internalisation of individual coated pits in living cells expressing a fluorescently tagged form of clathrin light chain (DsRed clathrin). The combination of these two approaches allowed the authors to distinguish the plasma-membrane-associated pool of clathrin from the bulk of the clathrin within the cell. They showed that transient recruitment of actin coincides with the inward movement of vesicles (Merrifield et al., 2002). Interestingly, actin is recruited during the internalisation of the majority of coated pits and vesicles, which indicates that its participation in mammalian endocytosis is widespread. Merrifield et al. also observed recruitment of the Arp2/3 complex to clathrin-coated pits, using this technique (Merrifield et al., 2004).

Further advances in imaging have allowed investigators to temporally distinguish components required for invagination from those required for scission. pH-sensitive probes have been developed that allow internalisation of cargo into individual clathrin-coated vesicles to be visualised, allowing us to follow the time course of invagination and identify the point of scission (Merrifield et al., 2005).

Actin regulation in the clathrin-coated-vesicle cycle

Treatment of cells with latrunculin A has shown that actin acts at multiple steps in the mammalian endocytic pathway. In living cells, the dynamic movement of clathrin spots and patches is inhibited in the presence of the drug (Merrifield et al., 2002; Yarar et al., 2005), and stage-specific assays in permeabilised cells indicate that invagination and scission are both affected by its sequestration of monomeric actin (Yarar et al., 2005).

The recruitment of actin during invagination is thought to provide the force that drives the invagination of the coated pit (Engqvist-Goldstein and Drubin, 2003; Merrifield, 2004; Yarar

et al., 2005). In contrast to the situation in yeast, the large GTPase dynamin appears to play a key role integrating actin dynamics with endocytosis, interacting with numerous endocytic and actin-binding proteins (Schafer, 2004). Whereas dynamin has a well-established role in the scission of clathrin-coated vesicles (Song and Schmid, 2003), live cell imaging experiments have indicated that dynamin is recruited during invagination (Ehrlich et al., 2004; Merrifield et al., 2002), which was predicted on the basis of biochemical experiments (Gad et al., 2000; Hill et al., 2001). This places dynamin in the appropriate spatial and temporal position during the coated-vesicle cycle to recruit actin-binding proteins to the invaginating pit.

As in yeast, activators of the Arp2/3 complex are recruited to coated pits in mammalian cells through interactions with coat components. A likely candidate to initiate actin polymerisation at this early stage is N-WASP. This has been shown to be recruited to coated pits in living cells (Merrifield et al., 2002) and knocking it down by RNAi reduces the rate of epidermal growth factor receptor (EGFR) uptake (Benesch et al., 2005; Innocenti et al., 2005). N-WASP could potentially be recruited by a variety of SH3-domain-containing proteins – cortactin, endophilin, syndapin and intersectin – that enhance its ability to activate Arp2/3 and that also bind to dynamin. Syndapin and endophilin have also been implicated in recruitment of N-WASP during coated-vesicle formation (Kessels and Qualmann, 2002; Otsuki et al., 2003), whereas intersectin has been implicated in the N-WASP-mediated uptake of the T-cell antigen receptor in T cells (McGavin et al., 2001). Another Arp2/3 activator, cortactin, is also recruited to coated pits, attaining maximal levels at scission (Merrifield et al., 2005). Although cortactin is recruited during coated-pit invagination, experimental evidence suggests that it is unlikely to act in the initial recruitment of actin in the clathrin-coated vesicle cycle, because it needs to bind F-actin in order to effectively bind and activate the Arp2/3 complex. Cortactin thus probably has a role in the dynamic turnover of actin filaments during clathrin-coated vesicle formation (Zhu et al., 2005).

Mammalian Abp1p (mAbp1), like its yeast orthologue Abp1, provides another important link between the endocytic pathway and the actin cytoskeleton. Early studies showed that mAbp1 binds to dynamin through its SH3 domain and that the two proteins interact in vivo. Overexpression of the SH3 domain of mAbp1 has a dominant-negative effect on endocytosis (Kessels et al., 2001). Furthermore, studies in mAbp1-knockout mice reveal defects in the immune response as a result of impaired T-cell endocytosis (Han et al., 2005). Mice lacking mAbp1 also suffer from behavioural abnormalities and exhibit reduced rates of endocytosis and synaptic vesicle recycling. Interestingly, analysis of these cells shows that the absence of mAbp1 affects events following scission of coated vesicles, specifically causing an increase in the number of endosomal intermediates and a significant reduction in the rate of synaptic vesicle repriming (Connert et al., 2006).

Another family recently implicated in the dynamic interplay between the endocytic pathway and the actin cytoskeleton is the PCH/F-BAR protein family (Itoh et al., 2005; Tsujita et al., 2006). The F-BAR domain of these proteins allows them to bind to phosphoinositides and to bend and tubulate membranes in liposome-based assays. Several family members can bind dynamin and co-localise with dynamin and actin at the cell surface. Overexpression of the F-BAR proteins causes tubules to form from the plasma membrane, an effect accentuated by treatment of cells with latrunculin A. Co-overexpression of wild-type but not mutant dynamin inhibits tubulation, leading to the suggestion that both dynamin and the rigidity of the actin cytoskeleton limit the ability of these proteins to tubulate membranes and therefore co-operate to regulate the degree of invagination of clathrin-coated pits or other early endocytic intermediates. Syndapin I is also a member of this family. Instead of acting downstream of dynamin in the endocytic process, it might therefore use its F-BAR domain to sense membrane curvature and generate invagination; its activity could subsequently be curtailed by dynamin binding (Anggono et al., 2006). This hypothesis is partially based on the fact that syndapin I only has one SH3 domain. However, recent studies have shown that syndapin I dimerises in vivo, which would allow it to bind both dynamin and N-WASP simultaneously (Kessels and Qualmann, 2006).

Orthologues of the yeast proteins that cap, bundle and stabilise actin all exist in mammalian cells (Table 1) but their relevance in endocytosis mostly remains to be explored. Coronin regulates phagosome formation in mammalian cells, apparently by recruitment of Arp2/3 to sites of actin remodelling (Yan et al., 2005), and in the filamentous fungi *Dictyostelium* (de Hostos, 1999). Similarly fimbrin has been observed at the phagocytic cup and in macropinosomes in *Dictyostelium* but, because of apparent functional redundancy, it has not yet been possible to establish its precise role (Pikzack et al., 2005). Cofilin is also required for endocytosis in mammalian cells (Nishimura et al., 2006) but further studies will be required to define where and how it acts.

Recent studies have identified a conserved motif that mediates the interaction of members of the CIN85/CD2AP protein family with capping protein and shown that CD2AP inhibits the activity of capping protein in vitro (Bruck et al., 2006). CIN85 and CD2AP are probably the mammalian orthologues of Sla1p, although their primary roles may be cell-type specific – they appear to be required for specific cellular events (Dikic, 2002). For example, CIN85 acts as an adaptor/scaffolding molecule to recruit signalling molecules following the activation of receptor tyrosine kinases in HEK293T cells (Haglund et al., 2002) and CD2AP has very specific roles in the regulation of T-cell activation (Shaw, 2005) and kidney glomerular function (Wolf and Stahl, 2003). Both interact with components of the endocytic machinery and actin cytoskeleton – CIN85 interacts with endophilin, synaptojanin and Huntingtin-interacting protein 1 (HIP1) and Hip1-related protein (HIP1R) (Kowanetz et al., 2004); CD2AP interacts with Rab4, AP2, cortactin and actin (Cormont et al., 2003) – and disruption of this leads to reduced downregulation of the T-cell receptor in vivo (Hutchings et al., 2003). It will be interesting to investigate whether targeted disruption of the conserved motif linking this protein family to capping protein affects endocytosis in vivo.

HIP1R, the mammalian orthologue of Sla2p, provides a further link between endocytic vesicles and the actin cytoskeleton. HIP1R binds to clathrin light chains and actin and thus could bridge endocytic vesicles to the actin cytoskeleton (Chen and Brodsky, 2005; Legendre-Guillemin et

al., 2005). Indeed, cells treated with siRNA to knock down HIP1R, and fibroblasts from mice lacking the related HIP1 protein, have reduced rates of coated-vesicle formation (Engqvist-Goldstein et al., 2004). Interestingly, however, in the absence of HIP1R, actin, dynamin and cortactin accumulate at sites of endocytosis, which suggests that HIP1R also modulates actin turnover at endocytic sites (Engqvist-Goldstein et al., 2004).

Actin as a facilitator of endocytosis

Although drugs such as latrunculin A can inhibit endocytosis in mammalian cells, they tend to reduce the rate of internalisation rather than blocking it completely. Indeed, endocytosis in some cell types is apparently unaffected by these treatments (Fujimoto et al., 2000). This strongly suggests that, in contrast to yeast, actin has a facilitatory rather than an essential role in endocytosis in mammalian cells. Actin could, however, become essential, depending on the context in which the cell finds itself. Subcellular localisation of signalling molecules frequently determines their output (Miaczynska et al., 2004). Furthermore, recent studies have suggested that there are subpopulations of clathrin-coated pits that select cargo (e.g. signalling molecules) destined to be degraded rapidly by the cell for delivery to a motile population of endosomes (Lakadamyali et al., 2006). A number of endocytic molecules act as 'alternative' cargo adaptors that, either independently or in concert with the classical AP2 adaptor protein complex, select specific cargo for incorporation into clathrin-coated pits (Traub, 2003). Both HIP1R and HIP1 share characteristics of alternative adaptors in that they bind clathrin and phosphoinositides through their ANTH domains (Brett et al., 2006) and HIP1 may act as a cargo receptor for the AMPA receptor (Metzler et al., 2003). Cargo adaptors could thus determine the rate at which particular cargo traverses the endocytic pathway by modulation of actin dynamics.

Flux through the endocytic pathway must therefore play a key role in allowing cells to migrate, proliferate or differentiate during development and actin may well be differentially recruited by coated pits. In this context the diversity of proteins that can apparently link endocytic vesicles to the actin cytoskeleton in multicellular organisms may allow cells to respond to different environments by altering the rate at which cargo moves through the endocytic pathway. Certain proteins, such as syndapins and CIN85/CD2AP, may have additional cargo-specific roles in multicellular organisms. Indeed recent studies have indicated that syndapin is the relevant binding partner for dynamin in synaptic vesicle recycling (Anggono et al., 2006).

If the requirement for actin in endocytosis in mammalian cells increases in certain cellular contexts, then those proteins that link endocytic vesicles to the actin cytoskeleton should be subject to tight regulation. For example, the interaction between syndapin I and dynamin is modulated by phosphorylation (Anggono et al., 2006). Somewhat surprisingly, studies have not yet uncovered a major role for the Ark/Prk orthologues in mammalian cells in the connections between actin and endocytosis. AAK1 is likely to enhance recruitment of cargo into coated pits (Conner and Schmid, 2002; Ricotta et al., 2002), and the DnaJ domain rather than the kinase domain of GAK has been implicated in uncoating (Umeda et al., 2000; Ungewickell et al., 1997). However, many of the key proteins involved in the link between endocytosis and the actin cytoskeleton are phosphoproteins and so phosphorylation is probably a major regulatory mechanism.

Conclusions and future perspectives

Although the last 2-3 years have seen dramatic advances in our understanding of the endocytic process and the role of actin, studies will now need to refine the experimental systems to address the contributions of the plethora of non-essential components in both yeast and mammalian cells. The ability to measure more subtle differences in endocytic rates and to define the temporal order of recruitment of endocytic components will allow us to examine apparently redundant protein functions. These studies will be especially powerful when combined with siRNA knockdown and rescue approaches.

Fundamental questions raised by the recent work focus on the differences between constitutive and cargo-mediated endocytosis and the role of lipids in the regulation of actin-binding proteins during the invagination and scission stages of the process. Thus, a key issue is whether individual cargo molecules can directly influence actin dynamics to facilitate their uptake. Scaffolding molecules such as Sla1p in yeast and CIN85 in mammalian cells are good candidates to integrate signals for individual cargoes, and CIN85 has already been implicated in the uptake of specific cargoes including IgE, EGFR and the h-Met receptor (Molfetta et al., 2005; Petrelli et al., 2002; Soubeyran et al., 2002). Interestingly, myosin VI, which has been implicated in endocytosis from the apical membrane of polarised cells (Buss et al., 2002), interacts with the Dab2 'alternative' adaptor, which mediates the specific uptake of the LDL receptor family (Dance et al., 2004; Morris et al., 2002). This suggests a further molecular link between cargo uptake and modulation of actin dynamics. In yeast the recent report that myosin phosphorylation is required for ligand-induced but not for constitutive endocytosis of the pheromone receptor Ste2p (Grosshans et al., 2006) indicates that further levels of regulation are likely to be identified, possibly for many specific endocytic events.

Finally, researching the interplay between lipids and proteins will be vital to our understanding of actin's role in endocytosis. The importance of protein-lipid interactions for endocytosis is clear, and without the appropriate lipid environment, membrane curvature, which is crucial for vesicle formation, cannot occur. However, membrane lipids also regulate the activity of several actin-binding proteins, including capping protein (Amatruda and Cooper, 1992). In addition, the Arf family of small GTPases integrate signals to coordinately regulate membrane traffic and actin dynamics through responses to PtdIns(4,5)P_2 levels in the membrane. Mammalian Arf6 functions at the plasma membrane to regulate both clathrin- and non-clathrin-mediated uptake whereas Arf1 coordinates trafficking through the trans-Golgi network, using many of the same molecular players (e.g. N-WASP and cortactin) (D'Souza-Schorey and Chavrier, 2006). Yeast Arf3p (the orthologue of Arf6) has also been implicated in the endocytic process (Costa et al., 2005), as have the synaptojanins, which deplete PIP2 levels possibly to facilitate the final stages of invagination and scission (Cremona et al., 1999; Bottcher et al., 2006; Stefan et al., 2005).

The advances in our mechanistic knowledge of endocytosis have come about from the use of multi-disciplinary approaches

using both yeast and mammalian cells. These studies have now given us the robust foundation from which we can progress to investigate both fundamental and more specific questions concerning this essential and ubiquitous eukaryotic process.

We thank Ewald Hettema and Karen Smillie for critical reading of this manuscript and Iwona Smaczynsla-de Rooij for the Abp1p images used. K.R.A. is supported by a Medical Research Council (MRC) Senior Research Fellowship (G117/394). E.S. is supported by grants from the MRC (program grant: G0300452) and BBSRC (BBS/B/04072).

References

Adams, A. E. M. and Pringle, J. R. (1984). Relationship to actin and tubulin distribution to bud growth in wild type and morphogenetic mutant *Saccharomyces cerevisiae*. *J. Cell Biol.* **98**, 934-945.

Amatruda, J. F. and Cooper, J. A. (1992). Purification, characterization, and immunofluorescence localization of Saccharomyces-cerevisiae capping protein. *J. Cell Biol.* **117**, 1067-1076.

Anggono, V., Smillie, K. J., Graham, M. E., Valova, V. A., Cousin, M. A. and Robinson, P. J. (2006). Syndapin I is the phosphorylation-regulated dynamin I partner in synaptic vesicle endocytosis. *Nat. Neurosci.* **9**, 752-760.

Aspenstrom, P. (2004). The mammalian verprolin homologue WIRE participates in receptor-mediated endocytosis and regulation of the actin filament system by distinct mechanisms. *Exp. Cell Res.* **298**, 485-498.

Ayscough, K. R. (2000). Endocytosis and the development of cell polarity in yeast require a dynamic F-actin cytoskeleton. *Curr. Biol.* **10**, 1587-1590.

Ayscough, K. R. (2005). Coupling actin dynamics to the endocytic process in Saccharomyces cerevisiae. *Protoplasma* **226**, 81-88.

Ayscough, K. R., Stryker, J., Pokala, N., Sanders, M., Crews, P. and Drubin, D. G. (1997). High rates of actin filament turnover in budding yeast and roles for actin in establishment and maintenance of cell polarity revealed using the actin inhibitor latrunculin-A. *J. Cell Biol.* **137**, 399-416.

Badour, K., Zhang, J. Y., Shi, F., McGavin, M. K. H., Rampersad, V., Hardy, L. A., Field, D. and Siminovitch, K. A. (2003). The Wiskott-Aldrich syndrome protein acts downstream of CD2 and the CD2AP and PSTPIP1 adaptors to promote formation of the immunological synapse. *Immunity* **18**, 141-154.

Benesch, S., Polo, S., Lai, F. P., Anderson, K. I., Stradal, T. E., Wehland, J. and Rottner, K. (2005). N-WASP deficiency impairs EGF internalization and actin assembly at clathrin-coated pits. *J. Cell Sci.* **118**, 3103-3115.

Bottcher, C., Wicky, S., Schwarz, H. and Singer-Kruger, B. (2006). Sjl2p is specifically involved in early steps of endocytosis intimately linked to actin dynamics via the Ark1p/Prk1p kinases. *FEBS Lett.* **580**, 633-641.

Brett, T. J., Legendre-Guillemin, V., McPherson, P. S. and Fremont, D. H. (2006). Structural definition of the F-actin-binding THATCH domain from HIP1R. *Nat. Struct. Mol. Biol.* **13**, 121-130.

Bruck, S., Huber, T. B., Ingham, R. J., Kim, K., Niederstrasser, H., Allen, P. M., Pawson, T., Cooper, J. A. and Shaw, A. S. (2006). Identification of a novel inhibitory actin capping protein binding motif in CD2 associated protein. *J. Biol. Chem.* **281**, 19196-19203.

Buss, F., Luzio, J. P. and Kendrick-Jones, J. (2002). Myosin VI, an actin motor for membrane traffic and cell migration. *Traffic* **3**, 851-858.

Chen, C. Y. and Brodsky, F. M. (2005). Huntingtin-interacting protein 1 (Hip1) and Hip1-related protein (Hip1R) bind the conserved sequence of clathrin light chains and thereby influence clathrin assembly in vitro and actin distribution in vivo. *J. Biol. Chem.* **280**, 6109-6117.

Conner, S. D. and Schmid, S. L. (2002). Identification of an adaptor-associated kinase, AAK1, as a regulator of clathrin-mediated endocytosis. *J. Cell Biol.* **156**, 921-929.

Conner, S. D. and Schmid, S. L. (2003). Regulated portals of entry into the cell. *Nature* **422**, 37-44.

Connert, S., Wienand, S., Thiel, C., Krikunova, M., Glyvuk, N., Tsytsyura, Y., Hilfiker-Kleiner, D., Bartsch, J. W., Klingauf, J. and Wienands, J. (2006). SH3P7/mAbp1 deficiency leads to tissue and behavioral abnormalities and impaired vesicle transport. *EMBO J.* **25**, 1611-1622.

Cope, M., Yang, S., Shang, C. and Drubin, D. G. (1999). Novel protein kinases Ark1p and Prk1p associate with and regulate the cortical actin cytoskeleton in budding yeast. *J. Cell Biol.* **144**, 1203-1218.

Cormont, M., Meton, I., Mari, M., Monzo, P., Keslair, F., Gaskin, C., McGraw, T. E. and Le Marchand-Brustel, Y. (2003). CD2AP/CMS regulates endosome morphology and traffic to the degradative pathway through its interaction with Rab4 and c-Cbl. *Traffic* **4**, 97-112.

Costa, R., Warren, D. T. and Ayscough, K. R. (2005). Lsb5p interacts with actin regulators Sla1p and Las17p, ubiquitin and Arf3p to couple actin dynamics to membrane trafficking processes. *Biochem. J.* **387**, 649-658.

Cremona, O., Di Paolo, G., Wenk, M. R., Luthi, A., Kim, W. T., Takei, K., Daniell, L., Nemoto, Y., Shears, S. B., Flavell, R. A. et al. (1999). Essential role of phosphoinositide metabolism in synaptic vesicle recycling. *Cell*. **99**, 179-188

D'Souza-Schorey, C. and Chavrier, P. (2006). ARF proteins: roles in membrane traffic and beyond. *Nat. Rev. Mol. Cell Biol.* **7**, 347-358.

Dance, A. L., Miller, M., Seragaki, S., Aryal, P., White, B., Aschenbrenner, L. and Hasson, T. (2004). Regulation of myosin-VI targeting to endocytic compartments. *Traffic* **5**, 798-813.

de Hostos, E. L. (1999). The coronin family of actin-associated proteins. *Trends Cell Biol.* **9**, 345-350.

Dikic, I. (2002). CIN85/CMS family of adaptor molecules. *FEBS Lett.* **529**, 110-115.

Doyle, T. and Botstein, D. (1996). Movement of yeast cortical actin cytoskeleton visualized *in vivo*. *Proc. Natl. Acad. Sci. USA* **93**, 3886-3891.

Duncan, M. C., Cope, M., Goode, B. L., Wendland, B. and Drubin, D. G. (2001). Yeast Eps15-like endocytic protein, Pan1p, activates the Arp2/3 complex. *Nat. Cell Biol.* **3**, 687-690.

Ehrlich, M., Boll, W., Van Oijen, A., Hariharan, R., Chandran, K., Nibert, M. L. and Kirchhausen, T. (2004). Endocytosis by random initiation and stabilization of clathrin-coated pits. *Cell* **118**, 591-605.

Engqvist-Goldstein, A. E. and Drubin, D. G. (2003). Actin assembly and endocytosis: from yeast to mammals. *Annu. Rev. Cell Dev. Biol.* **19**, 287-332.

Engqvist-Goldstein, A. E., Zhang, C. X., Carreno, S., Barroso, C., Heuser, J. E. and 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.

Fazi, B., Cope, M., Douangamath, A., Ferracuti, S., Schirwitz, K., Zucconi, A., Drubin, D. G., Wilmanns, M., Cesareni, G. and Castagnoli, L. (2002). Unusual binding properties of the SH3 domain of the yeast actin-binding protein Abp1 – Structural and functional analysis. *J. Biol. Chem.* **277**, 5290-5298.

Friesen, H., Murphy, K., Breitkreutz, A., Tyers, M. and Andrews, B. (2003). Regulation of the yeast amphiphysin homologue Rvs167p by phosphorylation. *Mol. Biol. Cell* **14**, 3027-3040.

Fujimoto, L. M., Roth, R., Heuser, J. E. and Schmid, S. L. (2000). Actin assembly plays a variable, but not obligatory role in receptor-mediated endocytosis in mammalian cells. *Traffic* **1**, 161-171.

Gad, H., Ringstad, N., Low, P., Kjaerulff, O., Gustafsson, J., Wenk, M., Di Paolo, G., Nemoto, Y., Crun, J., Ellisman, M. H. et al. (2000). Fission and uncoating of synaptic clathrin-coated vesicles are perturbed by disruption of interactions with the SH3 domain of endophilin. *Neuron* **27**, 301-312.

Geli, M. I. and Riezman, H. (1996). Role of type I myosins in receptor-mediated endocytosis in yeast. *Science* **272**, 533-535.

Goode, B. L., Wong, J. J., Butty, A. C., Peter, M., McCormack, A. L., Yates, J. R., Drubin, D. G. and Barnes, G. (1999). Coronin promotes the rapid assembly and cross-linking of actin filaments and may link the actin and microtubule cytoskeletons in yeast. *J. Cell Biol.* **144**, 83-98.

Goode, B. L., Rodal, A. A., Barnes, G. and Drubin, D. G. (2001). Activation of the Arp2/3 complex by the actin filament binding protein Abp1p. *J. Cell Biol.* **153**, 627-634.

Gourlay, C. W., Dewar, H., Warren, D. T., Costa, R., Satish, N. and Ayscough, K. R. (2003). An interaction between Sla1p and Sla2p plays a role in regulating actin dynamics and endocytosis in budding yeast. *J. Cell Sci.* **116**, 2551-2564.

Grosshans, B. L., Grotsch, H., Mukhopadhyay, D., Fernandez, I. M., Pfannstiel, J., Idrissi, F. Z., Lechner, J., Riezman, H. and Geli, M. I. (2006). TEDS site phosphorylation of the yeast myosins I is required for ligand-induced but not for constitutive endocytosis of the G protein-coupled receptor Ste2p. *J. Biol. Chem.* **281**, 11104-11114.

Haglund, K., Shimokawa, N., Szymkiewicz, I. and Dikic, I. (2002). Cbl-directed monoubiquitination of CIN85 is involved in regulation of ligand-induced degradation of EGF receptors. *Mol. Biol. Cell* **99**, 12191-12196.

Han, J., Shui, J. W., Zhang, X., Zheng, B., Han, S. and Tan, T. H. (2005). HIP-55 is important for T-cell proliferation, cytokine production, and immune responses. *Mol. Cell. Biol.* **25**, 6869-6878.

Hill, E., van Der Kaay, J., Downes, C. P. and Smythe, E. (2001). The role of dynamin and its binding partners in coated pit invagination and scission. *J. Cell Biol.* **152**, 309-323.

Huang, B., Zeng, G. S., Ng, A. Y. J. and Cai, M. J. (2003). Identification of novel recognition motifs and regulatory targets for the yeast actin-regulating kinase Prk1p. *Mol. Biol. Cell* **14**, 4871-4884.

Huckaba, T. M., Gay, A. C., Pantalena, L. F., Yang, H. C. and Pon, L. A. (2004). 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**, 519-530.

Humphries, C. L., Balcer, H. I., D'Agostino, J. L., Winsor, B., Drubin, D. G., Barnes, G., Andrews, B. J. and Goode, B. L. (2002). Direct regulation of Arp2/3 complex activity and function by the actin binding protein coronin. *J. Cell Biol.* **159**, 993-1004.

Hutchings, N. J., Clarkson, N., Chalkley, R., Barclay, A. N. and Brown, M. H. (2003). Linking the T cell surface protein CD2 to the actin-capping protein CAPZ via CMS and CIN85. *J. Biol. Chem.* **278**, 22396-22403.

Iida, K., Moriyama, K., Matsumoto, S., Kawasaki, H., Nishida, E. and Yahara, I. (1993). Isolation of a yeast essential gene, Cof1, that encodes a homolog of mammalian cofilin, a low-Mr actin-binding and depolymerizing protein. *Gene* **124**, 115-120.

Innocenti, M., Gerboth, S., Rottner, K., Lai, F. P., Hertzog, M., Stradal, T. E., Frittoli, E., Didry, D., Polo, S., Disanza, A. et al. (2005). Abi1 regulates the activity of N-WASP and WAVE in distinct actin-based processes. *Nat. Cell Biol.* **7**, 969-976.

Itoh, T., Erdmann, K. S., Roux, A., Habermann, B., Werner, H. and De Camilli, P. (2005). Dynamin and the actin cytoskeleton cooperatively regulate plasma membrane invagination by BAR and F-BAR proteins. *Dev. Cell* **9**, 791-804.

Jackson, A. P., Flett, A., Smythe, C., Hufton, L., Wettey, F. R. and Smythe, E. (2003).

Clathrin promotes incorporation of cargo into coated pits by activation of the AP2 adaptor micro2 kinase. *J. Cell Biol.* **163**, 231-236.
Jonsdottir, G. A. and Li, R. (2004). Dynamics of yeast Myosin I: evidence for a possible role in scission of endocytic vesicles. *Curr. Biol.* **14**, 1604-1609.
Kaksonen, M., Sun, Y. and Drubin, D. G. (2003). A pathway for association of receptors, adaptors, and actin during endocytic internalization. *Cell* **115**, 475-487.
Kaksonen, M., Toret, C. P. and Drubin, D. G. (2005). A modular design for the clathrin- and actin- mediated endocytosis machinery. *Cell* **123**, 305-320.
Kessels, M. M. and Qualmann, B. (2002). Syndapins integrate N-WASP in receptor-mediated endocytosis. *EMBO J.* **21**, 6083-6094.
Kessels, M. M. and Qualmann, B. (2004). The syndapin protein family: linking membrane trafficking with the cytoskeleton. *J. Cell Sci.* **117**, 3077-3086.
Kessels, M. M., Engqvist-Goldstein, A. E., Drubin, D. G. and 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.
Kim, K., Yamashita, A., Wear, M. A., Maeda, Y. and Cooper, J. A. (2004). Capping protein binding to actin in yeast: biochemical mechanism and physiological relevance. *J. Cell Biol.* **164**, 567-580.
Kim, K., Galletta, B. J., Schmidt, K. O., Chang, F. S., Blumer, K. J. and Cooper, J. A. (2006). Actin-based motility during endocytosis in budding yeast. *Mol. Biol. Cell* **17**, 1354-1363.
Kovacs, E. M., Makar, R. S. and Gertler, F. B. (2006). Tuba stimulates intracellular N-WASP-dependent actin assembly. *J. Cell Sci.* **119**, 2715-2726.
Kowanetz, K., Husnjak, K., Holler, D., Kowanetz, M., Soubeyran, P., Hirsch, D., Schmidt, M. H., Pavelic, K., De Camilli, P., Randazzo, P. A. et al. (2004). CIN85 associates with multiple effectors controlling intracellular trafficking of epidermal growth factor receptors. *Mol. Biol. Cell* **15**, 3155-3166.
Kübler, E. and Riezman, H. (1993). Actin and fimbrin are required for the internalization step of endocytosis in yeast. *EMBO J.* **12**, 2855-2862.
Lakadamyali, M., Rust, M. J. and Zhuang, X. (2006). Ligands for clathrin-mediated endocytosis are differentially sorted into distinct populations of early endosomes. *Cell* **124**, 997-1009.
Lappalainen, P. and Drubin, D. G. (1997). Cofilin promotes rapid actin filament turnover in vivo. *Nature* **388**, 78-82.
Legendre-Guillemin, V., Metzler, M., Lemaire, J. F., Philie, J., Gan, L., Hayden, M. R. and McPherson, P. S. (2005). Huntingtin interacting protein 1 (HIP1) regulates clathrin assembly through direct binding to the regulatory region of the clathrin light chain. *J. Biol. Chem.* **280**, 6101-6108.
Lombardi, R. and Riezman, H. (2001). Rvs161p and Rvs167p, the two yeast amphiphysin homologs, function together in vivo. *J. Biol. Chem.* **276**, 6016-6022.
Madania, A., Dumoulin, P., Grava, S., Kitamoto, H., Scharer-Brodbeck, C., Soulard, A., Moreau, V. and Winsor, B. (1999). The Saccharomyces cerevisiae homologue of human Wiskott-Aldrich syndrome protein Las17p interacts with the Arp2/3 complex. *Mol. Biol. Cell* **10**, 3521-3538.
McGavin, M. K., Badour, K., Hardy, L. A., Kubiseski, T. J., Zhang, J. and Siminovitch, K. A. (2001). The intersectin 2 adaptor links Wiskott Aldrich Syndrome protein (WASp)-mediated actin polymerization to T cell antigen receptor endocytosis. *J. Exp. Med.* **194**, 1777-1787.
Merrifield, C. J. (2004). Seeing is believing: imaging actin dynamics at single sites of endocytosis. *Trends Cell Biol.* **14**, 352-358.
Merrifield, C. J., Feldman, M. E., Wan, L. and Almers, W. (2002). Imaging actin and dynamin recruitment during invagination of single clathrin-coated pits. *Nat. Cell Biol.* **4**, 691-698.
Merrifield, C. J., Qualmann, B., Kessels, M. M. and Almers, W. (2004). 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**, 13-18.
Merrifield, C. J., Perrais, D. and Zenisek, D. (2005). Coupling between clathrin-coated-pit invagination, cortactin recruitment, and membrane scission observed in live cells. *Cell* **121**, 593-606.
Metzler, M., Li, B., Gan, L., Georgiou, J., Gutekunst, C. A., Wang, Y., Torre, E., Devon, R. S., Oh, R., Legendre-Guillemin, V. et al. (2003). Disruption of the endocytic protein HIP1 results in neurological deficits and decreased AMPA receptor trafficking. *EMBO J.* **22**, 3254-3266.
Miaczynska, M., Pelkmans, L. and Zerial, M. (2004). Not just a sink: endosomes in control of signal transduction. *Curr. Opin. Cell Biol.* **16**, 400-406.
Mochida, J., Yamamoto, T., Fujimura-Kamada, K. and Tanaka, K. (2002). The novel adaptor protein, Mti1p, and Vrp1p, a homolog of Wiskott-Aldrich syndrome protein-interacting protein (WIP), may antagonistically regulate type I myosins in *Saccharomyces cerevisiae*. *Genetics* **160**, 923-934.
Molfetta, R., Belleudi, F., Peruzzi, G., Morrone, S., Leone, L., Dikic, I., Piccoli, M., Frati, L., Torrisi, M. R., Santoni, A. et al. (2005). CIN85 regulates the ligand-dependent endocytosis of the IgE receptor: a new molecular mechanism to dampen mast cell function. *J. Immunol.* **175**, 4208-4216.
Moon, A. L., Janmey, P. A., Louie, K. A. and Drubin, D. G. (1993). Cofilin is an essential component of the yeast cortical cytoskeleton. *J. Cell Biol.* **120**, 421-435.
Morris, S. M., Arden, S. D., Roberts, R. C., Kendrick-Jones, J., Cooper, J. A., Luzio, J. P. and Buss, F. (2002). Myosin VI binds to and localises with Dab2, potentially linking receptor-mediated endocytosis and the actin cytoskeleton. *Traffic* **3**, 331-341.
Morton, W. M., Ayscough, K. R. and McLaughlin, P. J. (2000). Latrunculin alters the actin-monomer subunit interface to prevent polymerization. *Nat. Cell Biol.* **2**, 376-378.
Naqvi, S. N., Zahn, R., Mitchell, D. A., Stevenson, B. J. and Munn, A. L. (1998). The WASp homologue Las17p functions with the WIP homologue End5p/verprolin and is essential for endocytosis in yeast. *Curr. Biol.* **8**, 959-962.
Newpher, T. M., Smith, R. P., Lemmon, V. and Lemmon, S. K. (2005). In vivo dynamics of clathrin and its adaptor-dependent recruitment to the actin-based endocytic machinery in yeast. *Dev. Cell* **9**, 87-98.
Nishimura, Y., Yoshioka, K., Bernard, O., Bereczky, B. and Itoh, K. (2006). A role of LIM kinase 1/cofilin pathway in regulating endocytic trafficking of EGF receptor in human breast cancer cells. *Histochem. Cell Biol.* doi:10.1007/s00418-006-0198-x.
Olusanya, O., Andrews, P. D., Swedlow, J. R. and Smythe, E. (2001). Phosphorylation of threonine 156 of the mu2 subunit of the AP2 complex is essential for endocytosis in vitro and in vivo. *Curr. Biol.* **11**, 896-900.
Otsuki, M., Itoh, T. and Takenawa, T. (2003). Neural Wiskott-Aldrich syndrome protein is recruited to rafts and associates with endophilin A in response to epidermal growth factor. *J. Biol. Chem.* **278**, 6461-6469.
Peter, B. J., Kent, H. M., Mills, I. G., Vallis, Y., Butler, P. J. G., Evans, P. R. and McMahon, H. T. (2004). BAR domains as sensors of membrane curvature: the amphiphysin BAR structure. *Science* **303**, 495-499.
Petrelli, A., Gilestro, G. F., Lanzardo, S., Comoglio, P. M., Migone, N. and Giordano, S. (2002). The endophilin-CIN85-Cbl complex mediates ligand-dependent downregulation of c-Met. *Nature* **416**, 187-190.
Pikzack, C., Prassler, J., Furukawa, R., Fechheimer, M. and Rivero, F. (2005). Role of calcium-dependent actin-bundling proteins: characterization of Dictyostelium mutants lacking fimbrin and the 34-kilodalton protein. *Cell Motil. Cytoskeleton* **62**, 210-231.
Qualmann, B. and Kessels, M. M. (2002). Endocytosis and the cytoskeleton. *Int. Rev. Cytol.* **220**, 93-144.
Raths, S., Rohrer, J., Crausaz, F. and Riezman, H. (1993). end3 and end4: two mutants defective in receptor-mediated endocytosis in Saccharomyces cerevisiae. *J. Cell Biol.* **120**, 55-65.
Ricotta, D., Conner, S. D., Schmid, S. L., von Figura, K. and Honing, S. (2002). Phosphorylation of the AP2 mu subunit by AAK1 mediates high affinity binding to membrane protein sorting signals. *J. Cell Biol.* **156**, 791-795.
Rodal, A. A., Manning, A. L., Goode, B. L. and Drubin, D. G. (2003). Negative regulation of yeast WASp by two SH3 domain-containing proteins. *Curr. Biol.* **13**, 1000-1008.
Rodal, A. A., Sokolova, O., Robins, D. B., Daugherty, K. M., Hippenmeyer, S., Riezman, H., Grigorieff, N. and Goode, B. L. (2005). Conformational changes in the Arp2/3 complex leading to actin nucleation. *Nat. Struct. Mol. Biol.* **12**, 26-31.
Roth, T. F. and Porter, K. R. (1964). Yolk protein uptake in the oocyte of the mosquito Aedes Aegypti. L. *J. Cell Biol.* **20**, 313-332.
Salazar, M. A., Kwiatkowski, A. V., Pellegrini, L., Cestra, G., Butler, M. H., Rossman, K. L., Serna, D. M., Sondek, J., Gertler, F. B. and De Camilli, P. (2003). Tuba, a novel protein containing bin/amphiphysin/Rvs and Dbl homology domains, links dynamin to regulation of the actin cytoskeleton. *J. Biol. Chem.* **278**, 49031-49043.
Sauvonnet, N., Dujeancourt, A. and Dautry-Varsat, A. (2005). Cortactin and dynamin are required for the clathrin-independent endocytosis of gammac cytokine receptor. *J. Cell Biol.* **168**, 155-163.
Schafer, D. A. (2004). Regulating actin dynamics at membranes: a focus on dynamin. *Traffic* **5**, 463-469.
Shaw, A. S. (2005). T-cell activation and immunologic synapse. *Immunol. Res.* **32**, 247-252.
Shupliakov, O., Bloom, O., Gustafsson, J. S., Kjaerulff, O., Low, P., Tomilin, N., Pieribone, V. A., Greengard, P. and Brodin, L. (2002). Impaired recycling of synaptic vesicles after acute perturbation of the presynaptic actin cytoskeleton. *Proc. Natl. Acad. Sci. USA* **99**, 14476-14481.
Sirotkin, V., Beltzner, C. C., Marchand, J. B. and Pollard, T. D. (2005). Interactions of WASp, myosin-I, and verprolin with Arp2/3 complex during actin patch assembly in fission yeast. *J. Cell Biol.* **170**, 637-648.
Smythe, E. and Ayscough, K. R. (2003). The Ark1/Prk1 family of protein kinases – Regulators of endocytosis and the actin cytoskeleton. *EMBO Rep.* **4**, 246-251.
Song, B. D. and Schmid, S. L. (2003). A molecular motor or a regulator? Dynamin's in a class of its own. *Biochemistry* **42**, 1369-1376.
Soubeyran, P., Kowanetz, K., Szymkiewicz, I., Langdon, W. Y. and Dikic, I. (2002). Cbl-CIN85-endophilin complex mediates ligand-induced downregulation of EGF receptors. *Nature* **416**, 183-187.
Soulard, A., Lechler, T., Spiridonov, V., Shevchenko, A., Shevchenko, A., Li, R. and Winsor, B. (2002). Saccharomyces cerevisiae Bzz1p is implicated with type I myosins in actin patch polarization and is able to recruit actin-polymerizing machinery in vitro. *Mol. Cell. Biol.* **22**, 7889-7906.
Soulard, A., Friant, S., Fitterer, C., Orange, C., Kaneva, G., Mirey, G. and Winsor, B. (2005). The WASP/Las17p-interacting protein Bzz1p functions with Myo5p in an early stage of endocytosis. *Protoplasma* **226**, 89-101.
Stamenova, S., Dunn, R., Adler, A. and Hicke, L. (2004). The Rsp5 ubiquitin ligase binds to and ubiquitinates members of the yeast CIN85-endophilin complex, Sla1-Rvs167. *J. Biol. Chem.* **279**, 16017-16025.
Stefan, C. J., Padilla, S. M., Audhya, A. and Emr, S. D. (2005). The phosphoinositide phosphatase Sjl2 is recruited to cortical actin patches in the control of vesicle formation and fission during endocytosis. *Mol. Cell. Biol.* **25**, 2910-2923.
Sun, Y. D., Martin, A. C. and Drubin, D. G. (2006). Endocytic internalization in budding yeast requires coordinated actin nucleation and myosin motor activity. *Dev. Cell* **11**, 33-46.
Toshima, J., Toshima, J. Y., Martin, A. C. and Drubin, D. G. (2005). Phosphoregulation of Arp2/3-dependent actin assembly during receptor-mediated endocytosis. *Nat. Cell Biol.* **7**, 246-254.
Toshima, J. Y., Toshima, J., Kaksonen, M., Martin, A. C., King, D. S. and Drubin,

D. G. (2006). Spatial dynamics of receptor-mediated endocytic trafficking in budding yeast revealed by using fluorescent alpha-factor derivatives. *Proc. Natl. Acad. Sci. USA* **103**, 5793-5798.
Traub, L. M. (2003). Sorting it out: AP-2 and alternate clathrin adaptors in endocytic cargo selection. *J. Cell Biol.* **163**, 203-208.
Tsujita, K., Suetsugu, S., Sasaki, N., Furutani, M., Oikawa, T. and Takenawa, T. (2006). Coordination between the actin cytoskeleton and membrane deformation by a novel membrane tubulation domain of PCH proteins is involved in endocytosis. *J. Cell Biol.* **172**, 269-279.
Umeda, A., Meyerholz, A. and Ungewickell, E. (2000). Identification of the universal cofactor (auxilin 2) in clathrin coat dissociation. *Eur. J. Cell Biol.* **79**, 336-342.
Ungewickell, E., Ungewickell, H. and Holstein, S. E. (1997). Functional interaction of the auxilin J domain with the nucleotide- and substrate-binding modules of Hsc70. *J. Biol. Chem.* **272**, 19594-19600.
Waddle, J., Karpova, T., Waterson, R. and Cooper, J. (1996). Movement of cortical actin patches in yeast. *J. Cell Biol.* **132**, 861-870.
Warren, D. T., Andrews, P. D., Gourlay, C. G. and Ayscough, K. R. (2002). Sla1p couples the yeast endocytic machinery to proteins regulating actin dynamics. *J. Cell Sci.* **115**, 1703-1715.
Watson, H. A., Cope, M., Groen, A. C., Drubin, D. G. and Wendland, B. (2001). In vivo role for actin-regulating kinases in endocytosis and yeast epsin phosphorylation. *Mol. Cell. Biol.* **12**, 3668-3679.
Wendland, B., McCaffery, J. M., Xiao, Q. and Emr, S. D. (1996). A novel fluorescence-activated cell sorter-based screen for yeast endocytosis mutants identified a yeast homologue of mammalian Eps15. *J. Cell Biol.* **135**, 1485-1500.
Wesp, A., Hicke, L., Palecek, J., Lombardi, R., Aust, T., Munn, A. L. and Riezman, H. (1997). End4p/Sla2p interacts with actin-associated proteins for endocytosis in Saccharomyces cerevisiae. *Mol. Biol. Cell* **8**, 2291-2306.
Winder, S. J. and Ayscough, K. R. (2005). Actin binding proteins. *J. Cell Sci.* **118**, 651-654.
Winder, S. J., Jess, T. and Ayscough, K. R. (2003). SCP1 encodes an actin-bundling protein in yeast. *Biochem. J.* **375**, 287-295.
Wolf, G. and Stahl, R. A. (2003). CD2-associated protein and glomerular disease. *Lancet* **362**, 1746-1748.
Yan, M., Collins, R. F., Grinstein, S. and Trimble, W. S. (2005). Coronin-1 function is required for phagosome formation. *Mol. Biol. Cell* **16**, 3077-3087.
Yarar, D., Waterman-Storer, C. M. and Schmid, S. L. (2005). A dynamic actin cytoskeleton functions at multiple stages of clathrin-mediated endocytosis. *Mol. Biol. Cell* **16**, 964-975.
Yoshiuchi, S., Yamamoto, T., Sakane, H., Kadota, J., Mochida, J., Asaka, M. and Tanaka, K. (2006). Identification of novel mutations in ACT1 and SLA2 that suppress the actin-cable-overproducing phenotype caused by overexpression of a dominant active form of Bni1p in Saccharomyces cerevisiae. *Genetics* **173**, 527-539.
Zeng, G. H., Yu, X. W. and Cai, M. J. (2001). Regulation of yeast actin cytoskeleton-regulatory complex Pan1p/Sla1p/End3p by serine/threonine kinase Prk1p. *Mol. Biol. Cell* **12**, 3759-3772.
Zhang, C. X., Engqvist-Goldstein, A. E., Carreno, S., Owen, D. J., Smythe, E. and Drubin, D. G. (2005). Multiple roles for cyclin G-associated kinase in clathrin-mediated sorting events. *Traffic* **6**, 1103-1113.
Zhu, J., Zhou, K., Hao, J. J., Liu, J., Smith, N. and Zhan, X. (2005). Regulation of cortactin/dynamin interaction by actin polymerization during the fission of clathrin-coated pits. *J. Cell Sci.* **118**, 807-817.