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The role of motor proteins in endosomal sorting

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Abstract

Microtubule motor proteins play key roles in the spatial organization of intracellular organelles as well as the transfer of material between them. This is well illustrated both by the vectorial transfer of biosynthetic cargo from the endoplasmic reticulum to the Golgi apparatus as well as the sorting of secretory and endocytic cargo in the endosomal system. Roles have been described for dynein and kinesin motors in each of these steps. Cytoplasmic dynein is a highly complex motor comprised of multiple subunits that provide functional specialization. The family of human kinesins includes over 40 members. This complexity provides immense functional diversity yet little is known of the specific requirements and functions of individual motors during discrete membrane trafficking steps. Here, we describe some of the latest findings in this area that seek to define the mechanisms of recruitment and control of activity of microtubule motors in spatial organization and cargo trafficking through the endosomal network.

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Introduction

Eukaryotic cells are compartmentalized into distinct organelles that are composed of specific cohorts of proteins and lipids. Precise transport mechanisms are therefore required to direct molecules to the right compartment, to generate and maintain their identity and their specific functions. An elegant example of the complexity and functional specialization of related membranous organelles comes from the endosomal network. This system is comprised not only of distinct organelles but also of discrete membrane domains within organelles; it also underpins essential cell functions including the control of growth factor and morphogen signaling, cell adhesion and migration, and functional specializations such as the formation of dendritic spines and primary cilia. In the present review, we focus on the role of microtubule-based motor proteins in endosomal sorting and give an update on their close relationships with different key players of endosomal sorting including sorting nexins and Rab proteins.

Endosomal sorting

Endocytosis is the process by which cells internalize molecules from the cell surface. Endocytic vesicles initially mature and either homotypically fuse, or fuse with pre-existing organelles known historically as early endosomes (EE), now more commonly referred to as sorting endosomes (SE) [1]. Here sorting events direct cargoes either to recycling pathways back to the plasma membrane or to a degradative pathway via late endosomes and lysosomes. Other cargoes are ultimately delivered to the *trans*-Golgi network (TGN). In its simplest form, this system can be considered to comprise sorting endosomes, recycling endosomes, multi-vesicular bodies and lysosomes [2]. These primary endosomal trafficking pathways are summarized in figure 1. The machinery used to drive these pathways can be defined according to the localization of key molecular markers including the phosphoinositide phospholipids, Rab proteins and the sorting nexins (SNXs) [3].

After their incorporation in the sorting endosome (SE), receptors are sent back to the plasma membrane, to the late endosome or to the endocytic recycling compartment (ERC). There are two main recycling pathways back to the plasma membrane from the SE. Some cargoes are recycled directly to the plasma membrane [4] whereas others are recycled via the ERC [5]. Rab proteins are small GTPases and play essential regulatory roles in intracellular trafficking pathways. Rab4 and Rab5 are localized to a fast recycling pathway whereas a slower recycling pathway is defined by Rab4 and Rab11 [6]. The ERC plays also a role in the retrograde transport to the TGN [1]. A key player in endosomal sorting is the retromer complex, a multi-protein complex that associates with endosomes and mediates retrograde transport from the SE to the TGN [7]. In mammals, the retromer pathway includes a sorting nexin (SNX) dimer (SNX1 or SNX2 with SNX5 or SNX6) and a cargo recognition trimer Vps26-Vps29-Vps35 [7, 8]. Sorting of cargo to the TGN occurs via a tubulation event where the membrane deformation potential of the SNX proteins is central [8]. Increasing evidence suggests that members of the SNX family are key factors in defining the organization and dynamics of the endosomal network. Their ability to deform membranes is likely to be central to their ability to mediate selective trafficking through this network.

Microtubule-based motor proteins in the endosomal system

Evidence exists that different cargoes are sorted into distinct populations of clathrin coated vesicles, a notable example being the sorting of G-protein coupled receptors [9]. Other work has suggested that cargoes destined for degradation are targeted preferentially to rapidly maturing SE, while those destined for recycling are targeted to all vesicles [10]. One potential mechanism driving cargo sorting within this system is the integration between certain endocytic adaptors and microtubule-dependent motility. Considerable further evidence also implicates motors in endocytic sorting.

Microtubule-based motor proteins are required for intracellular movements, organization and positioning of membrane-bound organelles (recently reviewed in [11]). In *Dictyostelium*, dynein and kinesin have been shown to be both required for the bidirectional movements of endosomes [12]. Kinesin-1, involved in the early endosome movements, and kinesin-2, requisite for early and late endosome movements, are indeed the major motors [13]. Opposing motors dynein and kinesin-3 have been shown to be both required for retrograde and anterograde movements of SE – the direction of transport being driven by dynein binding to the organelle [14].

KIF13A or kinesin-3 has been shown to interact with the clathrin-adaptor-1 or AP-1 and this interaction promotes the formation of the peripheral recycling compartment in melanocytes [15]. KIF16B (also called SNX23) is another member of kinesin-3 subfamily that drives SE movements towards the cell periphery and is also essential for modulating the balance between receptor recycling and degradation. Interestingly, this specific kinesin, a member of the sorting nexin family, is localized to the phosphatidylinositol-3-phosphate (PtdIns-3P)-enriched early endosome membranes via its Phox-homology (PX) domain [16] suggesting a mechanism of direct coupling of the motor to the membrane.

Opposing motors are also contributing to the separation of tubular and vesicular endosomal subdomains hence being key players during the sorting process. EGF receptor, destined to the degradation pathway and transferrin receptor, destined to the recycling pathway, enter the same sorting endosome and microtubule-based motor dynein is required for the efficient separation of these cargoes within the SE [17]. With such body of evidence placing both dynein and kinesin motor proteins at the early endosome, with specific roles for different family members, microtubule-based motors are suggested to play a key role in defining the specificity of cargo sorting during endosomal sorting.

Given that transport from the cell cortex to the juxtannuclear region is minus-end directed, a key player in these trafficking events is clearly the dynein motor. A core role for this motor in the motility of endosomal and lysosomal compartments is well supported in the literature [18-22]. More recent data has focused on the individual components of dynein and how this single motor could define such a complex array of trafficking pathways. The multisubunit nature of the dynein complex leads on to postulate about the potential for selective targeting of this motor to different membranes. Furthermore, data also suggests that its partner complex, dynactin [23], is also involved in directing either the localization or activity of the dynein complex through interaction with other membrane bound protein complexes [21, 22, 24]. One focus for this work has been the dynein light intermediate chains (LIC1 and LIC2). Homodimerization of these subunits [25] therefore specifies LIC1- or LIC2-containing subpopulations of dynein. Work from our own lab using siRNA depletion of

these subunits revealed LIC1 to act primarily in the organization of the ER/Golgi interface, notably in maintaining the structure of the Golgi. In contrast LIC2 was implicated in positioning of recycling endosomes [26]. It is important to note here that our work was focused on the use of transferring as a reporter of the recycling endosomes and did not include any analysis of other endosomal markers [26]. However, other work has defined a role for LIC1 and LIC2 in dynein recruitment to late endosomes and lysosomes [27]. Data supports the idea of LIC specificity – pericentrin binds to LIC1 but not LIC2 [25] while LIC2 but not LIC1 appears to interact with PAR3 to define a functional complex involved in cell polarization [28]. Other work has shown a differential localization of LIC1 and LIC2 as cell progress through mitosis [29]. Rab11 also appears to direct dynein recruitment through its effector FIP3, but here no LIC specificity is evident [30, 31]. These data could be reconciled in many ways. It is highly likely that only one (or at most a few) dyneins are involved in organelle movement (Steinberg's lab recently showed that in *Ustilago maydis*, a single dynein is involved in the translocation of early endosomes [14]). Cell type differences as well as the efficacy of siRNA depletions might explain differences in the effects on Golgi organization on LIC depletion. Our more recent data supports a selective role for the LICs in individual membrane trafficking steps, even within the complex endosomal network (SDH and DJS, unpublished data).

The importance of bidirectional transport

Nearly all cargos are transported bidirectionally and little is known about the coordination between opposing motors. For many cargos, interference with their plus- or minus-directed motor protein completely disrupts their movement suggesting a strong link between dynein and kinesin. Bidirectional movements have been described previously for ER exit sites (ERES) and were proved being driven by microtubule-associated motor proteins [32]. Whether bidirectional movements are due to a motor coordination or a tug-of-war between opposing motors is still hotly discussed and likely depends on the system under investigation [33, 34]. With multiple examples of specific associations of motor proteins with certain cargo (e.g. [14, 34]), one might consider these associations being the key role of microtubule-based motors in defining the specificity of cargo sorting during endosomal sorting.

Rab proteins and microtubule-based motor proteins

Rab proteins are crucial endocytic regulators. These small GTP-binding proteins cycle between an inactive GDP-bound state and an active GTP-bound state. In their active state, they are interacting and recruiting proteins known as Rab effectors. The latter have many roles including vesicle tethering, fusion, budding and motility [35]. Major populations of endosome have been identified that label with a mosaic of Rab4, Rab5, Rab7, and Rab11 positive. Rab4 and Rab5 define early / sorting endosomes whereas the Rab11 is classically used to describe the juxtannuclear recycling endosome [35]. Rab7 and Rab9 are late endosomal markers. The early endosome matures into late endosome via a process of morphological changes. Contents from early endosome to late endosome switch between different markers (Rab5 to Rab7) and the composition of the phospholipids layer also undergoes changes (PtdIns-3P to PtdIns-3,5-P₂) [36]. Rab proteins and their effectors are not only required for budding and fusion events but they also regulate movements of vesicles and organelles along microtubules and actin filaments see [37]. An increasing number of evidence shows associations between these small GTPase and microtubule-based motors (Table 1). Rab proteins are

therefore determinants on the directionality of vesicle movements. Table 1 provides some principal examples of Rab motor coupling; many other Rabs are known to couple to kinesin family members as well as dynein and also myosin superfamily motors (for some nice examples, see [37]).

Sorting nexins and microtubule-based motor proteins

The composition in phosphoinositides at the SE is controlled at least in part by Rab5. The phosphoinositides play a major role in defining endosomal identity through specifying recruitment of effector molecules, including motor proteins (Rab5 for example, see Table 1). This defined identity is also crucial for the selective recruitment of sorting nexins (SNXs). Thus the relationship between Rabs and SNXs is likely to dictate a further layer of complexity in terms of motor coupling.

Sorting nexins

The sorting nexin family is a group of cytoplasmic and membrane-associated proteins involved in protein and membrane trafficking [3]. Sorting nexins are all characterized by the presence of a specific type of domain, Phox-homology domain or PX domain, a sequence of 100 to 130 amino-acids [38]. These particular domains are phosphoinositide-binding motifs that help the association between their host protein and the membranes enriched in these lipids. PX domains have been shown to mostly interact with PtdIns-3P, lipids present on the cytosolic face of endosomes. This ability to bind certain lipids is a key part of the mechanism of targeting these proteins to cellular membranes. Some sorting nexins also contain a BAR domain (Bar/Amphiphysin/Rvs) which binds to highly curved membrane, or can impose curvature upon membranes inducing the generation of tubulovesicular elements. These proteins form a sub-family called SNX-BARs [8] where the PX and BAR domains cooperate in membrane targeting through coincidence detection (e.g. see [39, 40]). The ability of sorting nexins to associate with PtdIns-3P enriched membrane of the early endocytic network, drive membrane deformation, and couple to microtubule motors, makes them central players in driving endosomal dynamics [3].

Coordination of endosomal sorting and motor proteins

Endosomal sorting is thought to be orchestrated by complex interactions between sorting nexins, cargo, and many other components. Cargo sorting occurs within tubular subdomains of interconnected organelle membranes. Tubulation here provides a potential mechanism for geometric sorting where cargo segregates into tubules purely on the basis of membrane area. One of the tubular elements enriched in SNX-BARs is the endosome-to-TGN transport carrier (ETC) that is characterized by the presence of SNX1. SNX1 and SNX4 may be both in the same early endosome compartment but they generate distinct tubules [3]. It is possible that interactions between dynein and different SNXs together coordinate endosomal sorting [36]; there might also be roles for other motor proteins in SNX-mediated sorting events. Both SNX1 and SNX4 couple to dynein yet operate within discrete transport pathways. SNX1 is a retromer component dictating transport of cargo from endosome to TGN, while SNX4 is involved in transferrin receptor recycling (also called the bulk recycling pathway). Interestingly, SNX4 has also been shown to interact with clathrin and dynein; after the release of clathrin, dynein could bind SNX4 and mediate retrograde transport to the Golgi, hence the suggested role for clathrin as a regulator of SNX4-dependent transport [41]. One possible explanation for this is that the SNXs couple via distinct adaptor molecules, SNX4 via KIBRA to dynein [40] while SNX1 binds dynactin [42].

Molecular motors are thought to drive fission events as well as transporting cargo-loaded carrier to the recipient membrane. Mammalian retromer function includes SNX1/SNX2 and SNX5/SNX6; these proteins could act to generate a tubular subdomain within the early endosome providing a nucleation point for tubule formation. Indeed, suppression of dynactin can induce the generation of very long retromer tubules unable to separate from the early endosome [42]. Tubule-based sorting has also recently been shown to occur to segregate the beta-2 adrenergic receptor, from bulk recycling proteins and the degrading delta-opioid receptor using an actin, and sequence-dependent mechanism [43]. Whether specific motifs within the cytoplasmic domains of cargo molecules also engage or modulate SNX/motor function remains to be defined. As well as tubulation, the association of sorting nexins and motor proteins might help drive membrane fission by providing longitudinal tension. This concept of longitudinal tension as a driving force for membrane fission is also a feature of dynamin function [44].

It is highly likely that other SNX proteins have key roles in the endosomal network. For example, SNX8 localizes to early endosome and partially colocalizes with the retromer complex, its suppression has a direct effect on the retrograde transport endosome-to-TGN trafficking [45], but little is known about the biology and biochemistry of this protein [36]. Whether SNX8 couples to motor proteins remains to be seen. Open questions remain about how many of the SNX proteins might couple to motors and notably whether they couple solely to dynein or to other motors (such as kinesin family members) in addition. GFP-tagging and siRNA depletion means that we have the tools to more generate a “motor map” of the endocytic network and derive more functional data relating to the specific dynein components that are required and the kinesin family members that might also be involved.

Conclusion and perspectives

Highlighted in this review are the multiple specific interactions between specific motor proteins and cargo, SNX and Rab proteins. We suggest these associations being the key mechanism of specific sorting, transports and tethering – placing microtubule-based motor proteins as active players in these specific mechanisms.

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Figure Legends

Figure 1: Endosomal trafficking pathways

This schema summarizes the primary endosomal trafficking pathways. The different compartments are indicated in grey and transport steps represented by arrows. Clathrin coat is depicted in red. Endocytosis pathways are represented in purple, recycling pathways in green, degradation pathway in orange and retromer transports in blue. Other pathways and coat proteins exist but are not depicted in this figure.

Table 2: Some key examples of coupling of Rabs to motors

Rab1A	Rab1a regulates the motility of early endocytic vesicles by recruiting the motor KIFC1 to these vesicles [46].
Rab4	Rab4, for instance, interacts with kinesin-2 and directly with microtubules – interactions that are crucial for the exocytosis of specific cargos [47].
Rab5	Rab5 regulates the entry of cargo from the plasma membrane, the generation of phosphatidylinositol-3-phosphate (PtdIns-3P), and the motility of the SE on actin and microtubules tracks. The major role of Rab5 is to promote membrane tethering and fusion of endocytic vesicles with the SE [6, 35]. Rab5 is also clearly implicated in motility of early endosomes: Rab5 and PtdIns-3P are both required for the recruitment of Kif16B to early endosomes [16]. Dynein dependent transport of Rab5-positive endosomes is also important in neuronal branching [48].
Rab7	Rab7 associates with late endosome / lysosome compartments and controls transport in these compartments. Involved in the regulation of EGF and EGF-R degradation, along with Rab5, Rab7 is also as a key regulator in the recruitment of the retromer complex at the early endosome [49]. Rab7 effector, involved in late endosome motility (RILP), recruits dynein motor and control lysosomal transport by preventing further cycling of Rab7 – consequently, late endosome and lysosome are transported by microtubule-based motors towards microtubule-minus-ends [50].
Rab11	The Rab11 effector protein FIP3 binds dynein light chain 2 and form a complex with Rab11 and dynein that mediates transport to the ERC [30, 31].

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