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Regulating cytoskeleton-based vesicle motility

Heidi Hehnly and **Mark Stamnes**
Department of Molecular Physiology & Biophysics Roy J. and Lucille A. Carver College of Medicine The University of Iowa Iowa City, IA 52242

Abstract

During vesicular transport, the assembly of the coat complexes and the selection of cargo proteins must be coordinated with the subsequent translocation of vesicles from the donor to an acceptor compartment. Here we review recent progress toward uncovering the molecular mechanisms that connect transport vesicles to the protein machinery responsible for cytoskeleton-mediated motility. An emerging theme is that vesicle cargo proteins, either directly or through binding interactions with coat proteins, are able to influence cytoskeletal dynamics and motor protein function. Hence, a vesicle's cargo composition may help direct its intracellular motility and targeting.

Keywords

Vesicular transport; actin; microtubules; dynein; kinesin; myosin

1. Introduction

As the molecular basis for transport vesicle assembly and vesicle fusion become clearer, recent research efforts are focusing on the motility events that translocate vesicles from a donor to an acceptor compartment. Both the microtubule and actin cytoskeletons make essential contributions to intracellular vesicle and organelle motility. Microtubules serve as tracks for transport via the motor proteins dynein and kinesin [1,2]. Microtubules are polarized with minus ends often localized at the juxtanuclear centrosome and plus ends present at the cell periphery. Hence, the minus-end-directed motor, dynein, is implicated in motility toward the cell interior while the plus-end-directed family of motors, kinesins, often mediates movement toward the cell surface. Actin contributes to vesicle motility in distinct ways [2,3]. First, actin serves as a track for the myosin family of motor proteins. Second, actin polymerization itself can propel vesicles in a manner related to the extrusion of leading edge during cell motility and the “comet-tail” motility of some pathogenic bacteria.

Regulatory mechanisms must exist to coordinate the cytoskeleton-based translocation machinery with the events of vesicle assembly (Figure 1). In the case of motor-protein-mediated vesicle motility, regulatory processes must ensure that the motors bind or are active only on the assembled vesicles and not on the donor organelle (Figure 1A). This implies that a motor-binding site is created or activated during the process of vesicle assembly. Furthermore, temporal coordination of vesicle assembly and motor-mediated motility must

Correspondence to: Mark Stamnes.

Department of Molecular Physiology & Biophysics Roy J. and Lucille A. Carver College of Medicine The University of Iowa Iowa City, IA 52242 Phone: 319-335-7858 FAX: 319-335-7330 Email: mark-stamnes@uiowa.edu

occur so that translocation away from the donor organelle occurs only after the completion of cargo packaging and vesicle scission (Figure 1B). Premature motor function could lead to the translocation of empty vesicles or the formation of tubular rather than vesicular carriers. Finally, a given type of vesicle may mediate multiple trafficking steps within the cell. For example, COPI vesicles are implicated in bidirectional transport at the Golgi complex [4] and in transport among endosomal compartments [5]. These various transport steps may require distinct interactions with the cytoskeleton or motor proteins. Thus, directional regulation may be required to select the correct motor protein for each step (Figure 1C).

Details about the molecular basis for spatial, temporal, and directional regulation of cytoskeleton-dependent vesicle motility have emerged from recent studies. One important line of investigation has involved the use of time-lapse microscopy to determine the order in which various coat components and cytoskeletal proteins associate and dissociate from sites of clathrin-coated vesicle budding. Other research is yielding insight into protein interactions involved in signaling pathways that function to coordinate cargo selection and vesicle assembly with cytoskeletal dynamics and motor protein recruitment. Also, structure function analysis of motor proteins is leading to the identification of specific motifs that direct motors to vesicular cargo. These recent studies indicate that cargo binding may play a direct role in regulating motor activity. The goal of this review is to describe recent progress in these research areas. We will focus on studies that are revealing how components of transport vesicles (cargo, GTP-binding proteins, coat proteins, coat-bound accessory proteins) interact with or regulate cytoskeletal proteins and molecular motors. We hope to provide a more complete picture of the molecular interface between cytoskeletal regulation and vesicular transport.

2. Cytoskeletal dynamics at sites of clathrin-mediated endocytosis

The role and regulation of actin during endocytosis have been covered in several excellent recent review articles [6-9]. Thus, we will provide an overview of actin function during clathrin vesicle budding from the plasma membrane before focusing on very recent studies that are examining a multifaceted role for the accessory actin-binding protein Hip1R/Sla2p in connecting clathrin-vesicle assembly to the regulation of actin dynamics. We will also describe new evidence indicating that cargo proteins are able to influence endocytic vesicle release and motility.

2.1 Correlation between actin polymerization and endocytic vesicles is revealed by time-lapse microscopy

Time-lapse epifluorescence microscopy studies in yeast and total internal reflection fluorescence (TIRF) microscopy imaging of the cell-surface in higher eukaryotic cells reveal that there is a burst of actin polymerization that occurs at the sites of clathrin vesicle assembly [10-12]. Clathrin vesicle formation requires a time course of 1-2 minutes with actin polymerization occurring as a relatively late step in both yeast and mammalian cells that correlates with the scission event and the movement of vesicles away from the plasma membrane. Actin polymerization at sites of vesicle assembly occurs by recruitment and activation of the Arp2/3 complex [6-9]. Actin polymerization facilitates endocytosis in mammalian cells but is essential for endocytosis in yeast. Actin polymerization is regulated in part through the recruitment of accessory proteins including Sla2p and Pan1p. Proteins are recruited as several distinct modules to clathrin vesicles in yeast [6,13]. The clathrin coat is recruited first followed by proteins Sla2p, and Pan1p that are involved in regulating actin dynamics. Arp2/3-dependent actin polymerization occurs together with the recruitment of the actin-binding protein Abp1p at a later time point immediately before vesicle movement. The time-resolved recruitment of distinct protein modules likely ensures that cargo selection and vesicle assembly are properly coordinated with latter events such as the scission reaction and motility.

2.2 Hip1R and Sla2p are key proteins linking clathrin coats to actin dynamics

The Huntingtin-interacting-protein-related (Hip1R) protein and its yeast ortholog, Sla2p, are clathrin-vesicle associated proteins that are involved in connecting vesicle assembly with the regulation of actin dynamics. These proteins bind directly to the clathrin light chain through a central coiled-coil domain [14-17] and to actin filaments through a C-terminal talin-like domain [15,18]. Sla2p was found to regulate actin assembly in a manner that is dependent on its interaction with clathrin [17]. Sla2p binds in the form of patches on the cell surface in the absence of clathrin but these Sla2p patches are associated with abnormal comet-tail-like actin structures.

Recent studies reveal that Sla2p and Hip1R may affect actin dynamics in multiple ways that are directed through interactions with other actin-binding proteins. One recently identified binding partner for Sla2p is the yeast actin-binding protein, Pan1p [19]. Pan1p is an activator of the Arp2/3 complex during endocytosis. Pan1p arrives at endocytic vesicles prior to actin polymerization and thus must be kept inactive until the correct time for vesicle scission and motility. Pan1p's activity is inhibited when bound to Sla2p indicating that Sla2p is important for specifying the timing of Pan1p-dependent Arp2/3 activity [19]. According to this model, vesicle-associated actin polymerization would commence upon the completion of vesicle assembly by an unknown signal that causes the disruption of the Sla2p/Pan1p complex.

The mammalian ortholog, Hip1R is also able to bind to an actin binding protein, cortactin, and block cortactin/N-WASP-dependent activation of Arp2/3 [20]. Interestingly, the Hip1R/cortactin complex was found to have an additional actin plus-end capping activity. Thus, Hip1R through its binding interaction with cortactin may regulate actin polymerization in multiple ways. It is proposed that Hip1R regulates Arp2/3-dependent actin polymerization at the neck of a budding vesicle while capping actin through its binding interaction with cortactin at the vesicle surface. This actin structure may assist in pushing vesicles away from the cell surface. The studies with Sla2p and Hip1R reveal that they might carry out multiple functions that are modified through interactions with the clathrin coat and other coat-bound accessory proteins.

2.3 Endocytic cargo proteins influence downstream vesicle motility

Several degraded endocytic cargo proteins such as the low-density lipoprotein (LDL) and epidermal growth factor (EGF) rapidly segregate from the recycled cargo protein transferrin receptor during endocytosis [21]. Endocytosis via these two subclasses of early endosomes displays distinct properties [21]. For example, internalization of transferrin required the clathrin adaptor AP-2 whereas internalization of the degraded cargo was not affected by AP-2 knockdown. Importantly, the LDL/EGF-containing endosomes underwent more rapid microtubule-based motility and maturation (Rab7 acquisition) when compared to the endosomes that contained transferrin receptor. While these proteins might be recruited into distinct preexisting endosomes, a second interesting possibility is that the cargo proteins influence the properties of clathrin vesicles and their interactions with the microtubule cytoskeleton. Studies using G-protein coupled receptors [22] and the bacterial toxin, Shiga toxin [23], indicate that cargo can influence interactions between endocytic vesicles and the cytoskeleton.

Some G-protein-coupled receptor proteins are present in clathrin-coated pits that display a prolonged surface residence time when compared to clathrin vesicles involved in transferrin uptake [22]. This may help optimize cargo packaging or facilitate an aspect of receptor signaling. The increased surface residence time occurs because of a delay in dynamin-mediated scission and internalization of the vesicles. The delayed internalization involved a PDZ-ligand domain present at the C-terminus of some G-protein coupled receptors (Figure 2). Appending a PDZ-ligand domain from the β-adrenergic receptor onto the δ-opioid receptor caused the δ-

opioid receptor to undergo delayed internalization [22]. A chimeric opioid receptor containing the actin-binding domain of ezrin, an ezrin/radixin/moesin (ERM)-family protein, also underwent delayed internalization. The G-protein coupled receptors, therefore, appear to modulate internalization kinetics by recruiting PDZ-domain-containing proteins and ERM-family actin binding proteins. It is proposed that cargo-dependent binding interactions between the vesicles and cortical actin play a role in specifying the timing of scission and internalization (Figure 2).

Shiga toxin, secreted by pathogenic E. coli, is another example of an endocytic cargo protein that influences cytoskeletal dynamics and vesicle motility. This toxin undergoes retrograde transport from endosomes to the Golgi apparatus. It is then transported from the Golgi apparatus to the endoplasmic reticulum (ER) where it enters the cytosol. Shiga toxin requires dynein-mediated motility along microtubules for retrograde transport from endosomes to the Golgi complex [23]. Upon binding to its glycolipid receptor at the cell surface, the B subunit of the toxin causes an increase in both actin polymerization and microtubule assembly [23,24]. In addition to increasing the rate of microtubule assembly, Shiga toxin bound to the cell surface increases the rate of microtubule-dependent organelle and vesicle motility such as the transport of transferrin from early endosomes to the juxtanuclear recycling compartment [23]. These findings raise the interesting possibility that the Shiga toxin-dependent changes in cytoskeletal dynamics serve to facilitate the toxin's intracellular transport from endosomes to the juxtanuclear Golgi complex.

The ability of vesicle coat and cargo proteins to influence cytoskeletal dynamics and vesicle motility is not unique to endocytosis but is recapitulated during microtubule-dependent vesicular transport at the Golgi complex as reviewed in the next section.

3. Protein transport at the Golgi complex involves interdependent use of microtubule motors and cargo regulated actin dynamics

3.1 Microtubule motor proteins direct transport to and from the Golgi apparatus

Protein transport at the Golgi complex utilizes both microtubule- and actin-associated motor proteins [1,3,25,26]. The cytoskeleton is also necessary for Golgi morphology and intracellular positioning. Disrupting microtubules or blocking dynein function causes a dramatic redistribution of Golgi stacks from a compact juxtanuclear structure localized at the centrosome to dispersed punctate structures localized near ER-exit sites [1,25]. Because of the Golgi apparatus' position at the centrosome (microtubule-organizing center), it is expected that transport toward the Golgi apparatus should be mediated by minus end-directed microtubule motors (dynein) and transport away from the Golgi should be mediated by plus-end-directed motors (kinesins). This has now largely been confirmed.

Anterograde post-Golgi transport involves conventional kinesins, kinesin-1. The regulation of kinesin-based post-Golgi vesicular transport is not understood, although kinesin-mediated endosome motility is regulated by multiple Rab proteins [1]. Retrograde transport from the Golgi apparatus to the ER or ER/Golgi intermediate compartment (ERGIC) appears to involve multiple classes of motor proteins [1,3,25,26]. Retrograde transport or retrieval of cargo proteins containing KDEL or dibasic ER retrieval signals is mediated by COPI vesicles. Microinjecting inhibitory antibodies into cells revealed a role for kinesin-1 in retrograde COPI-dependent transport [27]. Interestingly, RNAi-mediated knockdown of kinesin-2 expression showed that this kinesin also contributes to COPI-mediated Golgi-to-ER transport [28]. Dynein has also been implicated in COPI-independent retrograde transport from the Golgi complex [1,29]. While it is difficult to picture how a minus-end directed motor mediates transport away

from the Golgi apparatus toward the ER, this might reflect the fact that the morphological relationships among Golgi exit sites, the ERGIC, and ER entry sites are poorly understood.

Protein transport toward the Golgi apparatus is mediated by dynein through its adaptor complex dynactin. This has been shown for ER-to-Golgi transport via COPI-coated vesiculotubular clusters [30], and recently for endosome-to-Golgi trafficking of Shiga toxin [23]. Multiple protein factors have now been identified that may mediate binding interactions between the dynein/dynactin complex and Golgi vesicles. The proteins ZW10 and RINT-1 are involved in dynein function both during mitotic chromosome segregation and cytoplasmic transport in non-mitotic cells [31-34]. Interestingly, these proteins bind the ER-Golgi SNARE protein syntaxin 18, and are involved in several dynein-mediated trafficking steps including ER-to-Golgi transport. The golgin family of tethering proteins, including lava-lamp, tGolgin-1, and BicaudalD, also serve to regulate dynein recruitment or function at the Golgi apparatus [1, 25]. The GTP-binding protein Rab6 regulates BicaudalD interactions with the dynein/dynactin complex [29]. Many details regarding the precise roles of these proteins and mechanisms for regulated dynein binding to vesicles remain to be clarified. Surprisingly, clues regarding how dynein recruitment is regulated during COPI vesicle formation have emerged from studies on ARF1-dependent actin polymerization.

3.2 The cargo-sensitive binding interaction between coatomer and Cdc42 regulate actin dynamics and dynein recruitment on Golgi vesicles

Besides (or as part of) triggering COPI vesicle assembly, the GTP-binding protein ARF1 triggers actin polymerization on Golgi membranes [35]. Efforts to understand ARF1-dependent actin dynamics have converged with studies characterizing Cdc42 regulation and effectors at the Golgi complex. Cdc42 function is involved in protein transport at the Golgi complex [3, 36,37]. Together, these studies have elucidated a signaling pathway that connects ARF1 to Arp2/3-dependent actin polymerization (Figure 3) [35]. Upon ARF1 activation, Cdc42 is recruited to Golgi membranes through a binding interaction with the γ-COP subunit of the COPI vesicle coat protein, coatomer [35,38]. Importantly, this interaction is sensitive to the putative vesicle cargo receptor, p23, such that coatomer cannot be simultaneously bound to Cdc42 and p23 (Figure 3) [35,38]. A Golgi-localized Cdc42 GTPase activating protein (GAP), ARHGAP10, has been identified that is specific for ARF1-dependent Cdc42 function [39]. Interestingly, coatomer is required for Cdc42-regulated protein targeting during cell-polarity determination in yeast indicating that signaling through a coatomer/Cdc42 complex may occur in all eukaryotic cells [40].

ARF1 and Cdc42-dependent actin polymerization on Golgi membranes involves the previously characterized effectors WASP and Arp2/3 [41,42]. Reconstitution of ARF1-dependent actin polymerization on isolated Golgi membranes shows that two distinct pools of ARF1-dependent actin can be defined based on sensitivity to toxins and their interactions with two related actin-binding proteins mAbp1 and drebrin [35,43]. In addition to its role in endocytosis, the actin-binding protein cortactin is involved in post-Golgi transport and its binding to Golgi membranes also is regulated by ARF1 [44]. The binding of mAbp1 to Golgi membranes, unlike drebrin, is dependent on the coatomer/Cdc42 binding interaction. The ability to bind specifically to actin polymerized upon activation of the coatomer/Cdc42 complex is conferred by the actin-binding domains of mAbp1 [43]. These properties of mAbp1 make it a good candidate effector to function downstream of coatomer-bound Cdc42.

Attempts to identify other Cdc42-dependent vesicle-associated actin-binding proteins led to the unexpected observation that dynein recruitment to COPI vesicles is regulated by the coatomer/Cdc42 complex [36]. When the coatomer/Cdc42 binding interaction is disrupted by the p23 cargo receptor, there is an ARF1-dependent increase in vesicle associated dynein (Figure 3). Thus, coatomer-bound Cdc42 acts as a negative regulator of dynein recruitment.

This is consistent with the finding that the expression of constitutively-active Cdc42 disrupts the dynein-dependent translocation of coatomer-coated vesiculotubular clusters from ER exit sites to the Golgi complex. Cdc42 may affect multiple microtubule-dependent transport steps at the Golgi complex because inhibiting Cdc42 with the small molecule secramine blocks exit of anterograde directed cargo [37]. The ability of a cargo protein to block Cdc42 signaling and thus stimulate motor recruitment may provide a mechanism to ensure that vesicle motility is inhibited until the completion of vesicle assembly (Figure 1B).

The effects of Cdc42 on dynein recruitment involved changes in actin dynamics [36]. This indicates that there is crosstalk between actin and microtubules at the Golgi apparatus. This may imply a “handoff” type mechanism wherein an actin-dependent motility event precedes and helps regulate the subsequent microtubule motor-dependent vesicle translocation. Another possibility is that actin assembled on transport vesicles serves as a signaling scaffold to organize regulatory proteins involved in the later steps of vesicle formation such as scission and microtubule-dependent motility.

4. Structural analysis of myosin and kinesin cargo-binding domains reveals possible cargo-sensitive regulation

Actin-dependent myosin motors and microtubule-dependent kinesin motors are both comprised of large protein families. These motors share N-terminal head domains that mediate the ATP-sensitive binding to microfilaments or microtubules and C-terminal tail domains implicated in cargo binding. The yeast myosin 5 isoform, myo2p, mediates motility of multiple types of intracellular cargo. The characterization of tryptic fragments and a recently solved crystal structure reveal that the globular tail domain is divided into two distinct subdomains (Figure 4A) [45,46]. One of these domains binds to vacuoles and mediates vacuole inheritance during yeast budding. The second subdomain mediates transport vesicle motility. The crystal structure reveals that the vacuole-binding surface on subdomain I and the binding surface for vesicles on subdomain II are offset by 180 degrees. Thus two distinct cargo-binding motifs on the globular cargo tail are distant from each other and simultaneously exposed. This might also allow specific regulation for vesicle versus organelle motility (i.e. Figure 1A).

Cargo selection by kinesin motors also involves the tail domains composed of kinesin heavy- and light-chain subunits. Splicing variants of the light chain confer the ability of kinesin to bind to multiple types of cargo [47]. A longer splice variant (KLC1D) is responsible for Golgi membrane motility whereas a shorter variant (KLC1B) mediates ER motility. These studies show that myosins and kinesins expand the repertoire of cargo interactions through the presence of multiple binding surfaces within their tertiary structure (myosin 5), or alterations in their primary structure as generated by splice variants.

Kinesins exist in an inactive folded form and an active extended form. Analysis of kinesin truncation and point mutations indicate that in the folded conformation, a motif on the tail domain inhibits ATPase activity in the head domain [48,49]. Recently, structures of myosin 5 obtained by negative-staining electron microscopy reveal similar folded and unfolded states [50,51]. The myosin 5 globular tail domain also inhibits the ATPase activity of the head domain. Based on these studies, a model has been proposed wherein the motor remains in the inactive folded form until the binding of an appropriate cargo protein triggers a conformation change and activates ATP-dependent translocation (Figure 4B). This concept has important implications because it could allow motor-protein recruitment and motor function to be regulated separately. Future studies will be required to test whether this type of regulation plays a role during motor-based motility of secretory organelles and vesicles.

5. Conclusions

Over the past five years, research interest in the function and regulation of cytoskeleton-based motility in the secretory pathway has greatly increased. This interest together with improved techniques such as evanescent-field time-lapse microscopy and the reconstitution of organelle-associated cytoskeletal regulation in cell-free systems is leading to rapid advancement. Thus, the types of motor proteins and cytoskeletal filaments used for each trafficking step are now becoming defined. Each of the three major classes of transport vesicle coats COPII [52], COPI [35,36], and clathrin [6-9] form binding interactions with regulatory or structural components of the cytoskeleton-based motility machinery. Multiple types of coat-bound accessory proteins participate at the interface between vesicle formation and the cytoskeleton. It has been shown that cytoskeletal dynamics and motor protein function are regulated through the Rab, ARF and Rho families of GTP-binding proteins. Additional progress should clarify the specific contributions of individual proteins in the spatial and temporal regulation of motility as well as how these proteins adopt distinct functions as part of complexes.

An important theme that is emerging during the recent progress in this area is that the cargo proteins may not simply be passive “passengers” within vesicles, but in addition have the ability to direct cytoskeletal function and intracellular motility. As outlined above, cargo can direct interactions with actin to regulate the timing of endocytic vesicle formation. Cargo-regulated actin dynamics at the Golgi can ensure that motor proteins are only recruited upon completion of cargo packaging and coat assembly. Binding interactions between motor proteins and cargo may function not only for recruitment but also as part of the mechanism for regulating the head domain's interaction with cytoskeletal filaments. It seems likely that these represent only initial observations, and that the rapid research progress into the interface between transport vesicles and the cytoskeleton will soon offer many more examples of cargo-based regulatory mechanisms.

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List of Abbreviations

COPI, coat-protomer I; TIRF, total internal reflection fluorescence; HIP, huntingtin interacting protein, low-density lipoprotein; EGF, epidermal growth factor; ERM, ezrin/radixin/moesin; ER, endoplasmic reticulum; ERGIC, ER/Golgi intermediate compartment.

References

1. Caviston JP, Holzbaur EL. Microtubule motors at the intersection of trafficking and transport. Trends Cell Biol 2006;16:530–7. [PubMed: 16938456]
2. Soldati T, Schliwa M. Powering membrane traffic in endocytosis and recycling. Nat Rev Mol Cell Biol 2006;7:897–908. [PubMed: 17139330]
3. Egea G, Lazaro-Dieguez F, Vilella M. Actin dynamics at the Golgi complex in mammalian cells. Curr Opin Cell Biol 2006;18:168–178. [PubMed: 16488588]
4. Orci L, Stamnes M, Ravazzola M, Amherdt M, Perrelet A, Sollner TH, Rothman JE. Bidirectional transport by distinct populations of COPI-coated vesicles. Cell 1997;90:335–49. [PubMed: 9244307]
5. Gu F, Aniento F, Parton RG, Gruenberg J. Functional dissection of COP-I subunits in the biogenesis of multivesicular endosomes. J Cell Biol 1997;139:1183–95. [PubMed: 9382865]
6. Kaksonen M, Toret CP, Drubin DG. Harnessing actin dynamics for clathrin-mediated endocytosis. Nat Rev Mol Cell Biol 2006;7:404–14. [PubMed: 16723976]

7. Perrais D, Merrifield CJ. Dynamics of endocytic vesicle creation. Dev Cell 2005;9:581–92. [PubMed: 16256734]
8. Smythe E, Ayscough KR. Actin regulation in endocytosis. J Cell Sci 2006;119:4589–98. [PubMed: 17093263]
9. Toret CP, Drubin DG. The budding yeast endocytic pathway. J Cell Sci 2006;119:4585–7. [PubMed: 17093262]
10. Kaksonen M, Sun Y, Drubin DG. A pathway for association of receptors, adaptors, and actin during endocytic internalization. Cell 2003;115:475–87. [PubMed: 14622601]
11. Kochubey O, Majumdar A, Klingauf J. Imaging clathrin dynamics in Drosophila melanogaster hemocytes reveals a role for actin in vesicle fission. Traffic 2006;7:1614–27. [PubMed: 17014698]
12. Merrifield CJ, Feldman ME, Wan L, Almers W. Imaging actin and dynamin recruitment during invagination of single clathrin-coated pits. Nat Cell Biol 2002;4:691–8. [PubMed: 12198492]
13. Kaksonen M, Toret CP, Drubin DG. A modular design for the clathrin- and actin-mediated endocytosis machinery. Cell 2005;123:305–20. [PubMed: 16239147]
14. Legendre-Guillemin V, Metzler M, Lemaire JF, Philie J, Gan L, Hayden MR, McPherson PS. Huntingtin interacting protein 1 (HIP1) regulates clathrin assembly through direct binding to the regulatory region of the clathrin light chain. J Biol Chem 2005;280:6101–8. [PubMed: 15533941]
15. Legendre-Guillemin V, Wasiak S, Hussain NK, Angers A, McPherson PS. ENTH/ANTH proteins and clathrin-mediated membrane budding. J Cell Sci 2004;117:9–18. [PubMed: 14657269]
16. Newpher TM, Idrissi FZ, Geli MI, Lemmon SK. Novel function of clathrin light chain in promoting endocytic vesicle formation. Mol Biol Cell 2006;17:4343–52. [PubMed: 16870700]
17. Newpher TM, Lemmon SK. Clathrin is important for normal actin dynamics and progression of Sla2p-containing patches during endocytosis in yeast. Traffic 2006;7:574–88. [PubMed: 16643280]
18. Brett TJ, Legendre-Guillemin V, McPherson PS, Fremont DH. Structural definition of the F-actin-binding THATCH domain from HIP1R. Nat Struct Mol Biol 2006;13:121–30. [PubMed: 16415883]
19. Toshima J, et al. Negative Regulation of Yeast Eps15-like Arp2/3 Complex Activator, Pan1p, by the Hip1R-related Protein, Sla2p, during Endocytosis. Mol Biol Cell. 2006
20. Le Clainche C, Pauly BS, Zhang CX, Engqvist-Goldstein AE, Cunningham K, Drubin DG. A Hip1R-cortactin complex negatively regulates actin assembly associated with endocytosis. Embo J. 2006(in Press)
21. Lakadamyali M, Rust MJ, Zhuang X. Ligands for clathrin-mediated endocytosis are differentially sorted into distinct populations of early endosomes. Cell 2006;124:997–1009. [PubMed: 16530046]
22. Puthenveedu MA, von Zastrow M. Cargo regulates clathrin-coated pit dynamics. Cell 2006;127:113–24. [PubMed: 17018281]
23. Hehnly H, Sheff D, Stamnes M. Shiga toxin facilitates its retrograde transport by modifying microtubule dynamics. Mol Biol Cell 2006;17:4379–89. [PubMed: 16885418]
24. Takenouchi H, Kiyokawa N, Taguchi T, Matsui J, Katagiri YU, Okita H, Okuda K, Fujimoto J. Shiga toxin binding to globotriaosyl ceramide induces intracellular signals that mediate cytoskeleton remodeling in human renal carcinoma-derived cells. J Cell Sci 2004;117:3911–22. [PubMed: 15265987]
25. Allan VJ, Thompson HM, McNiven MA. Motoring around the Golgi. Nat Cell Biol 2002;4:E236–42. [PubMed: 12360306]
26. Palmer KJ, Watson P, Stephens DJ. The role of microtubules in transport between the endoplasmic reticulum and Golgi apparatus in mammalian cells. Biochem Soc Symp 2005:1–13. [PubMed: 15649125]
27. Lippincott-Schwartz J, Cole NB, Marotta A, Conrad PA, Bloom GS. Kinesin is the motor for microtubule-mediated Golgi-to-ER membrane traffic. J Cell Biol 1995;128:293–306. [PubMed: 7844144]
28. Stauber T, Simpson JC, Pepperkok R, Vernos I. A role for kinesin-2 in COPI-dependent recycling between the ER and the Golgi complex. Curr Biol 2006;16:2245–51. [PubMed: 17113389]
29. Fuchs E, Short B, Barr FA. Assay and properties of rab6 interaction with dynein-dynactin complexes. Methods Enzymol 2005;403:607–18. [PubMed: 16473624]

30. Murshid A, Presley JF. ER-to-Golgi transport and cytoskeletal interactions in animal cells. Cell Mol Life Sci 2004;61:133–45. [PubMed: 14745493]

31. Arasaki K, Tani K, Yoshimori T, Stephens DJ, Tagaya M. Nordihydroguaiaretic Acid Affects Multiple Dynein-dynactin Functions in Interphase and Mitotic Cells. Mol Pharmacol 2007;71:454–60. [PubMed: 17105871]

32. Arasaki K, Taniguchi M, Tani K, Tagaya M. RINT-1 regulates the localization and entry of ZW10 to the syntaxin 18 complex. Mol Biol Cell 2006;17:2780–8. [PubMed: 16571679]

33. Vallee RB, Varma D, Dujardin DL. ZW10 Function in Mitotic Checkpoint Control, Dynein Targeting and Membrane Trafficking: Is Dynein the Unifying Theme? Cell Cycle 2006;5epublished ahead of print

34. Varma D, Dujardin DL, Stehman SA, Vallee RB. Role of the kinetochore/cell cycle checkpoint protein ZW10 in interphase cytoplasmic dynein function. J Cell Biol 2006;172:655–62. [PubMed: 16505164]

35. Stamnes M. Regulating the actin cytoskeleton during vesicular transport. Curr Opin Cell Biol 2002;14:428–33. [PubMed: 12383793]

36. Chen JL, Fucini RV, Lacomis L, Erdjument-Bromage H, Tempst P, Stamnes M. Coatomer-bound Cdc42 regulates dynein recruitment to COPI vesicles. J Cell Biol 2005;169:383–389. [PubMed: 15866890]

37. Pelish HE, et al. Secramine inhibits Cdc42-dependent functions in cells and Cdc42 activation in vitro. Nat Chem Biol 2006;2:39–46. [PubMed: 16408091]

38. Chen JL, Xu W, Stamnes M. In vitro reconstitution of ARF-regulated cytoskeletal dynamics on Golgi membranes. Methods Enzymol 2005;404:345–58. [PubMed: 16413281]

39. Dubois T, Paleotti O, Mironov AA, Fraisier V, Stradal TE, De Matteis MA, Franco M, Chavrier P. Golgi-localized GAP for Cdc42 functions downstream of ARF1 to control Arp2/3 complex and F-actin dynamics. Nat Cell Biol 2005;7:353–64. [PubMed: 15793564]

40. Rida PC, Surana U. Cdc42-dependent localization of polarisome component Spa2 to the incipient bud site is independent of the GDP/GTP exchange factor Cdc24. Eur J Cell Biol 2005;84:939–49. [PubMed: 16325503]

41. Chen JL, Lacomis L, Erdjument-Bromage H, Tempst P, Stamnes M. Cytosol-derived proteins are sufficient for Arp2/3 recruitment and ARF/coatomer-dependent actin polymerization on Golgi membranes. FEBS Lett 2004;566:281–6. [PubMed: 15147909]

42. Matas OB, Martinez-Menarguez JA, Egea G. Association of Cdc42/N-WASP/Arp2/3 signaling pathway with Golgi membranes. Traffic 2004;5:838–46. [PubMed: 15479449]

43. Xu W, Stamnes M. The actin-depolymerizing factor homology and charged/helical domains of drebrin and mAbp1 direct membrane binding and localization via distinct interactions with actin. J Biol Chem 2006;281:11826–33. [PubMed: 16452483]

44. Cao H, Weller S, Orth JD, Chen J, Huang B, Chen JL, Stamnes M, McNiven MA. Actin and Arf1-dependent recruitment of a cortactindynamin complex to the Golgi regulates post-Golgi transport. Nat Cell Biol 2005;7:483–92. [PubMed: 15821732]

45. Pashkova N, Catlett NL, Novak JL, Wu G, Lu R, Cohen RE, Weisman LS. Myosin V attachment to cargo requires the tight association of two functional subdomains. J Cell Biol 2005;168:359–64. [PubMed: 15684027]

46. Pashkova N, Jin Y, Ramaswamy S, Weisman LS. Structural basis for myosin V discrimination between distinct cargoes. Embo J 2006;25:693–700. [PubMed: 16437158]

47. Wozniak MJ, Allan VJ. Cargo selection by specific kinesin light chain 1 isoforms. Embo J 2006;25:5457–68. [PubMed: 17093494]

48. Hackney DD, Stock MF. Kinesin's IAK tail domain inhibits initial microtubule-stimulated ADP release. Nat Cell Biol 2000;2:257–60. [PubMed: 10806475]

49. Seiler S, Kirchner J, Horn C, Kallipolitou A, Woehlke G, Schliwa M. Cargo binding and regulatory sites in the tail of fungal conventional kinesin. Nat Cell Biol 2000;2:333–8. [PubMed: 10854323]

50. Li XD, Jung HS, Mabuchi K, Craig R, Ikebe M. The globular tail domain of myosin Va functions as an inhibitor of the myosin Va motor. J Biol Chem 2006;281:21789–98. [PubMed: 16757473]

51. Thirumurugan K, Sakamoto T, Hammer JA 3rd, Sellers JR, Knight PJ. The cargo-binding domain regulates structure and activity of myosin 5. Nature 2006;442:212–5. [PubMed: 16838021]

52. Watson P, Forster R, Palmer KJ, Pepperkok R, Stephens DJ. Coupling of ER exit to microtubules through direct interaction of COPII with dynactin. Nat Cell Biol 2005;7:48–55. [PubMed: 15580264]

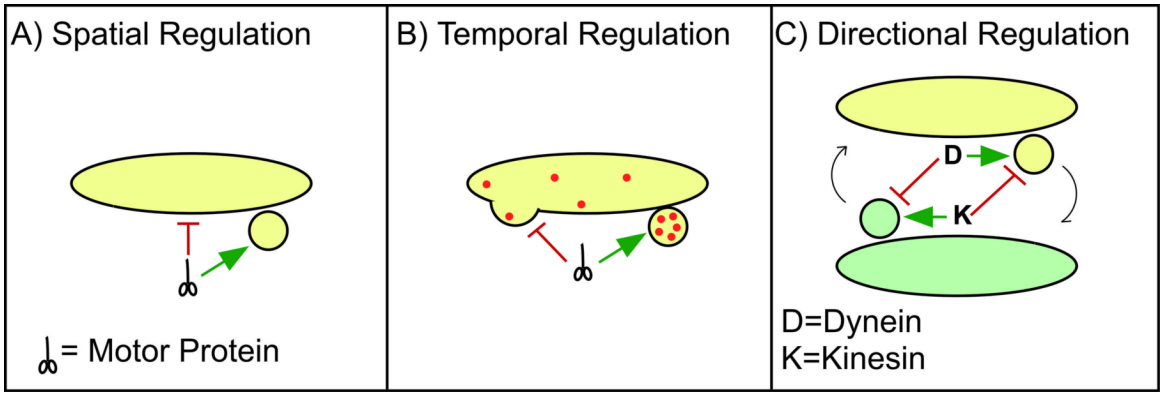

Figure 1. Motor protein-mediated motility of organelles and transport vesicles requires spatial, temporal, and directional regulation

(A) Mechanisms for spatial regulation must exist to ensure that while a nascent vesicle binds to a motor and becomes motile, the donor organelle does not. (B) Temporal regulation should occur in order to coordinate motor recruitment with other steps in vesicle formation. Motor-based motility should be blocked until vesicle coat assembly, cargo packaging, and vesicle scission have been completed (C) In the cases where vesicles or cargo are recycled or undergo bidirectional transport, regulatory processes must ensure that the correct motor is functional. For example, during recycling between the ER and the Golgi apparatus coatomer-coated vesiculotubular clusters undergo dynein-mediated anterograde transport, whereas coatomer-coated COPI vesicles mediating retrograde transport from the Golgi apparatus utilize kinesin motors.

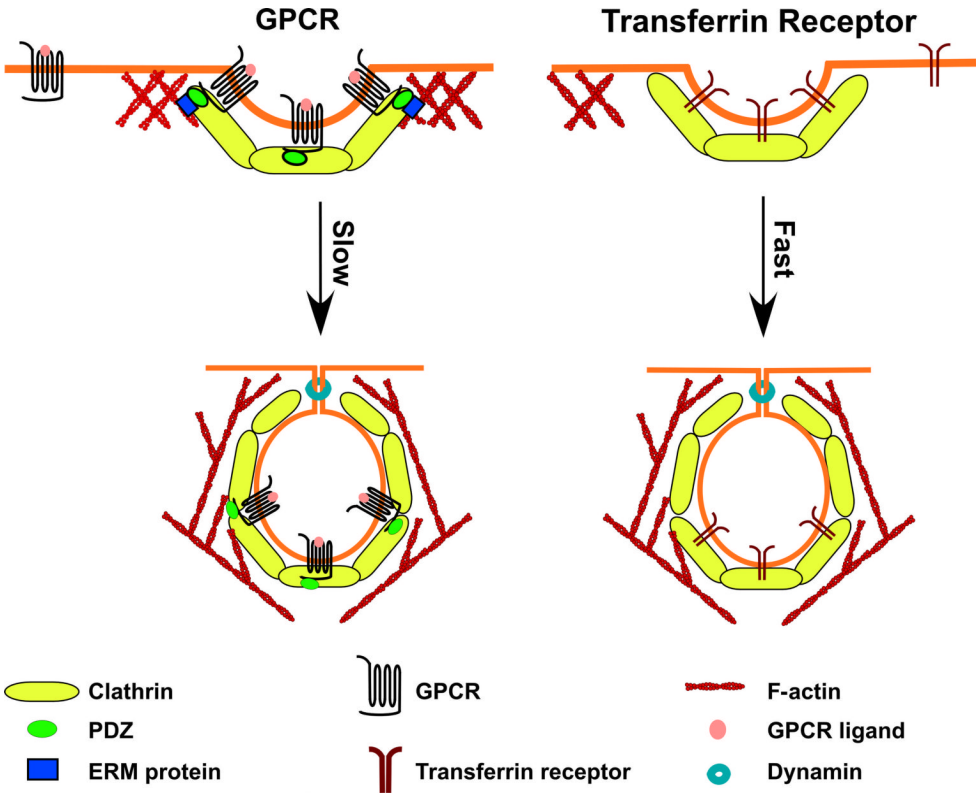

Figure 2. Some activated G-protein coupled receptors undergo delayed internalization by directing interactions between clathrin-coated vesicles and the actin cytoskeleton

G-protein coupled receptors (GPCRs) are recruited to clathrin-coated pits after ligand binding (Left). For GPCRs that contain PDZ-ligand domains, an anchor between the forming vesicle and the actin cytoskeleton may be formed via PDZ-domain-containing proteins and the ERM-family of actin binding proteins. This creates clathrin-coated pits with delayed internalization and extended cell-surface residence times [22]. The transferrin receptor (Right) is also internalized through clathrin-coated pits. However, transferrin lacks a PDZ domain and does not form ERM-dependent interactions with cortical actin. Thus for transferring containing vesicles, the scission and internalization steps proceed rapidly.

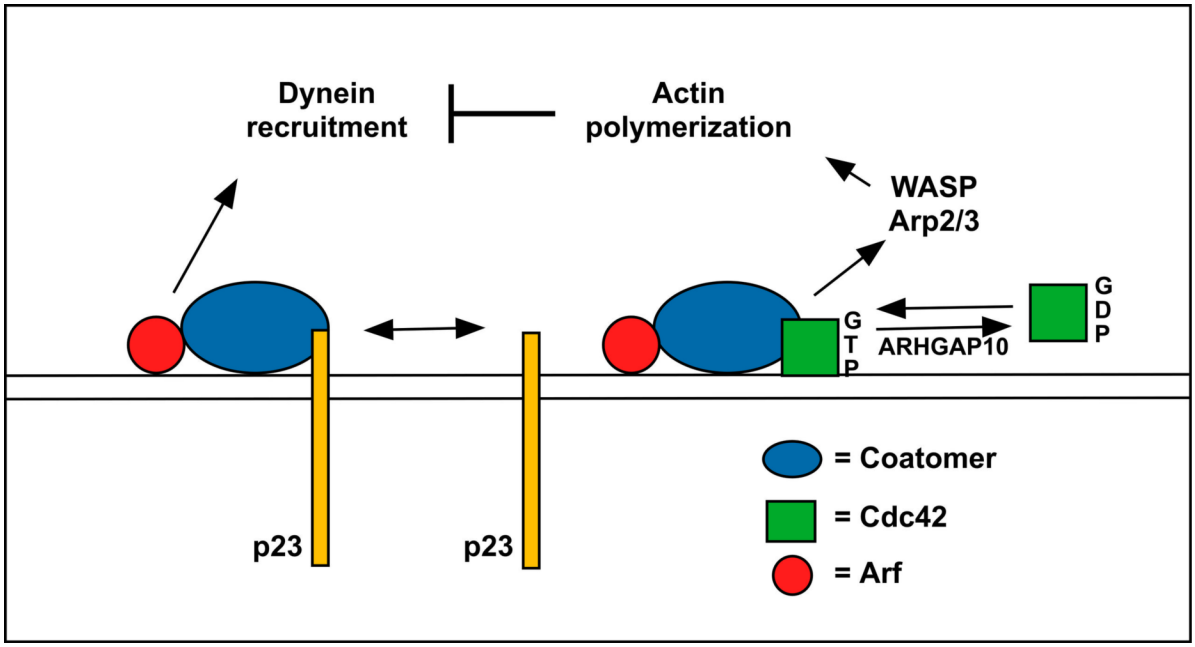

Figure 3. Actin dynamics and dynein recruitment on COPI vesicles is regulated through a cargo-sensitive binding interaction between coatomer and Cdc42

Actin polymerization on Golgi vesicles is stimulated by the ARF1-dependent recruitment of a complex between the COPI-coat protein, coatomer and the Rho-family GTP-binding protein, Cdc42 [35]. ARF1-dependent Cdc42 function at the Golgi apparatus is specifically regulated by the GTPase activating protein, ARHGAP10 [39]. Active Cdc42 also inhibits dynein recruitment in an actin-dependent manner [36]. Coatomer cannot be simultaneously bound to Cdc42 and the p23 putative cargo receptor. Thus, the presence of p23 acts to block actin polymerization and stimulate dynein recruitment. This signaling may ensure that vesicle motility does not commence until the completion of vesicle assembly and cargo packaging.

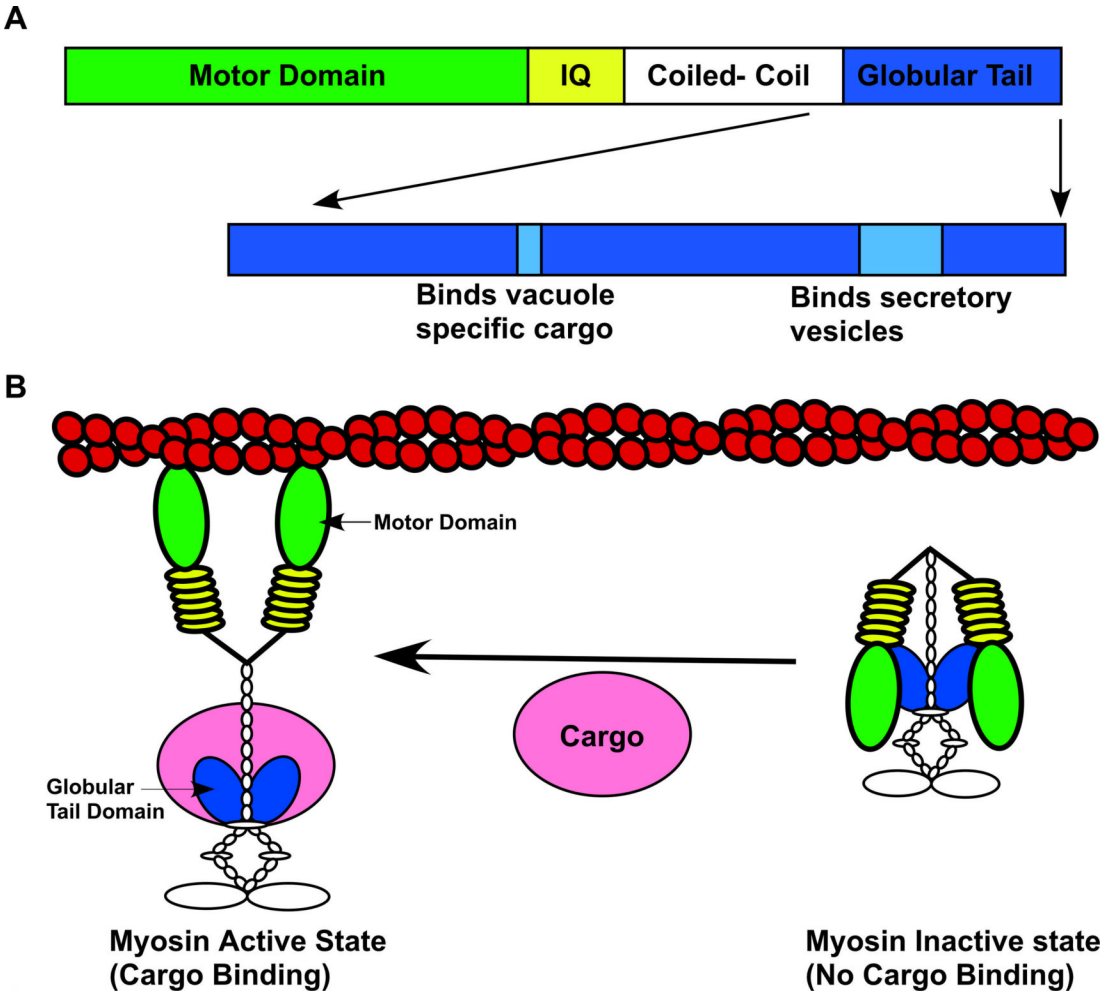

Figure 4. Cargo binding to the globular tail domain of myosin 5a regulates its structure and activity

(A) The heavy chain of myosin 5 contains an N-terminal head domain and a C-terminal globular tail domain. The head domain contains the binding sites for actin and ATP. The globular tail domain contains the cargo-binding sites. Structural analysis of the yeast myosin 5 protein, Myo2p, reveals that the globular tail contains two distinct cargo-binding modules one for transport vesicles and a second for vacuoles [45,46]. (B) The globular tail of myosin 5a can bind and inhibit the ATPase activity of the N-terminal head domain. It is proposed that cargo binding to the globular tail domain of myosin 5 causes a conformation change leading to the activation of the motor [50,51].