Novel regulators of endosome dynamics, MHCII antigen presentation and chemosensitivity

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Chapter 1:
ER contact sites direct late endosome transport

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Abstract

Endosomes shuttle select cargoes between cellular compartments and, in doing so, maintain intracellular homeostasis and enable interactions with the extracellular space. Directionality of endosomal transport critically impinges on cargo fate, as retrograde (microtubule minus-end-directed) traffic delivers vesicle contents to the lysosome for proteolysis, while the opposing anterograde (plus-end-directed) movement promotes recycling and secretion. Intriguingly, the endoplasmic reticulum (ER) is emerging as a key player in spatiotemporal control of late endosome and lysosome transport, through the establishment of physical contacts with these organelles. Earlier studies have described how minus-end-directed motor proteins become discharged from vesicles engaged at such contact sites. Now, Raiborg et al. implicate ER-mediated interactions, induced by protrudin, in loading plus-end-directed motor kinesin-1 onto endosomes, thereby stimulating their transport toward the cell's periphery. In this review, we recast the prevailing concepts on bidirectional late endosome transport and discuss the emerging paradigm of inter-compartmental regulation from the ER-endosome interface viewpoint.

Introduction

The endocytic compartment is both a critical mediator of intracellular homeostasis and a front line negotiator between the cell and its environment. Captured by plasma membrane invagination and subsequent pinching off of a nascent endosome, internalized cargo progresses through a variety of vesicle maturation stages, each defined by a unique repertoire of resident markers and small GTPases from the Rab, Arl and Arf families. When in their active GTP-bound state, the latter function as scaffolds for assembly of transport and fusion machineries on target vesicles [1]. The first stop along the maturation journey is the early endosome (EE) sorting platform, from where cargo is either recycled or targeted downstream to the late endosome (LE) stage. LEs in turn fuse with or mature into degradative lysosomes (Ly), characterized by an acidic luminal pH and proteolytic enzymes warranting cargo degradation and redistribution of catabolized materials [2]. In addition to extracellular contents and those derived from the plasma membrane, newly synthesized proteins can gain direct access to the LE compartment through the vesicular arm of the trans-Golgi network [3]. Moreover, cytosolic materials and even whole organelles, once incorporated into autophagic vesicles, can be targeted for proteolysis in the lysosome [4]. Within this dynamic membrane system, consisting of a wide variety of specialized vesicles, LEs encounter traffic from the endocytic, biosynthetic and autophagic components alike, and thus occupy its logistic epicenter. In this review, we discuss recent insights into the transport mechanisms of LEs and Lys (hereafter collectively referred to as LEs, unless stated otherwise), with special emphasis on the influence of the ER in this context.

LE transport is orchestrated by Rab7 and its effector proteins

To accommodate long-range endosome movement, required for communication between distant membranes, microtubule-based motor proteins drive cargo either towards (minus-end) or away from (plus-end) the microtubule-organizing center

(MTOC) [5]. Because the balance between retrograde dynein and anterograde kinesin motor binding determines the net directionality of movement and maturation of affected endosomes [2,5], LE transport is inherently linked to cargo localization and fate [6]. Interestingly, most endosomes inside the cell move bi-directionally in a stop-and-go manner, rather than linearly towards either the periphery or the perinuclear region [5,7]. This implies either that specific motor proteins are likely retained for short periods of time, or that additional regulatory mechanisms are in place to monitor and influence switching between opposing transport machineries. Currently, formidable evidence is building up in support of the latter hypothesis, where different effectors responsible for recruitment of opposing motor functions have been shown to utilize a common and potentially competitive mode of LE recognition.
The LE compartment is decorated by the GTPase Rab7, which recruits effectors facilitating endosome transport along microtubule tracks as well as their fusion with lysosomes and autophagosomes (RILP and PLEKHM1, respectively [8-10]). For LEs to move into the perinuclear area of the cell, Rab7 calls on RILP [11,12], an effector that binds to the dynactin subunit of the dynein motor complex [13]. Concurrent association of RILP with the HOPS complex then induces fusion with the lysosome, thereby coupling transport to maturation processes [8]. Conversely, to move into the cell's periphery, Rab7 turns to its effector FYCO1 [14], which attracts the light chain-2 of kinesin-1 (KLC2), thereby linking the plus-end-directed motor complex to LEs [6]. Alternatively, kinesin-1 can be targeted to LEs by the Arl8 effector, SKIP [15]. It is currently unclear whether the GTPases Rab7 and Arl8 occupy the same LEs or distinguish between their respective subpopulations. However, for most LEs, Rab7 appears to be the key that turns both sides, where RILP leads to dynactin and dynein recruitment, while FYCO1 results in acquisition of kinesin-1. In principle, Rab7 binding to either of the above then dictates which way the endosome will go. Both effectors recognize Rab7 using a similar motif, implying that their binding is mutually exclusive on a given Rab7 molecule [16]. This raises the question of which factors govern effector binding to Rab7 and thus effectively decide the direction of endosome movement along microtubules. It has long been known that in an in vitro setting, the net force generated by dynein and kinesin molecules found on the same vesicle decides the contest [5] (reviewed in [7]). Similarly, following the law of mass action, relative amounts of opposing Rab7 effectors determine overall directionality of transport, as shown by overexpression of FYCO1 or RILP [12,14]. Although straightforward in the abstract, coordinating transport as a function of overall effector expression levels would disallow control of LEs on the individual basis and would be incompatible with fast switching of direction. To supervise and fine-tune endosomal transport in the cell's complex environment, elegant regulatory mechanisms have evolved to coordinate association and activation of Rab7 effector proteins.

ER determines directionality of LE transport

Its presence throughout the cell positions the ER as an ideal candidate to accommodate general scaffolding functions for dynamic processes involving endosomes in every corner of the cytoplasm. This is supported by the observation that LEs make recurring contacts with the ER along their transport routes, with contact propensity and dwelling time found to increase with endosome maturation [17]. In the case of

LE transport, its regulatory focal point rests on Rab7, which mediates recruitment of both dynein and kinesin motors to the LE membrane. Similarly, its association with negative regulators of directional transport helps guard the dynamic character of endosomes. The first negative regulator of LE motility ever reported is the oxysterol-binding protein ORP1L [18]. Preferentially associating to Rab7 in the presence of RILP [13], ORP1L can block RILP-mediated recruitment of the dynein motor complex in response to changes in LE cholesterol content [19-21]. Prior to ending up in the LEs, cholesterol must either be endocytosed from the extracellular environment in the form of LDL or synthesized de novo in the ER and subsequently delivered to endosomes (reviewed in [22]). When the cholesterol-sensing domain of ORP1L recognizes cholesterol molecules, it clamps down on the LE membrane in a closed conformation, compatible with minus-end transport. However, under low endosomal cholesterol conditions the cholesterol-sensing domain of ORP1L becomes exposed and allows the neighboring FFAT motif to bind the integral ER protein VAP-A, yielding extensive contact sites between LE and the ER (Figure 1). As a result, dynactin is displaced from RILP, and transport of endosomes towards the minus-end is inhibited. Now, the LE is stabilized at a membrane contact site (MCS) with the ER [19], poised for a change of direction. Factors influencing LE cholesterol levels thus effectively influence directionality of LE transport.

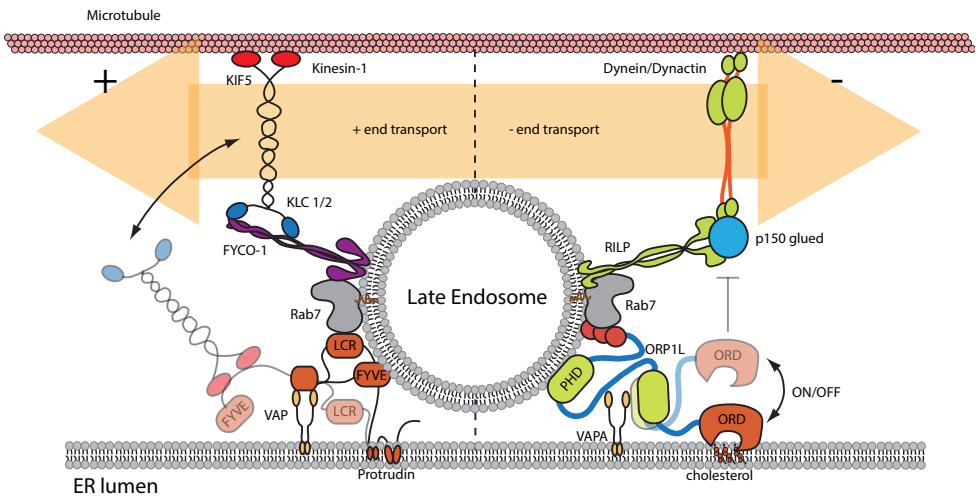

Figure 1: ORP1L and protrudin control motor loading onto Rab7. *Rab7 associates with its effectors, FYCO1 and RILP, which in turn recruit kinesin-1 or dynactin/dynein to respectively mediate plus- or minus-end-directed transport. In the former case, ER-localized protrudin binds VAP-A and kinesin-1 subunit KIF5. Through coincident recognition of PI3P by its FYVE domain and Rab7 with its low complexity region (LCR), protrudin instigates the formation of an ER-LE membrane contact site (MCS), where transfer of kinesin-1 onto FYCO1 provides a plus-end pulse. On the other side, ORP1L inhibits minus-end transport when in complex with VAP-A. At high cholesterol concentrations, the cholesterol-binding domain of ORP1L (ORD) interacts with the LE membrane, allowing minus-end transport to proceed. However, cholesterol depletion exposes the FFAT motif of ORP1L for binding ER-localized VAP-A. VAP-A subsequently binds the dynactin complex, thereby removing dynein from RILP and blocking minus-end transport. Thus, MCSs formed by both ORP1L and protrudin positively contribute to transport of endosomes towards the cell's periphery.*

In a new chapter on LE transport mechanisms, Raiborg et al. demonstrate that plus-end movement of Rab7-positive LEs also falls under ER control, through the actions of an ER-localized protein, protrudin [6]. Previously linked to neurite outgrowth—a process requiring concerted trafficking of vesicle membranes [23]—protrudin is now shown to directly promote LE transport towards and subsequent deposition at the neural protrusion site. Situated in the ER membrane, protrudin is shown to coincidentally engage Rab7 and the LE lipid phosphatidylinositol 3-phosphate (PI3P) [24], leading to ER-LE contact site formation. Biochemical and cell biological analyses by Raiborg et al. demonstrate that, through an interaction with the KIF5 subunit of kinesin-1 [25], protrudin facilitates loading of the plus-end motor complex onto FYCO1 (Figure 1). Observing FYCO1-positive vesicles briefly pause at the ER prior to resuming plus-end-directed motion, the authors suggest that protrudin orchestrates a hand-over of kinesin-1 to FYCO1 at Rab7-mediated contact sites. Taken together with the established functions of the ORP1L/VAP-A system at ER-LE contact sites, these new findings by Raiborg et al. underscore the breadth of ER involvement in the regulation of LE dynamics.

Intriguingly, the above indicates that PI3P is instrumental in targeting LEs for plus-end transport, since localization of both FYCO1 and protrudin to endosomes requires their respective PI3P-binding FYVE domains [6,14]. While EEs are known to contain high concentrations of PI3P, their maturation into LEs is associated with conversion of PI3P into PI(3,5)P. The resulting decrease in PI3P abundance on the LE limiting membranes [24,26] suggests that dephosphorylation of PI(3,5)P [27] may play a role in recruitment of FYCO1 and protrudin to these vesicles. Taken together with the well-established interplay between cholesterol and minus-end-directed LE motility [20,28], the new findings by Raiborg et al. expand the notion that proteins and lipids actively cooperate at the ER-LE interface, integrating cargo selection and motor acquisition, and suggest that cholesterol and PI3P act as opposing lipids in the regulation of minus- versus plus-end-directed LE transport.

Mechanisms and rationale for ER-curated motor engagement with endosomes

It is becoming abundantly clear that cells have evolved complex inter-compartmental controls over motility of endosomes. But what advantage does ER-based regulation in this context serve? One option is that due to their limited processivity, kinesin and dynein motors require continuous external triggers to remain bound. In vitro, kinesin and dynein travel an average distance of 600 nm and 1900 nm along microtubules, respectively [29,30]. Particularly in the former case, these intrinsic parameters are insufficient to afford direct transit between the perinuclear region and the periphery of the cell, and intermittent contacts with protrudin may promote continuity of transport. Moreover, on long journeys, vesicles may encounter crossed microtubule tracks or road blocks in the form of (macro-)molecules and complexes. Reloading motors onto vesicles that have past such barriers could then be critical to maintain a chosen course. Observations of endosome transport in living cells by Raiborg et al. reveal that vesicles captured by protrudin had already been moving in a directional manner en route to the ER, implying active presence of kinesin-1 prior to protru-

din engagement. Authors speculate that protrudin could reload or replace the motor upon contact with the endosome. In addition, the contact site can be envisioned to spatially restrict transport by directing vesicle transport to specific areas of the cell. For instance, during neurite outgrowth, plus-end transport is harnessed specifically in the direction of the growing protrusion, which presumably necessitates limitation of outward vesicle mobility elsewhere in the cell. Selective protrudin activation along microtubules directed into the protrusion site could be implemented to steer endosome transport in accordance with cellular demand. This concept can be further generalized to serve in sequestration of vesicle-associated cargo at specific regions in the cell, enabling localized signaling or polarized secretion [31].
While restricting directionality of cargo traffic may be required in special circumstances, under steady state conditions LE transport rarely follows direct trajectories. Instead, individual vesicles move in a bidirectional manner, switching frequently between plus- and minus-end motility [5,7]. Raiborg et al. note that by way of ORP1L and protrudin systems, both sides of bidirectional transport fall under the control of the ER. This raises the possibility that the two mechanisms may operate simultaneously at the same MCS. If so, the former could promote the release of minus-end-directed dynein and allow the latter to mediate the switch by recruiting plus-end motor machinery. Intriguingly, like ORP1L, protrudin contains an FFAT motif, which binds VAP-A and is required for protrusion formation [32]. Within the ER membrane, VAP-A exists as a dimer [33] and could therefore simultaneously accommodate both ORP1L and protrudin to facilitate motor exchange at the LE-ER contact sites. Alternatively, given its ER location, protrudin could compete away the available VAP-A, thereby inhibiting ORP1L-mediated contact site formation and inducing endosome release. In either scenario, the ER-LE contact sites can be envisioned as comprehensive navigation platforms, coordinating competing directionalities of endosome transport between the MTOC and the cell's periphery.

Roles of ER-LE contact sites in endosome biology beyond transport

The study of mechanisms underlying endosomal transport has produced invaluable understanding of basic molecular principles and yielded numerous tools for manipulation of complex intracellular systems in real time. Remarkably, control of LE transport by the ER highlights the power of cross-compartmental regulation in vesicle biology, and new questions on the topic continue to emerge. MCSs offer a stable platform upon which various complex molecular events may unfold in a spatially and temporally regulated fashion. For instance, recent work has shown that ER-endosome contact sites determine positioning and timing of endosome fission—a process required for compartmentalization within the highly interconnected endosomal system [34,35]. Fission or physical separation of one endosome carrying diverse cargoes results in two new vesicles, each containing cargoes destined for a specific fate [36]. Both position and timing of fission are shaped by the tubular ER, which wraps around the former [34] by an unknown molecular mechanism. Interestingly, fission requires activities of motor proteins to stretch the tubules containing select cargoes away from the scission site [37], and protrudin has been shown to

localize predominantly to tubular ER [38], opening the door to a potential role for this protein in the fission process. Besides those observed in fission, tubule-shaped LEs can also form during autophagic lysosome reformation, as well as upon activation of macrophages and dendritic cells [39-41]. In the latter case, tubulation has been shown to depend on RILP and FYCO1 [42], allowing the possibility that this process may fall under control of the ER. Resolving the molecular composition and function of ER-endosome contact sites and integrating their elements within the larger molecular networks in charge of endosome dynamics presents an exciting new frontier in endosome biology.

In working to shape endosome dynamics, can the ER also extract symbiotic benefits in return? ER-endosome contact sites could serve to fuel the former organelle with nutrients and metabolites. For instance, cholesterol is derived largely from the extracellular environment via endocytosis and must be subsequently transported to the ER [43]. One route for this is the formation of an MCS between lysosomal NPC-1 and ER localized ORP5, which interact with each other and facilitate cholesterol transport, probably directly via its contact site [44]. Additionally, cholesterol transport could potentially be mediated by MCSs formed between VAP-A and MLN64/STARD3 or MENTHO/STARD3NL, both cholesterol-binding proteins found on endosomes [45,46]. Evolution of nutrient exchange between the ER and endosomes argues that vesicle cargo can play an active role in contact site formation. This notion is supported by MCSs instigated between activated EGFR and the ER-localized phosphatase PTP1B, which serves to promote receptor sorting into the intra-luminal vesicles (ILVs) of LEs [47]. On the basis of such examples where cargo is directly involved in MCS formation, integrating various types of cargo may reshape our understanding of regulatory mechanisms underlying minus- and plus-end transport and subsequent maturation events.

Conclusions and outlook

The initial discoveries of MCSs between the ER and endosomes unveiled a new regulatory paradigm, where membrane compartments functionally influence one another in a dynamic manner. For simplicity's sake, it may be tempting to envision the ER as a mere facilitator of predetermined endosomal routes, and by extension cargo fates. However, the emerging understanding of molecular complexity underlying bidirectional transport paints a far more intricate picture. It appears that by providing pit stop opportunities to endosomes along the busy microtubule highways, the ER can actively influence what departure trajectories they should take. Sensitive molecular switches typically consist of positive and negative influences, held in delicate non-linear balance [7], and in the case of LE transport, the ER is a likely candidate to tip the scales. By assembling ORP1L and protrudin complexes in close proximity of one another, the ER could streamline spatiotemporal control of endosome dynamics, resulting in increased sensitivity and efficiency of long-range transport.

While a wide variety of ER-mediated MCSs have been discovered (reviewed in [48,49]), thus far only for late endosomes have they been linked to transport control. This raises the question as to whether the ER could offer the same to other organelles. Most immediately plausible candidates for transport under ER control are autophagosomes and the trans-Golgi network. Both of the above consist of vesicles

subject to long-range transport along microtubules, and the former has already been shown to acquire FYCO1 and Rab7 [14]. Additionally, mitochondria found in the tips of neurons travel vast distances with the help of the dynein motor complex in a manner dependent on the GTPase Miro, found at the ER-mitochondria interaction sites [50,51]. Do ER-assisted transport and membrane dynamics constitute common mechanisms of spatiotemporal organelle control? Thus far, endosomes lead the way to finding the answer.

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