

Review

Endocytic Traffic in Polarized Epithelial Cells: Role of the Actin and Microtubule Cytoskeleton

Gerard Apodaca

Renal-Electrolyte Division of the Department of Medicine,
Laboratory of Epithelial Biology, and Department of Cell
Biology and Physiology, University of Pittsburgh, Pittsburgh,
PA 15261, USA
gla6@pitt.edu

The cytoskeleton is required for multiple cellular events including endocytosis and the transfer of cargo within the endocytic system. Polarized epithelial cells are capable of endocytosis at either of their distinct apical or basolateral plasma membrane domains. Actin plays a role in internalization at both cell surfaces. Microtubules and actin are required for efficient transcytosis and delivery of proteins to late endosomes and lysosomes. Microtubules are also important in apical recycling pathways and, in some polarized cell types, basolateral recycling requires actin. The microtubule motor proteins dynein and kinesin and the class I unconventional myosin motors play a role in many of these trafficking steps. This review examines the endocytic pathways of polarized epithelial cells and focuses on the emerging roles of the actin cytoskeleton in these processes.

Key words: Actin, cytochalasin D, cytoskeleton, endocytosis, endosomes, epithelial cells, lysosomes, MDCK, microtubules, myosins, nocodazole

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The cytoskeleton is vital to the function of all eukaryotic cells and is required for mitosis, cytokinesis, cell motility, muscle contraction, maintenance of cell shape, endocytosis, and secretion (1–3). Epithelial cells, which have distinct apical and basolateral plasma membrane domains, exploit cytoskeletal elements to ensure efficient targeting of newly synthesized proteins from the *trans*-Golgi network to the appropriate cell surface domain (4). The cytoskeleton also plays a role in endocytosis, exit of cargo from early and late endosomes, and the transport, via transcytosis, of endocytosed proteins from one plasma membrane domain to the opposite (5). In addition to its function in protein targeting, the cytoskeleton is also important in protein sorting and can stabilize newly synthesized proteins at one plasma membrane domain or the other (1). The goal of this review is to examine the role of the cytoskeleton in the endocytic traffic of polarized epithelial cells. When appropriate, examples from non-epithelial cells will be discussed. While the function

of the microtubule cytoskeleton in these endocytic processes is well established, it is becoming increasingly clear that the actin cytoskeleton also plays a part. As such, much of this review will focus on the dependence of endocytosis on the actin cytoskeleton.

Endocytic Pathways in Epithelial Cells

Endocytosis is a diverse set of processes used by the cell to internalize specialized regions of the plasma membrane as well as small amounts of extracellular fluid (5). The best-understood form of endocytosis occurs at clathrin-coated pits and involves clathrin, AP-2 adaptor complexes, and the dynamin GTPase (5,6). Other types of endocytosis include that mediated by caveolae (7), small flask-shaped vesicles rich in cholesterol and signaling molecules, and so called clathrin-independent pathways that involve neither clathrin-coated pits nor caveolae (8). Finally, some cell types are capable of internalizing large amounts of fluid via macropinocytosis or large particulates by phagocytosis (5). In simple non-polarized cells, internalized membrane proteins and fluid are first delivered to Rab5 GTPase-positive and early endosome antigen 1 (EEA1)-positive early 'sorting' endosomes. EEA1 is a Rab5 effector protein involved in endosome–endosome tethering and fusion (9). While the vacuolar portion of the early endosome and associated fluid is thought to be delivered to (or mature into) late endosomes and ultimately lysosomes, the membrane-rich tubular portions of the early endosome deliver cargo to the pericentriolar recycling endosome where membranous proteins are recycled back to the cell surface (5). Other pathways connect recycling endosomes and late endosomes to the *trans*-Golgi network (10).

Polarized epithelial cells have added complexity because they can internalize macromolecules from either of their apical or basolateral plasma membrane domains (11). In Madin–Darby canine kidney (MDCK) cells, a model system used by many investigators to analyze membrane/protein traffic in polarized epithelial cells, endocytosis occurs by both clathrin-dependent and -independent mechanisms (12). The postendocytic fate (i.e., the trafficking steps that occur after internalization) of endocytosed markers varies. When fluid-phase markers are internalized from the apical or basolateral poles of these cells, they enter distinct populations of Rab5/EEA1-positive apical early endosomes (AEE; step 1A in Figure 1) or basolateral early endosomes (BEE; step 1B) (13,14). Endotubulin, an apical endosomal marker, is associated with the AEE (15), as is the cytoplasmic adaptor molecule syntenin (16). While the majority of apically internalized fluid is thought to recycle (step 3A) or transcytose (step 4), some is delivered to a

shared population of late endosomes (step 2A) and lysosomes (step 5) that receives the majority of basolaterally internalized fluid (step 2B) (11). Transferrin (Tf) and its receptor have a different fate. Following endocytosis and delivery to BEE (step 1B), the Tf/receptor complex is directed to a tubular supranuclear common recycling endosome (CE) that also receives apically internalized membrane markers (step 3C) (17). The Rab17 GTPase may be associated in part with this compartment (18). It is within the tubular evaginations of the CE that Tf/receptor complexes are packaged in 60-nm vesicles and recycled back to the basolateral cell surface (step 7B) (17). Some Tf/receptor complexes may recycle directly from the BEE (step 6B) (19). A fraction of the Tf/receptor complexes may be delivered from the CE to the AEE (step 7C) (14,20), from where they may recycle back to the basolateral pole of the cell (step 4).

IgA, a ligand for the polymeric immunoglobulin receptor (pIgR), is also internalized from the basolateral pole of the cell. Unlike Tf, endocytosed IgA is delivered to the apical surface of the cell by transcytosis where it is released into the apical secretions. Once internalized, pIgR-IgA complexes are delivered to BEE (step 1B) and then the CE (step 6A) (21,22). IgA-pIgR complexes, sorted from Tf, are subsequently delivered to the rab11-, rab 25-, and rab17-positive elements of the apical recycling endosome (ARE; step 7A) (14,21,23–25). Exit of IgA-pIgR from the ARE may be via C-shaped vesicles (step 8) (26). At the surface, a proteinase cleaves the pIgR, releasing it and bound IgA ligand into secretions. However, some pIgR-IgA complexes escape cleavage and are efficiently internalized and recycle at the apical plasma membrane (27). Apically internalized pIgR-IgA are delivered to the AEE (step 1A), they are sorted from internalized fluid, and then are subsequently delivered to the rab11-positive ARE (step 3B) (14) or to the Tf-rich CE (step 3C) (28), from which recycling occurs. The pathway taken by proteins transcytosing in the apical to basolateral direction is not well understood, but is likely to involve initial passage through the AEE. Readers interested in postendocytic traffic in other epithelial cell types are referred to a review by Van IJzendoorn et al. (29), and those interested in regulation of endocytic traffic are directed to a review by Mostov et al. (30).

Requirements for the Microtubule Cytoskeleton in Endocytic Traffic of Polarized Epithelial Cells

Microtubules are long filamentous elements that are important in organellar localization, as well as transport of cargo between organelles. Movement along microtubules is relatively rapid ($\sim 1 \mu\text{m/s}$) and can occur over long distances. Kinesins (generally involved in movement toward the fast-growing plus-end of microtubules) and dynein (involved in movement toward the slow-growing minus-end of microtubules) are mechanochemical motor proteins required for microtubule based motility (31,32).

Although receptor-mediated endocytosis is not generally affected by microtubule depolymerization (33), there are examples where treatment with microtubule depolymerizing agents (e.g., nocodazole) slows adsorptive and fluid-phase endocytosis (34–36). Transport from early endosomes to late endosomes (steps 2A and 2B) is blocked in nocodazole-treated baby hamster kidney cells (37), and as a result, degradation is impaired in these cells. In polarized MDCK cells, this block is thought to reflect microtubule-, kinesin-, and dynein-dependent transport of cargo between AEE, BEE, and late endosomes (38). Although the kinetics of recycling of Tf/receptor complexes are unaffected by microtubule disruption (our unpublished results and 39), transport of these markers from BEE to CE (step 6A) is apparently impaired (21). In this case, basolateral recycling may occur directly from BEE (step 6B). Transport between AEE and ARE requires microtubules (step 3B) (14), and apical recycling is inhibited by approximately 20–30% in nocodazole-treated cells (40). Apical to basolateral transcytosis of IgG does not require microtubules (41), although apical to basolateral transcytosis of human immunodeficiency virus apparently does (42). Microtubules are also crucial in the basolateral to apical transcytosis of several proteins including the pIgR (14,40,41). Transfer of cargo between BEE and the more apical endocytic compartments (i.e., CE/ARE; step 6A), a normally rapid and efficient process, is significantly inhibited in nocodazole-treated cells (21). The microtubule-based motor proteins involved in these recycling and transcytosis pathways are presently unknown.

Links Between the Actin Cytoskeleton and Internalization

Actin filaments have also been implicated in the localization of intracellular organelles and vesicular transport. In contrast to microtubules, actin filaments are generally shorter and actin-based transport is significantly slower ($\sim 0.1 \mu\text{m/s}$). Myosin motors are responsible for these movements. In addition to the well-characterized class II myosin of muscle and non-muscle cells, there exist at least thirteen additional classes of myosins, known collectively as unconventional myosins (43). Class I, class V, class VI, and class VII myosins have all been implicated in organellar movement (44).

Actin has been linked to endocytosis in several polarized tissue cell types, including endothelial cells (45,46), hepatocytes (47–49), MDCK cells (50,51), proximal tubule-derived opossum kidney cells (35), cultured intestinal epithelial cells (52), the intestinal Caco-2 and T84 cell lines (53–55), and toad urinary bladder cells (56). The majority of these studies employed cytochalasins, a large family of actin-disrupting agents (>20 members) that are used widely to study the role of actin filaments in different biological systems (57). The cytochalasins cap the barbed or rapidly growing ends of actin filaments, nucleate actin polymerization, and shorten actin filaments (57).

Apical endocytosis of the membrane markers vesicular stomatitis virus G-protein and cationized ferritin (which are internalized via clathrin-coated pits) and the fluid-phase marker lucifer yellow (step 1A) is blocked when polarized MDCK cells are treated with cytochalasin D (CD) (50). The mechanism of fluid-phase internalization was not assessed in this study. Relative to untreated control cells, CD-treated cells exhibit a doubling in the number of coated pits at the apical

surface (50). However, in these CD-treated cells, the ability of the coated pits to pinch off is apparently impaired and the vesicles are often connected to the plasma membrane by long necks. Basolateral endocytosis of both Tf (which is internalized via clathrin-coated pits) and lucifer yellow in these MDCK cells is unaffected by CD treatment (50). Similar results are observed in CD-treated Caco-2 cells; clathrin-dependent endocytosis at the apical surface is impaired, in-

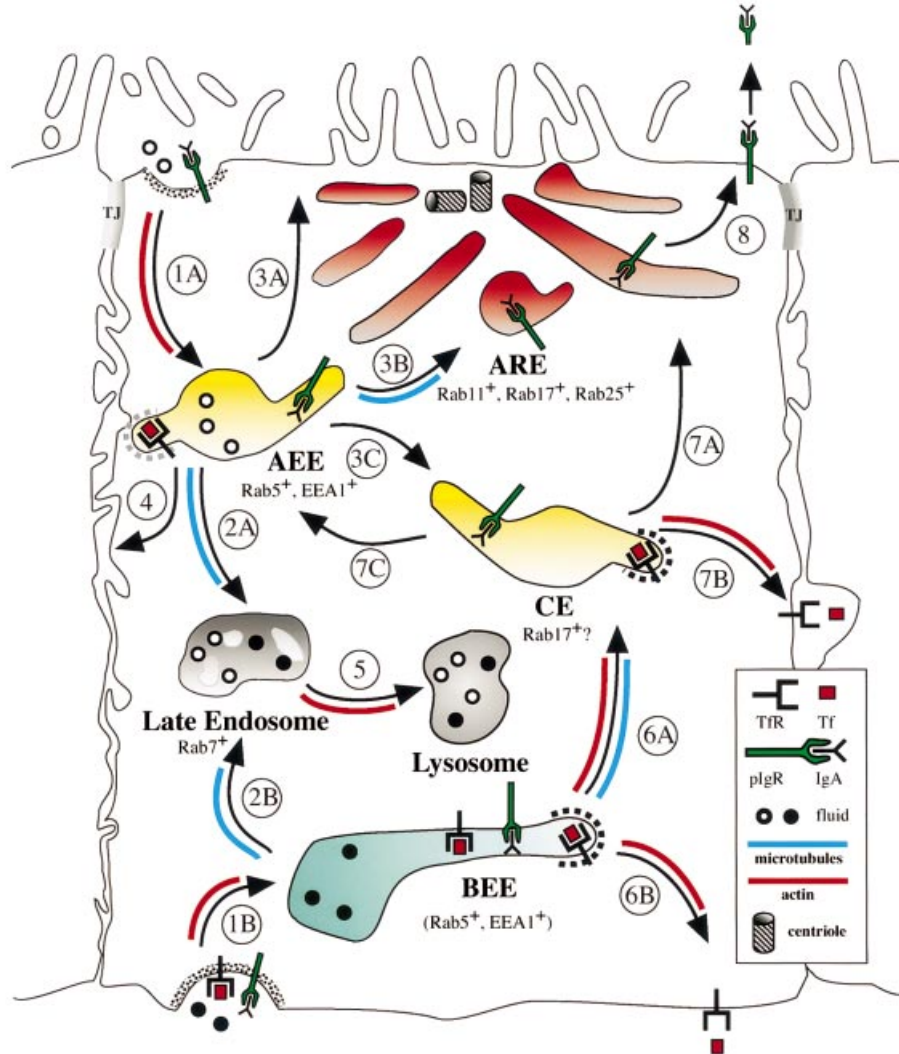


Figure 1: Model for endocytic traffic in polarized epithelial cells. Upon internalization, fluid and membrane are delivered to distinct AEE (step 1A) or BEE (step 1B). Endocytosis at both surfaces requires actin. Apically internalized fluid can recycle (step 3A), transcytose (step 4), or it can be delivered in a microtubule-dependent step to late endosomes (step 2A) and ultimately lysosomes (step 5). This latter step is actin-dependent. Basolaterally internalized fluid is primarily delivered to late endosomes (step 2B) and lysosomes (step 5). Apical recycling proteins are delivered from the AEE to the ARE (step 3B) or the CE (step 3C) before their ultimate release from the apical pole of the cell (step 8). Delivery between the AEE and ARE requires microtubules. The cytoskeletal requirements, if any, for delivery from the AEE to CE are presently unknown. Basolateral recycling proteins (i.e., receptor-bound Tf) as well as proteins transcytosing in the basolateral to apical direction (i.e., plgR-IgA) enter a shared BEE (step 1B). Although some receptor-bound Tf may recycle directly from this compartment (step 6B), a significant fraction is delivered to the CE along with the majority of the plgR-IgA (step 6A). This translocation step requires actin and microtubules. The majority of the receptor-bound Tf is thought to recycle from the CE (step 7B); however, a fraction may be delivered to the AEE (step 7C) and may recycle from this compartment (step 4). The transcytosing plgR-IgA complexes, as well as apical recycling plgR-IgA complexes, are delivered from the CE to the ARE (step 7A) and are ultimately released at the apical pole of the cell (step 8). Actin may be required for efficient recycling of receptor-bound Tf (step 6B and/or step 7B).

creased numbers of clathrin-coated pits are observed, and basolateral endocytosis is largely unaffected (53,54). Endocytosis of horseradish peroxidase (HRP) from the apical surface of cultured small-intestinal tissue (52), internalization of albumin at the apical pole of opossum kidney cells (35), and endocytosis of apically-delivered water channels in toad bladder epithelial cells (56) are also impaired by CD treatment.

The differential effects of cytochalasin treatment on apical and basolateral receptor-mediated endocytosis may indicate that actin is only required for internalization at the apical pole of the cell and that actin, therefore, is not an absolute requirement for receptor-mediated internalization (also see discussion below). There is recent evidence that endocytosis at the basolateral pole of MDCK and T84 cells (step 1B) may also involve actin. CD treatment stimulates endocytosis of fluid at the basolateral pole of T84 cells (55). In MDCK cells, treatment with jasplakinolide, which, like phalloidin, binds to and stabilizes actin filaments, selectively stimulates basolateral endocytosis of the fluid-phase markers FITC-dextran and HRP; apical endocytosis of these markers is unaffected (51). Jasplakinolide treatment did not appear to enhance receptor-mediated endocytosis of Tf and thus presumably increases clathrin-independent endocytosis. The reason for the differences between CD and jasplakinolide may reflect a difference in the pools of actin associated with the apical and basolateral poles of the cells (58) and/or the differential activity of these agents on these different actin pools.

In addition to the examples described above, actin is known to be required for phagocytosis (5), internalization of caveolae (7), and may also be important in the endocytosis of fluid and membrane in other mammalian cell types, including A431 cells (59–62), Hep2 cells (47), and cultured B-lymphoblastoid cells (63). Moreover, there is genetic evidence that actin and actin-based motor proteins are required for endocytosis in *Dictyostelium* (64) and *Saccharomyces cerevisiae* (65,66). Nevertheless, it should be noted that perturbation of the actin cytoskeleton does not affect endocytosis in all cell types. In macrophages, fluid-phase endocytosis is not altered by disruption of the actin cytoskeleton (34,67). In the specific case of clathrin-dependent endocytosis of Tf, the sensitivity of the process to CD and latrunculin A, which causes actin disassembly by sequestering actin monomers, depends on the cell type and whether the cells are grown on a solid substrate or in suspension culture (59). The results of these recent experiments indicate that actin does not have a compulsory role in receptor-mediated endocytosis. Instead, actin-regulated endocytosis may be important in specific cell types, at specific plasma membrane domains, or under specific growth conditions.

Role of Actin in Receptor-Mediated Endocytosis

The following section examines how actin may regulate internalization. Although its role in receptor-mediated endocytosis is emphasized, similar mechanisms are envisioned for

other pathways of internalization. The models presented are not mutually exclusive and it is likely that actin may play multiple roles in this process. Also, the function of the actin may vary in different cell types and at different plasma membrane domains. Because the plasma membrane of all cells is directly linked to an underlying submembranous actin-rich cytoskeleton (i.e., the cell cortex), it is not surprising that the actin cytoskeleton may regulate endocytic as well as exocytic events. Some receptors are associated transiently with the actin cytoskeleton (68). As a result, actin may regulate the entry of these receptors into forming endocytic vesicles (model 1, Figure 2).

Alternatively, actin may act as a molecular fence (model 2, Figure 2). Many regulated secretory events are accompanied by localized actin rearrangement or depolymerization that is thought to allow vesicle access to the plasma membrane. In fact, experimentally-induced actin disruption can stimulate vesicle fusion with the plasma membrane (69,70). In a similar fashion, endocytosis may require actin depolymerization or rearrangement to remove barriers to forming endocytic vesicles. There are data that indicate that the fence function of actin may play some role in endocytosis. When the plasma membrane of cells is examined by quick-freeze deep-etch techniques, few of the actin filaments in the plane of the membrane are observed in the immediate vicinity of clathrin-coated pits (59). However, previous studies have observed actin filaments that associate with clathrin-coated pits and extend into the cytoplasm (63). There are a small number of cell systems in which treatment with cytochalasins stimulates fluid-phase endocytosis (55,62,71). It was not determined in these studies if fluid was internalized via clathrin-dependent or -independent pathways. As described above in MDCK and Caco-2 cells, the number of coated pits at the apical surface of CD-treated cells is significantly increased by a factor of two (50,53). At least in these cell types, the ability to form coated pits at the apical plasma membrane may be modulated by the amount of polymerized actin associated with this domain. Although its mechanism of action is unclear, the ARF6 GTPase regulates apical endocytosis and apparently is capable of directing cortical actin rearrangements (72,73). There are, however, several observations that argue against this model. First, in the majority of cases, disruption of the actin cytoskeleton impairs, but does not stimulate, endocytosis (35,45–56,60,61,63,65). Second, stabilization of the actin cytoskeleton with jasplakinolide apparently enhances fluid-phase endocytosis at the basolateral pole of MDCK cells (51).

Actin and/or actin–myosin complexes may also play a role in specifying the sites of clathrin-coat assembly or regulating the invagination of the plasma membrane (model 3, Figure 2). Actin may act as a scaffolding which would be permissive for the assembly of a plasma membrane-associated endocytic machinery (74). An interesting link between receptor-mediated endocytosis and the actin cytoskeleton is the Huntingtin interacting protein 1-related (HIP1R), the mam-

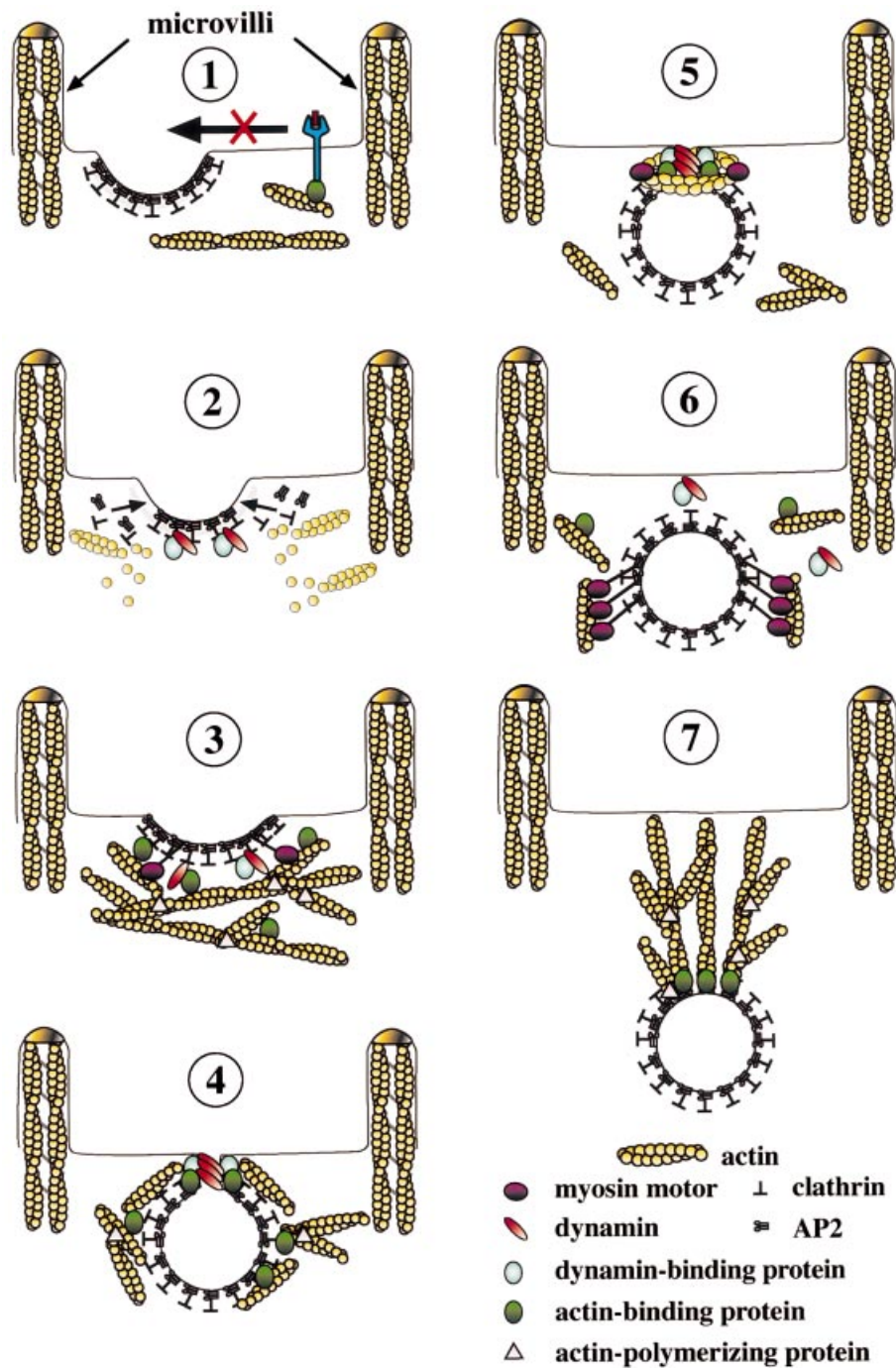


Figure 2: Models for role of the actin cytoskeleton in receptor-mediated endocytosis. (1) The actin cytoskeleton may constrain the lateral mobility of receptors, thereby regulating their entry into coated pits. (2) Actin may act as a molecular fence that regulates access to the plasma membrane. Presumably, localized actin depolymerization would be permissive for the formation of coated pits and their subsequent internalization. (3) Actin may act as a scaffold for the assembly of the endocytic machinery or may be required for efficient invagination of the plasma membrane. (4) Actin forms a scaffold that promotes the assembly of dynamin and associated components at the neck of deeply invaginated coated pits. (5) Myosin motors, in conjunction with actin, form a contractile ring that helps pinch off coated vesicles. This event may be regulated by the dynamin GTPase. (6) Myosin motor/actin complexes are required for vesicle detachment and movement into the cytoplasm. (7) Actin polymerization on the coated vesicle promotes vesicle detachment and movement by formation of a comet tail.

malian homolog of yeast sla2p (75). This protein associates with clathrin-coated pits and vesicles and binds F-actin via a C-terminal talin-like domain. It is an excellent candidate to link forming coated pits with the underlying actin scaffolding (model 3, Figure 2). Additional examples of proteins that associate with actin and endocytic vesicles are described in a recent review by Qualmann et al. (76). Alternatively, the formation of an endocytic vesicle requires that curvature be introduced into the membrane. Regulated actin polymerization at the sites of coated vesicle formation could play a role in this process (model 3, Figure 2).

Actin could modulate scission of vesicles (model 4, Figure 2). This would explain why CD treatment of MDCK cells results in the accumulation of long pits that are apparently incapable of pinching off and forming vesicles (50,53). Similarly, it would explain why late steps in the formation of coated vesicles are impaired by the addition of agents that sequester actin monomers (B4 thymosin and Dnase I) to an assay that reconstitutes clathrin-dependent endocytosis in perforated A431 cells (61). An important molecule involved in scission is dynamin (6). This GTPase regulates formation of vesicles derived from clathrin-coated pits as well as caveolae (6). In the case of receptor-mediated endocytosis, dynamin is thought to coordinate the assembly of numerous effector molecules at the neck of forming coated pits, ultimately leading to scission of the coated vesicle (6). Dynamin interacts with several proteins that may link this molecule with the actin cytoskeleton, including profilin (77) and syndapin (78). Profilin is an actin monomer-binding protein that regulates actin polymerization. Syndapin interacts with the neuronal isoform of the Wiskott–Aldrich syndrome protein, which regulates actin assembly (78).

In addition to actin and the actin-binding protein fimbrin, receptor-mediated endocytosis in yeast requires the product of the *MYO5* gene (66). Myo5p is a class I myosin motor. At present it remains to be established whether myosin motors play a role in endocytosis in mammalian cells. An intriguing class of myosins found in mammalian cells is the type VI subfamily (44). This class of myosin is associated with the Golgi and is found at the cell periphery (79). Type VI myosins are the only class of myosins that move toward the pointed end of actin filaments (80). Because the majority of actin filaments associated with coated pits and plasma membrane are oriented with their pointed ends directed towards the cytoplasm (63), type VI myosins could play a role in endocytic events. Although the function of myosin motors in endocytosis is speculative, they may be involved in membrane invagination (model 3, Figure 2) or dynamin-coordinated vesicle scission (model 5, Figure 2). The latter possibility would involve the formation of an actin ring, similar to that observed during cytokinesis, that would assist in the formation of the vesicle (81). Alternatively, myosin motors could be important in the detachment of vesicles from the plasma membrane and centripetal movement towards the cell's interior (model 6, Figure 2). In polarized epithelial cells, movement from the apical plasma membrane towards the cell interior requires

passage through the terminal web. Likewise, movement of vesicles away from the basal plasma membrane requires transit through the actin-rich layer of stress fibers present at the base of the cell. Both of these events may therefore require actin-based motility.

An alternative process that could direct endocytic vesicle movement through actin barriers involves the formation of a comet-like tail (so called 'rocket-based' motility; model 7, Figure 2). In cultured mast cells, newly-forming endocytic vesicles are propelled into the cytoplasm at the tips of actin comet tails (82), similar to those that propel *Listeria* bacteria in infected host cells. Actin tails also associate with endosomes and lysosomes in an *in vitro* system that reconstitutes actin assembly in *Xenopus* oocyte extracts (83). These later events require recruitment of neuronal Wiskott–Aldrich syndrome protein, and indicate that postendocytic trafficking events may also require associations between endosomes and the actin cytoskeleton.

Actin and its Function in Postendocytic Traffic

While a significant amount of research has focused on the early events in the endocytic process, less is understood about the role of the actin cytoskeleton in postendocytic trafficking events. CD treatment impairs the kinetics of ligand degradation in several systems (47,49,84). This is likely a defect in the transfer of cargo between late endosomes and lysosomes (step 5), as this process apparently requires actin and is altered in CD-treated Hep-2 cells (84). In MDCK cells, recycling pathways are generally unaltered by CD treatment; the kinetics of Tf recycling are unaffected and apical IgA recycling is only slightly slowed (85). Nonetheless, in Caco-2 cells Tf recycling is slowed in cells treated with the actin-monomer-binding toxin latrunculin A (steps 6B and 7B) (86). This discrepancy may reflect the different cell types used or the different mechanisms by which these two toxins disrupt the actin cytoskeleton.

Basolateral to apical transcytosis is significantly slowed in CD-treated MDCK cells (85). Like nocodazole (which inhibits transcytosis by ~60%), CD works early in the transcytotic pathway, inhibits transit between the BEE and the more apical endosomal compartments (step 6A), and slows transcytosis by ~45% (85). Tf movement between the BEE and CE is also impaired, but like nocodazole, CD treatment does not affect the kinetics of Tf recycling. When examined closely, filamentous actin is associated with endosomes below the level of the stress fibers (87). Likewise, actin and other actin-binding proteins are also associated with several endocytic compartments purified from rat liver (88). Perhaps this actin is important in facilitating the movement of these endosomes through the actin cortex. Order-of-addition experiments indicate that the CD-sensitive step precedes the nocodazole-sensitive step, and treatment with both CD and nocodazole inhibits transcytosis >95% (85). Apparently, postendocytic traffic of plgR-IgA complexes requires both an intact actin filament and microtubule cytoskeleton and

demonstrates that transcytosis, like other trafficking processes (47), is likely to require coordinated movement between both actin and microtubule cytoskeletons. Biosynthetic transport of vesicles is also likely to require sequential transport between microtubule and actin cytoskeletons (89).

As described above, transport between endosomes and lysosomes, and BEE and the CE/ARE, are examples of trafficking steps that are significantly disrupted in CD-treated cells. Moreover, Tf recycling is impaired in latrunculin A-treated Caco-2 cells (86). In each case, there is evidence that class I myosin motors may play a role in these processes. Myosin I α (MI α ; also known as myr1) is member of a subclass of myosin I motors that include brush-border myosin I (BBMI). Structurally these proteins are comprised of a large head domain (which has sites for both ATP and actin binding) and a smaller tail domain with both calmodulin-binding motifs and a positively-charged region that allows for binding to anionic lipids. MI α is associated with both endosomes and lysosomes (90). A truncated BBMI construct, which lacks the ATP-binding site, acts in a dominant negative fashion and promotes dissociation of endogenous MI α from endosomes and impairs the delivery of HRP to lysosomes, implicating class I myosins in this process (90).

New data indicate myr4 may be required for movement of cargo between early endosomes and the CE (20). Myr4, also a member of the class I myosins, is found on Tf-rich CE in MDCK cells. Anti-Myr4 antibodies inhibit an assay that reconstitutes fusion between AEE or BEE with Tf-labeled CE (20). Moreover the fusion assay is inhibited by treatments that prevent actin polymerization or induce depolymerization of existing filaments. Finally, two experiments point to a role for myosin motors in regulating Tf recycling. Overexpression of MI α or truncated versions of MI α that lack either the ATP-binding site or the entire motor domain result in the dispersion of Tf-containing compartments in the BW7G3 hepatoma cell line (91). In MDCK cells, expression of a truncated mutant of BBMI lacking the entire motor domain results in decreased recycling of Tf coupled with a large increase (> 3-fold) in apical release of this ligand (86).

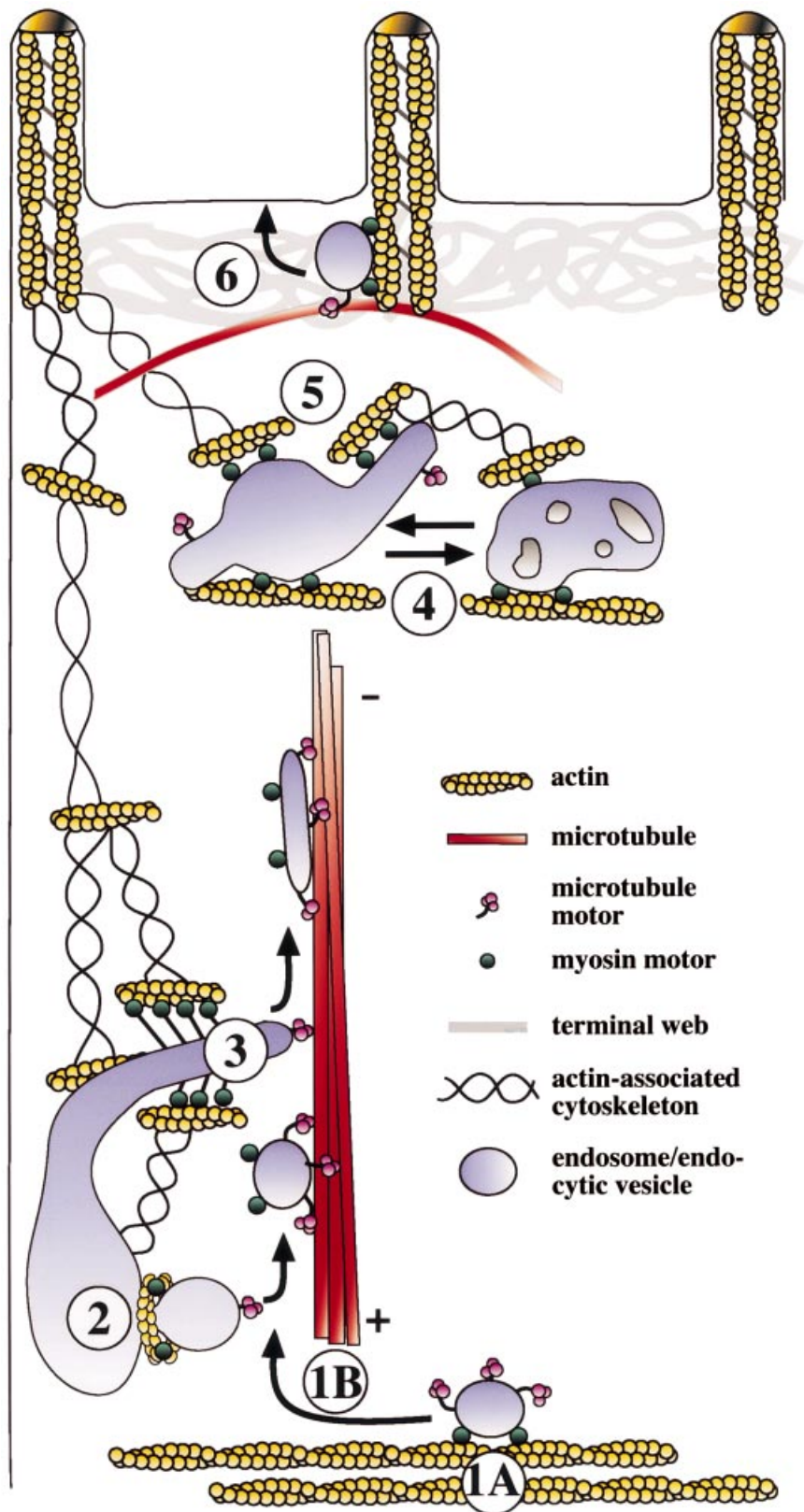
As in the case of endocytosis, several models have been proposed to explain the function of the actin cytoskeleton and myosin motor proteins in these postendocytic processes. Myosin motors are likely to be involved in movements of endosomal compartments. In the case of basal endosomes, they could control movement along or across stress fibers (model 1A, Figure 3) (87). These movements would propel the endosomes towards the long parallel bundles of lateral microtubules that could allow for transport towards the apical pole of the cell (model 1B, Figure 3). These lateral bundles of microtubules are oriented with their plus-ends directed toward the base of the cell (92). As such, the minus-end-directed motor dynein is likely to be important in this microtubule-based transport. Alternatively, a new family of kinesin motors has been described that moves in the minus-end direction (32).

Myosin motors may be important in vesiculation or the formation of tubular processes associated with endosomal elements (81). By forming a contractile ring (model 2, Figure 3), myosin motors in conjunction with actin could pinch off vesicles (81). Alternatively, tension generated by myosin motors on endosomal membranes could be important in the generation of tubules (model 3, Figure 3) (81). There are little data in this regard. Myosin-based motility could promote fusion by bringing endocytic compartments in close proximity (model 4, Figure 3) (84). This requirement would explain why transport between endosomes and lysosomes is blocked in CD-treated cells or cells expressing dominant negative mutants of type I myosins. As described above, expression of dominant negative myosin I constructs alters the distribution of endocytic compartments (86,91). By tethering organelles to the underlying actin cytoskeleton (model 5, Figure 3), myosin motors may be important in specifying the localization of intracellular compartments. In melanocytes, this mechanism is invoked to explain the myosin V-dependent capture of melanosomes at the periphery of dendritic processes (93). Finally, myosin motors may play a role in the delivery of endocytic vesicles through the terminal web, *en route* to the plasma membrane (model 6, Figure 3). Such a model has been proposed for apically targeted vesicles containing newly synthesized cargo (94).

Rho Family GTPases in Polarized Endocytic Traffic

Finally, a few words on actin and the Rho family of GTPases (95,96). Members of this family include Cdc42 (Cdc42Hs and G25K isoforms), TC-10, Rac (1, 2, and 3 isoforms), Rho (A, B, and C isoforms), RhoD, RhoE, and RhoG. The best studied members are RhoA, Rac1, and Cdc42. Initially, it was observed that each of these Rho family members regulated the formation of a distinct set of actin-rich structures (95): RhoA was important in the formation of stress fibers, Rac1 modulated lamellipodia formation, and Cdc42 promoted the formation of filopodia. In addition to the cytoskeleton, it is now known that these GTPases modulate multiple cellular events, including endocytosis (97). In non-polarized cells, receptor-mediated endocytosis is inhibited by constitutively active mutants of RhoA and Rac1, although these effects may be independent of actin assembly (98).

In polarized MDCK cells, expression of a constitutively active mutant of RhoA stimulates both apical and basolateral endocytosis, while a dominant negative mutant of RhoA impairs endocytosis at both cell surfaces (87). Mutants of Rac1 have the opposite effect on endocytosis; dominant negative Rac1 stimulates apical and basolateral endocytosis, while dominant active Rac1 inhibits the process (99). Cdc42 mutants also impair endocytosis in MDCK cells (R. Rojas and G. Apodaca, unpublished observations). These GTPases also have effects on postendocytic traffic. Dominant active RhoA impairs the exit of cargo from BEE (87), while dominant active Rac1 affects the exit of traffic out of the CE and/or ARE (99). Mutants of Cdc42 impair basolateral recycling of Tf



(100). While the downstream effector proteins that regulate these endocytic trafficking events are unknown, by altering the actin cytoskeleton they could have multiple effects on endocytic traffic in polarized epithelial cells.

Future Outlook

Evidence is mounting that, in addition to microtubules, the actin cytoskeleton plays a diversity of roles in endocytosis and endocytic traffic. Several questions remain to be answered. Do the effects of actin and microtubule depolymerizing/stabilizing agents reflect alterations in molecular motor/vesicle/cytoskeleton association? This is often the case as molecular motors have been implicated in many of the systems perturbed by agents such as nocodazole and CD. Which molecular motors are associated with each transport step? For example, in yeast and lower metazoans unconventional myosin motors are important in endocytosis (64–66); however, it is not yet known if they play a role in internalization in mammalian cells. What is the relationship between microtubule- and actin-based motility? Endosomes apparently can associate with both actin- and microtubule-based motor proteins. How transport between these two cytoskeletal elements is regulated is still unclear. What is the role of newly described proteins that link the cytoskeleton to endocytic pits and endosomes? Proteins such as HIP1R are found in coated pits and endosomes and bind actin (75), and yet their function remains elusive. Finally, how do the Rho family of GTPases regulate these processes? While these proteins can modulate the actin cytoskeleton, they also activate multiple downstream effector pathways that may act independently of the actin cytoskeleton. Many of these questions will be answered in genetically tractable organisms and through identification and characterization of new and old motor proteins, as well as approaches that take advantage of dominant negative protein constructs. Finally, advances in live-cell imaging, coupled with chimeric proteins containing the green fluorescent protein (or its derivatives) allow one to examine the relationship between endocytosis and the cytoskeleton in real time. Such approaches are already leading to a heightened understanding of these processes (74,82).

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References

1. Mays RW, Beck KA, Nelson WJ. Organization and function of the cytoskeleton in polarized epithelial cells: a component of the protein sorting machinery. *Curr Opin Cell Biol* 1994;6: 16–24.
2. Brown D, Stow JL. Protein trafficking and polarity in kidney epithelium: from cell biology to physiology. *Physiol Rev* 1996;76: 245–297.
3. Kelly RB. Microtubules, membrane traffic, and cell organization. *Cell* 1990;61: 5–7.
4. Mays RW, Beck KA, Nelson WJ. Organization and function of the cytoskeleton in polarized epithelial cells: a component of the protein sorting machinery. *Current Opin Cell Biol* 1994;6: 16–24.
5. Mukherjee S, Ghosh RN, Maxfield FR. Endocytosis. *Physiol Rev* 1997;77: 759–803.
6. Sever S, Damke H, Schmid SL. Garrotes, springs, ratchets, and whips: putting dynamin models to the test. *Traffic* 2000;1: 385–392.
7. Parton RG, Joggerst B, Simons K. Regulated internalization of caveolae. *J Cell Biol* 1994;127: 1199–1215.
8. Lamaze C, Schmid SL. The emergence of clathrin-independent pinocytotic pathways. *Curr Opin Cell Biol* 1995;7: 573–580.
9. Christoforidis S, McBride HM, Burgoyne RD, Zerial M. The Rab5 effector EEA1 is a core component of endosome docking. *Nature* 1999;397: 621–625.
10. Mallet WG, Maxfield FR. Chimeric forms of furin and TGN38 are transported with the plasma membrane in the trans-Golgi network via distinct endosomal pathways. *J Cell Biol* 1999;146: 345–359.
11. Bomsel M, Prydz K, Parton RG, Gruenberg J, Simons K. Endocytosis in filter-grown Madin–Darby canine kidney cells. *J Cell Biol* 1989;109: 3243–3258.
12. Eker P, Holm PK, van Deurs B, Sandvig K. Selective regulation of apical endocytosis in polarized Madin–Darby canine kidney cells by mastoparan and cAMP. *J Biol Chem* 1994;269: 18607–18615.
13. Bucci C, Wandinger-Ness A, Lutcke A, Chiariello M, Bruni CB, Zerial M. Rab5a is a common component of the apical and basolateral endocytic machinery in polarized epithelial cells. *Proc Natl Acad Sci USA* 1994;91: 5061–5065.
14. Leung S-M, Ruiz WG, Apodaca G. Sorting of membrane and fluid at the apical pole of polarized MDCK cells. *Mol Biol Cell* 2000;11: 2131–2150.
15. Gokay KE, Wilson JM. Targeting of an apical endosomal protein to endosomes in MDCK cells. *Traffic* 2000;1: 354–365.
16. Fialka I, Steinlein P, Ahorn H, Böck G, Burbelo PD, Haberfellner M, Lottspeich F, Paiha K, Pasquali C, Huber LA. Identification of syntenin as a protein of the apical early endocytic compartment in Madin–Darby canine kidney cells. *J Biol Chem* 1999;274: 26233–26239.
17. Odorizzi G, Pearse A, Domingo D, Trowbridge IS, Hopkins CR. Apical and basolateral endosomes of MDCK cells are interconnected and contain a polarized sorting mechanism. *J Cell Biol* 1996;135: 139–152.
18. Zacchi P, Stenmark H, Parton RG, Orioli D, Lim F, Giner A, Mellman I, Zerial M, Murphy C. Rab17 regulates membrane trafficking through apical recycling endosomes in polarized epithelial cells. *J Cell Biol* 1998;140: 1039–1053.
19. Sheff DR, Daro EA, Hull M, Mellman I. The receptor recycling pathway contains two distinct populations of early endosomes with different sorting functions. *J Cell Biol* 1999;145: 123–139.

Figure 3: Models for the function of actin/myosin complexes in postendocytic traffic in polarized epithelial cells. (1A) Myosin motor/actin complexes regulate the movement of endosomes/endocytic vesicles along or across stress fibers. (1B) These movements direct the endocytic vesicles to lateral bundles of microtubules that would allow for transport towards the apical pole of the cell. (2) Myosin motors in conjunction with actin may form a contractile ring that promotes vesicle budding. (3) Myosin motors associated with endosomes may generate tension on endosomal membranes, thereby generating tubules. (4) Myosin motors bring endocytic compartments in close proximity and promote fusion. (5) By tethering endocytic compartments to the actin cytoskeleton, myosin motors may be important in determining the localization of these organelles. (6) Myosin motors may be important in the navigation of vesicles through the terminal web, and therefore promote fusion with the apical plasma membrane.

20. Huber LA, Fialka I, Paiha K, Hunziker W, Sacks DB, Bähler M, Way M, Gagescu R, Gruenberg J. Both calmodulin and the unconventional myosin Myr4 regulate membrane trafficking along the recycling pathway of MDCK cells. *Traffic* 2000;1: 494–503.
21. Apodaca G, Katz LA, Mostov KE. Receptor-mediated transcytosis of IgA in MDCK cells is via apical recycling endosomes. *J Cell Biol* 1994;125: 67–86.
22. Brown PS, Wang E, Aroeti B, Chapin SJ, Mostov KE, Dunn KW. Definition of distinct compartments in polarized Madin–Darby canine kidney (MDCK) cells for membrane-volume sorting, polarized sorting and apical recycling. *Traffic* 2000;1: 124–140.
23. Casanova JE, Wang X, Kumar R, Bhartur SG, Navarre J, Woodrum JE, Altschuler YA, Ray GS, Godenring JR. Association of Rab25 and Rab11a with the apical recycling system of polarized Madin–Darby canine kidney cells. *Mol Biol Cell* 1999;10: 47–61.
24. Hunziker W, Peters PJ. Rab17 localizes to recycling endosomes and regulates receptor-mediated transcytosis in epithelial cells. *J Biol Chem* 1998;273: 15734–15741.
25. Barroso M, Sztul E. Basolateral to apical transcytosis in polarized cells is indirect and involves BFA and trimeric G protein sensitive passage through the apical endosome. *J Cell Biol* 1994;124: 83–100.
26. Gibson A, Futter CE, Maxwell S, Allchin EH, Shipman M, Kraehenbuhl JP, Domingo D, Odorizzi G, Trowbridge IS, Hopkins CR. Sorting mechanisms regulating membrane protein traffic in the apical transcytotic pathway of polarized MDCK cells. *J Cell Biol* 1998;143: 81–94.
27. Breitfeld PP, Harris JM, Mostov KM. Postendocytotic sorting of the ligand for the polymeric immunoglobulin receptor in Madin–Darby canine kidney cells. *J Cell Biol* 1989;109: 475–486.
28. Wang E, Brown PS, Aroeti B, Chapin SJ, Mostov KE, Dunn KW. Apical and basolateral pathways of MDCK cells meet in acidic common endosomes distinct from a nearly-neutral apical recycling endosome. *Traffic* 2000;1: 480–493.
29. Van IJzendoorn SCD, Maier O, Van Der Wouden JM, Hoekstra D. The subapical compartment and its role in intracellular trafficking and cell polarity. *J Cell Physiol* 2000;184: 151–160.
30. Mostov KE, Verges M, Altschuler Y. Membrane traffic in polarized epithelial cells. *Curr Opin Cell Biol* 2000;12: 483–490.
31. Vale RD. Intracellular transport using microtubule-based motors. *Ann Rev Cell Biol* 1987;3: 347–378.
32. Woehlke G, Schliwa M. Walking on two heads: the many talents of kinesin. *Nature Rev Mol Cell Biol* 2000;1: 50–58.
33. Matter K, Bucher K, Hauri H-P. Microtubule perturbation retards both the direct and the indirect apical pathway but does not affect sorting of plasma membrane proteins in intestinal epithelial cells (Caco-2). *EMBO J* 1990;9: 3163–3170.
34. Piasek A, Thyberg J. Effects of colchicine on endocytosis of horseradish peroxidase by rat peritoneal macrophages. *J Cell Sci* 1980;45: 59–71.
35. Gekle M, Mildnerberger S, Freudinger R, Schwerdt G, Silbernagl S. Albumin endocytosis in OK cells: dependence on actin and microtubules and regulation by protein kinases. *Am J Physiol* 1997;272: F668–F677.
36. Pratten MK, Lloyd JB. Effects of temperature, metabolic inhibitors and some other factors on fluid-phase and adsorptive pinocytosis by rat peritoneal macrophages. *Biochem J* 1979;180: 567–571.
37. Gruenberg J, Griffiths G, Howell K. Characterization of the early endosome and putative endocytic carrier vesicles in vivo and with an assay of vesicle fusion in vitro. *J Cell Biol* 1989;108: 1301–1316.
38. Bomsel M, Parton R, Kuznetsov SA, Schroer TA, Gruenberg J. Microtubule- and motor-dependent fusion in vitro between apical and basolateral endocytic vesicles from MDCK cells. *Cell* 1990;62: 719–731.
39. McGraw TE, Dunn KW, Maxfield FR. Isolation of a temperature-sensitive variant Chinese hamster ovary cell line with a morphologically altered endocytic recycling compartment. *J Cell Physiol* 1993;155: 579–594.
40. Breitfeld PP, McKinnon WC, Mostov KE. Effect of nocodazole on vesicular traffic to the apical and basolateral surfaces of polarized MDCK cells. *J Cell Biol* 1990;111: 2365–2373.
41. Hunziker W, Mâle P, Mellman I. Differential microtubule requirements for transcytosis in MDCK cells. *EMBO J* 1990;9: 3515–3525.
42. Bomsel M. Transcytosis of infectious human immunodeficiency virus across a tight human epithelial cell line barrier. *Nat Med* 1997;3: 42–47.
43. Mermall V, Post PL, Mooseker MS. Unconventional myosins in cell movement, membrane traffic, and signal transduction. *Science* 1998;279: 527–533.
44. Tuxworth RI, Titus MA. Unconventional myosins: anchors in the membrane traffic relay. *Traffic* 2000;1: 11–18.
45. Alexander JS, Jackson SA, Chaney E, Kevil CG, Haselton FR. The role of cadherin endocytosis in endothelial barrier regulation: involvement of protein kinase C and actin-cadherin interactions. *Inflammation* 1998;22: 419–433.
46. Smedsrod B, Johansson S, Pertoft H. Studies in vivo and in vitro on the uptake and degradation of soluble collagen $\alpha 1(I)$ chains in rat liver endothelial and Kupffer cells. *Biochem* 1985;228: 415–424.
47. Durrbach A, Louvard D, Coudrier E. Actin filaments facilitate two steps of endocytosis. *J Cell Sci* 1996;109: 457–465.
48. Kyle ME, Nakae D, Sakaida I, Miccadei S, Farber JL. Endocytosis of superoxide dismutase is required in order for the enzyme to protect hepatocytes from cytotoxicity of hydrogen peroxide. *J Biol Chem* 1988;263: 3784–3789.
49. Kaufman SS, Blain PL, Park JHY, Tuma DJ. Role of microfilaments in asialoglycoprotein processing in adult and developing liver. *Am J Physiol* 1990;259: G639–G645.
50. Gottlieb TA, Ivanov IE, Adesnik M, Sabatini DD. Actin microfilaments play a critical role in endocytosis at the apical but not the basolateral surface of polarized epithelial cells. *J Cell Biol* 1993;120: 695–710.
51. Shurety W, Stewart NL, Stow JL. Fluid-phase markers in the basolateral endocytic pathway accumulate in response to the actin assembly-promoting drug jasplakinolide. *Mol Biol Cell* 1998;9: 957–975.
52. Blok J, Scheven BAA, Mulder-Stapel AA, Ginsel LA, Daems WT. Endocytosis in absorptive cells of cultured human small-intestinal tissue: effect of cytochalasin B and D. *Cell Tissue Res* 1982;222: 113–126.
53. Shurety W, Bright NA, Luzio JP. The effects of cytochalasin D and phorbol myristate acetate on the apical endocytosis of ricin in polarised Caco-2 cells. *J Cell Sci* 1996;109: 2927–2935.
54. Jackman MR, Shurety W, Ellis JA, Luzio JP. Inhibition of apical but not basolateral endocytosis of ricin and folate in Caco-2 cells by cytochalasin D. *J Cell Sci* 1994;107: 2547–2556.
55. Song JC, Hrnjez BJ, Farokhzad OC, Matthews JB. PKC- ϵ regulates basolateral endocytosis in human T84 intestinal epithelia: role of F-actin and MARCKS. *Am J Physiol* 1999;277: C1239–C1249.
56. Dibas A, Mia A, Yorio T. Microfilament network is needed for the endocytosis of water channels and not for apical membrane insertion upon vasopressin action. *Proc Soc Exp Biol Med* 1999;223: 203–209.
57. Cooper JA. Effects of cytochalasin and phalloidin on actin. *J Cell Biol* 1987;105: 1473–1478.
58. Yao X, Chaponnier C, Gabbiani G, Forte JG. Polarized distribution of actin isoforms in gastric parietal cells. *Mol Biol Cell* 1995;6: 541–557.
59. Fujimoto LM, Roth R, Heuser JE, Schmid SL. Actin assembly plays a variable, but not obligatory role in receptor-mediated endocytosis in mammalian cells. *Traffic* 2000;1: 161–171.
60. Sandvig K, vanDeurs B. Selective modulation of the endocytic uptake of ricin and fluid phase markers without alteration in transferrin endocytosis. *J Biol Chem* 1990;265: 6382–6388.
61. Lamaze C, Fujimoto LM, Yin HL, Schmid SL. The actin cytoskeleton is required for receptor-mediated endocytosis in mammalian cells. *J Biol Chem* 1997;272: 20332–20335.
62. Haigler HT, McKanna JA, Cohen S. Rapid stimulation of pinocytosis in human carcinoma cells A-431 by epidermal growth factor. *J Cell Biol* 1979;83: 82–90.
63. Salisbury JL, Condeelis JS, Satir P. Role of coated vesicles, microfilaments, and calmodulin in receptor-mediated endocytosis by cultured B lymphoblastoid cells. *J Cell Biol* 1980;87: 132–141.

64. Novak KD, Peterson MD, Reedy MC, Titus MA. *Dictyostelium* myosin I double mutants exhibit conditional defects in pinocytosis. *J Cell Biol* 1995;131: 1205–1221.
65. Kübler E, Riezman H. Actin and fimbrin are required for the internalization step of endocytosis in yeast. *EMBO J* 1993;12: 2855–2862.
66. Geli MI, Riezman H. Role of type I myosins in receptor-mediated endocytosis in yeast. *Science* 1996;272: 533–535.
67. Klaus GG. Dissociation of pinocytosis and phagocytosis by peritoneal macrophages. *Exp Cell Res* 1973;79: 73–78.
68. Sako Y, Kusumi A. Compartmentalized structure of the plasma membrane for receptor movements as revealed by nanometer-level motion analysis. *J Cell Biol* 1994;125: 1251–1264.
69. Orci L, Gabbay KH, Malaisse WJ. Pancreatic beta-cell web: its possible role in insulin secretion. *Science* 1972;175: 1128–1130.
70. Valentijn K, Valentijn JA, Jamieson JD. Role of actin in regulated exocytosis and compensatory membrane retrieval: insights from an old acquaintance. *Biochem Biophys Res Commun* 1999;266: 652–661.
71. Verrey F, Groscurth P, Bolliger U. Cytoskeletal disruption in A6 kidney cells: impact on endo/exocytosis and NaCl transport regulation by antidiuretic hormone. *J Membr Biol* 1995;145: 193–204.
72. Radhakrishna H, Al-Awar O, Khachikian Z, Donaldson JG. ARF6 requirement for Rac ruffling suggests a role for membrane trafficking in cortical actin rearrangements. *J Cell Sci* 1999;112: 855–866.
73. Altschuler Y, Liu S-H, Katz L, Tang K, Hardy S, Brodsky F, Apodaca G, Mostov K. ADP-ribosylation factor 6 and endocytosis at the apical surface of Madin–Darby canine kidney cells. *J Cell Biol* 1999;147: 7–12.
74. Gaidarov I, Santini F, Warren RA, Keen JH. Spatial control of coated-pit dynamics in living cells. *Nature Cell Biol* 1999;1: 1–8.
75. Engqvist-Goldstein AEY, Kessels MM, Chopra VS, Hayden MR, Drubin DG. 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* 1999;147: 1503–1518.
76. Qualmann B, Kessels MM, Kelly RB. Molecular links between endocytosis and the actin cytoskeleton. *J Cell Bio* 2000;150: F111–F116.
77. Witke W, Podtelejnikov AV, DiNardo A, Sutherland JD, Gurniak CB, Dotti C, Mann M. In mouse brain profilin I and profilin II associate with regulators of the endocytic pathway and actin assembly. *EMBO J* 1998;17: 967–976.
78. Qualmann B, Roos J, DiGregorio PJ, Kelly RB. Syndapin I, a synaptic dynamin-binding protein that associates with the neural Wiskott–Aldrich syndrome protein. *Mol Biol Cell* 1999;10: 501–513.
79. Buss F, Kendrick-Jones J, Lionne C, Knight AE, Coté GP, Luzio JP. The localization of myosin VI at the Golgi complex and leading edge of fibroblasts and its phosphorylation and recruitment into membrane ruffles of A431 cells after growth factor stimulation. *J Cell Biol* 1998;143: 1535–1545.
80. Wells AL, Lin AW, Chen LQ, Safer D, Cain SM, Hasson T, Caragher BO, Milligan RA, Sweeney HL. Myosin VI is an actin-based motor that moves backwards. *Nature* 1999;401: 505–508.
81. Coudrier E, Durrbach A, Louvard D. Do unconventional myosins exert functions in dynamics of membrane compartments. *FEBS Lett* 1992;307: 87–92.
82. Merrifield CJ, Moss SE, Ballestrem C, Imhof BA, Giese G, Wunderlich I, Almers W. Endocytic vesicles move at the tips of actin tails in cultured mast cells. *Nature Cell Biol* 1999;1: 72–74.
83. Taunton J, Rowning BA, Coughlin ML, Wu M, Moon RT, Mitchison TJ, Larabell CA. Actin-dependent propulsion of endosomes and lysosomes by recruitment of N-WASP. *J Cell Biol* 2000;1489: 519–530.
84. van Deurs B, Holm PK, Kayser L, Sandvig K. Delivery to lysosomes in the human carcinoma cell line Hep-2 involves an actin filament-facilitated fusion between mature endosomes and preexisting lysosomes. *Eur J Cell Biol* 1995;66: 309–323.
85. Maples CJ, Ruiz WG, Apodaca G. Both microtubules and actin filaments are required for efficient postendocytic traffic of the polymeric immunoglobulin receptor in polarized Madin–Darby canine kidney cells. *J Biol Chem* 1997;272: 6741–6751.
86. Durrbach A, Raposo G, Tenza D, Louvard D, Coudrier E. Truncated brush border myosin I affects membrane traffic in polarized epithelial cells. *Traffic* 2000;1: 411–424.
87. Leung S-M, Rojas R, Maples C, Flynn C, Ruiz WG, Jou T-S, Apodaca G. Modulation of endocytic traffic in polarized MDCK cells by the small GTPase RhoA. *Mol Biol Cell* 1999;10: 4369–4384.
88. Pol A, Ortega D, Enrich C. Identification of cytoskeleton-associated proteins in isolated rat liver endosomes. *Biochem J* 1997;327: 741–746.
89. Fath KR, Burgess DR. Membrane mobility mediated by unconventional myosin. *Curr Opin Cell Biol* 1994;6: 131–135.
90. Raposo G, Cordonnier M-N, Tenza D, Menichi B, Durrbach A, Louvard D, Coudrier E. Association of myosin I alpha with endosomes and lysosomes in mammalian cells. *Mol Biol Cell* 1999;10: 1477–1494.
91. Durrbach A, Collins K, Matsudaira P, Louvard D, Coudrier E. Brush border myosin-I truncated in the motor domain impairs the distribution and the function of endocytic compartments in an hepatoma cell line. *Proc Natl Acad Sci USA* 1996;93: 7053–7058.
92. Bacallao R, Antony C, Dotti C, Karsenti E, Stelzer EHK, Simons K. The subcellular organization of Madin–Darby canine kidney cells during the formation of a polarized epithelium. *J Cell Biol* 1989;109: 2817–2832.
93. Wu X, Bowers B, Rao K, Wei Q, Hammer JA. Visualization of melanosome dynamics within wild-type and dilute melanocytes suggests a paradigm for myosin V function in vivo. *J Cell Biol* 1998;143: 1899–1918.
94. Fath KR, Trimburo GM, Burgess DR. Molecular motors are differentially distributed on Golgi membranes from polarized epithelial cells. *J Cell Biol* 1994;126: 661–675.
95. Hall A. Rho GTPases and the actin cytoskeleton. *Science* 1998;279: 509–514.
96. Van Aelst L, D'Souza-Schorey C. Rho GTPases and signaling networks. *Genes Devel* 1997;11: 2295–2322.
97. Ellis S, Mellor H. Regulation of endocytic traffic by Rho family GTPases. *T Cell Biol* 2000;10: 85–88.
98. Lamaze C, Chuang T-H, Terlecky LJ, Bokoch GM, Schmid SL. Regulation of receptor-mediated endocytosis by Rho and Rac1. *Nature* 1996;382: 177–179.
99. Jou T-S, Leung S-M, Fung LM, Ruiz WG, Nelson WJ, Apodaca G. Selective alterations in biosynthetic and endocytic protein traffic in Madin–Darby canine kidney epithelial cells expressing mutants of the small GTPase Rac1. *Mol Biol Cell* 2000;11: 287–304.
100. Kroschewski R, Hall A, Mellman I. Cdc42 controls secretory and endocytic transport to the basolateral plasma membrane of MDCK cells. *Nature Cell Biol* 1999;1: 8–13.