Published in final edited form as:

Curr Opin Cell Biol. 2010 August ; 22(4): 528–534. doi:10.1016/j.ceb.2010.05.008.

Transport at the Recycling Endosome

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Summary

The recycling endosome (RE) has long been considered as a sub-compartment of the early endosome that recycles internalized cargoes to the plasma membrane. The RE is now appreciated to participate in a more complex set of intracellular itineraries. Key cargo molecules and transport factors that act in these pathways are being identified. These advancements are beginning to reveal complexities in pathways involving the RE, and also suggest ways of further delineating functional domains of this compartment.

Introduction

Upon internalization from the plasma membrane (PM), cargoes that undergo endocytic recycling first encounter a sub-compartment of the early endosome, known as the sorting endosome (SE). Here, they can either recycle to the PM, or transit further to another sub-compartment, known as the recycling endosome (RE). Although the RE has been extensively characterized for its role in recycling to the PM [1,2], it also has been characterized in recent years to act in another major route, retrograde transport to the trans-Golgi network (TGN) [3,4], which results in access to the secretory pathways (Fig 1). The identification of cargoes and transport factors that act in these pathways is providing new insights into the complexity of RE. This advancement has been facilitated by a general consideration that a series of conserved mechanistic steps that are performed by distinct families of key effectors is now appreciated to underlie transport in different pathways within the cell. Initially, coat proteins act in coupling carrier formation with the proper sorting of cargoes into such carriers. Motor proteins then act in the translocation of these carriers, followed by tether complexes and soluble NSF attachment protein receptor (SNARE) proteins, which act in the docking and fusion, respectively, of carriers to another compartment [5,6].

Recent reviews have broadly surveyed the different classes of transport factors found to act in pathways involving the RE [2,4]. This review has a more focused goal, highlighting mechanistic insights that have been gathered in studying key classes of transport factors, and also pointing out new questions that have arisen as the result of this advancement. In particular, we will focus on coats, tethers, SNAREs, along with their key regulators, while

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motor proteins will be covered in other review in this issue. Studies on these transport factors not only have begun to shed insights into the complexity of pathways involving the RE, but also suggest the prospect of achieving a more precise delineation of the different sub-compartments that comprise the early endosome.

Coat components

In the endocytic recycling itinerary (Fig 1), the role of coat proteins has been uncertain for many years. Although a clathrin complex was initially suggested to act in recycling [7], subsequent studies that directly perturbed clathrin could not confirm such a role with clarity [8,9]. Early studies often examined the recycling of transferrin receptor (TfR) as the model system [1]. However, endocytosis of TfR from the PM requires clathrin [8,10], a circumstance that likely confounded the ability to detect with certainty a role for clathrin in the recycling of TfR. In recent years, other recycling cargoes have been characterized as additional model systems, in particular glucose transporter type 4 (GLUT4) [11] and integrin [12]. Endocytosis of these cargoes can occur without clathrin [13,14]. As such, studies on their recycling have allowed a better determination regarding whether clathrin acts in endocytic recycling. In particular, ACAP1 (Arfgap with Coil-coil and Ankyrin repeat Protein 1) has been found to act as an adaptor for a novel clathrin complex in recycling from the RE to the PM [14–16]. ACAP1 is also known as a GTPase-activating protein (GAP) for ADP-Ribosylation Factor 6 (ARF6) [17]. This dual role of ACAP1, functioning both as regulator (through its GAP activity) and effector (as a coat component) of ARF6, suggests that the novel clathrin complex shares mechanistic parallel to the COPI (Coat Protein I) and COPII complexes, in which ARF GAPs have also been shown to play such dual roles [18,19].

In polarized cells, a different adaptor was found to couple with clathrin for endocytic recycling. Whereas Adaptor Protein 1 (AP1) is expressed ubiquitously, a variant form known as AP1B is expressed only in polarized cells and has been shown to act in basolateral targeting [20]. Although AP1B was initially thought to act at the TGN for targeting to the basolateral PM of polarized cells, subsequent studies suggest that AP1B acts mainly at the RE (known as the common recycling endosome in polarized cells), for both basolateral recycling [21] and another itinerary that involves transport from the TGN to the RE and then to the basolateral surface [22,23].

In the other major pathway that involves the RE, retrograde transport to the TGN (Fig 1), coat proteins are also being identified (Table 1). Studies on model cargoes, such as shiga toxin and the mannose-6-phosphate receptor (MPR), have identified AP1 [24], epsinR and clathrin [25], GGA3 (Golgi-associated γ -ear-containing ARF binding protein 3) and PACS-1 (Phosphofurin acidic cluster sorting protein 1) [26], and also retromer [27,28] to be involved in transport from early endosomal compartments to the TGN. Why so many coat components? Some coat components are likely to act in a cargo-specific manner, and thereby contributing to defining the complexity of transport from early endosomal compartments to the TGN. Another likely explanation comes from the consideration that this transport involves multiple possible pathways: i) SE to RE, ii) RE to TGN, and iii) SE to TGN (Fig 1). As such, further studies will be needed to pinpoint more precisely the roles of different coat components.

Besides deforming membrane for carrier formation, coat proteins also play an integral role in cargo sorting. Distilled to its fundamental level, this process involves coat components recognizing specific sequences on cargoes, known as sorting signals [5]. Thus, along with advancements in identifying coat components, progress has also been made in identifying sorting signals on cargo proteins recognized by the different coats. For retrograde transport

to the TGN, a tri-peptide motif dominated by aromatic and hydrophobic residues has been shown to be recognized by both retromer and AP1 [29]. In contrast, PACS-1 recognizes cargo sequences that are characterized by acidic clusters [30]. For recycling to the PM, sorting signals have also been identified. These cargo sequences can be either predominantly positive-charged or hydrophobic/aromatic residues [14,15]. In light of the diverse nature of these recycling sorting signals, and that ACAP1 has been demonstrate to bind directly to these different sequences [14,15], a future goal will be to determine how ACAP1 can accommodate such flexibility in achieving cargo sorting.

Recent studies have also revealed an interesting twist for how clathrin acts. The classic form of the clathrin heavy chain (CHC) is expressed ubiquitously. In contrast, a variant form, which is encoded on human chromosome 22 (and thus referred as CHC22), is expressed only in certain human cells [31]. A recent study suggests that the classic form of clathrin acts prior to CHC22 in transport from endosomes to the TGN [32]. However, because this transport is suggested to occur in sorting endosomal compartment, an apparent discrepancy is that CHC22 colocalizes better with a late endosome marker, rather than sorting endosome markers [32]. Another puzzling observation regarding CHC22 has been its suggested role in GLUT4 trafficking, which involves association with AP1 [33]. However, GLUT4 recycling has been shown previously to involve classic clathrin in association with ACAP1 [14]. Thus, further studies will be needed to clarify these apparent discrepancies.

Fission factors

The final stage of carrier formation involves membrane fission. Whereas sorting nexins (SNXs) have been known to act in cargo sorting, some members of this protein family have also been shown in recent years to participate in membrane fission [34]. In the context of clathrin AP2-mediated endocytosis, SNX9 has been shown to regulate the role of dynamin in membrane fission, and also to participate more directly in membrane fission through its BAR (Bin, Amphiphysin, Rvs) domain that induces membrane curvature [35,36]. SNX18 has been shown recently to exhibit similar properties, and has been proposed to act in conjunction with the AP1 complex for endosomal retrograde transport to the TGN [37].

However, for endocytic recycling to the PM, a different class of proteins has been suggested to act in membrane fission. EHD1 (Eps15 Homology Domain 1) has been known to have a critical role in this pathway [38], but how it acts has been unclear for many years. A recent study reveals that a member of the EHD family is similar to dynamin, both structurally and functionally, implying a role in membrane fission [39]. As a mechanistic twist, rather than hydrolyzing GTP, as is the case for dynamin, EHD proteins appear to behave as ATPases [39]. A subsequent study has further supported the role of EHD proteins in membrane fission, and suggests that EHD1 acts in conjunction with amphiphysin for this process [40].

An actin-based process has also been shown to act in fission. The Arp2/3 complex is well known to act in the crosslinking of actin filaments to form branched networks. Activators of Arp2/3, known as WASH (Wiskott-Aldrich Syndrome Protein and SCAR Homolog), have been suggested to promote the fission of endosomal carriers for TfR recycling [41] and also for endosomal retrograde transport of MPR to the TGN [42]. WASH interacts with dynamin [41], and also with retromer [42]. Thus, as some SNX members are components of retromer [3,4], and also in light of a recent finding that SNX9 coordinates the actions of WASP (Wiskott-Aldrich Syndrome Protein) and dynamin for endocytosis at the PM [43], members of three protein families (SNX, Arp2/3 activators, and dynamin-like proteins) are likely to have conserved roles in coordinating membrane fission in endocytic pathways.

Tethers

Recognition of the target membrane is one of the key steps in determining the specificity of endocytic membrane transport. There is now general consensus that the initial step of this docking process involves tether complexes. Tethers can be classified into two types. One type is characterized by long coiled-coil domains, which usually form dimers and can mediate either the docking of carriers to a target organelle or homotypic fusion of organelles (Table 2). One of the better characterized tethers is EEA1, which acts in homotypic fusion of sorting endosomes [44]. Other examples include the golgins, which consist of four members (Golgin-97, Golgin-245, GCC88 and GCC185). Recent studies suggest that golgins participate in distinct retrograde pathways from endosomes to the TGN. GCC185 tethers Rab9-positive vesicles originating from late endosomes [45,46], while Golgin-97 and GCC88 have been proposed to tether vesicles derived from sorting endosome [47,48].

Another type of tethers exists as multimeric complexes. Several of them have been shown to act in endocytic transport, including the exocyst, the centralspindlin and GARP (Golgi-associated retrograde protein) complexes (Table 2). The exocyst was originally identified as an octameric complex consisting of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84 subunits [49,50]. The exocyst has been found to act in polarized post-Golgi transport to the PM in interphase and dividing cells [51–53]. In recent years, the RE has been revealed to participate in cytokinesis [54]. For this process, the exocyst works together with the centralspindlin complex to target and tether the RE to the midbody of dividing cells [51,55]. Thus, tethers can sometimes work synergistically, presumably to provide further fidelity in targeting. The GARP complex is also starting to be characterized. In yeast, it is composed of four subunits, Vps51p, Vps52p, Vps53p and Vps54p, but whether Vps51 exists in mammals has been unclear [56,57]. The GARP complex has been shown to mediate retrograde transport from endosomes to TGN [58]. Thus, as golgins also act in these pathways, a future goal will be to delineate more clearly the relative roles of golgins and GARP in endocytic pathways/events.

Small GTPases have emerged as critical regulators of tethers. Rab11, Ral and several Rac/Rho members have been shown to regulate the recruitment of the exocyst to target membrane [59,60]. GCC185 has been shown recently to bind Arl1, Rab6 and Rab9 [46,61]. It has been proposed that GCC185 binds to multiple small GTPases simultaneously in mediating the targeting of this tether to the TGN [46]. A later study has questioned this mechanism [62]. More recently, a review has offered potential reconciling explanations, which also suggest that future publications will be forthcoming in resolving this apparent controversy [63].

SNARE proteins

SNAREs represent the core machinery for the fusion of transport carriers with their target compartment. Two main classes of SNAREs have been identified, VAMPs (vesicle associated SNAREs) and syntaxins (target membrane SNAREs). Various VAMPs and syntaxins form cognate SNARE complexes, which mediate distinct endocytic pathways. In pathways that involve the sorting endosome, VAMP4 has been shown to form a complex with syntaxin 6 for retrograde transport from endosomes to TGN [58]. VAMP3 and syntaxin 13 have shown to act in recycling to the PM by forming a complex with SNAP-23 [64].

All syntaxins possess an N-terminal α -helix (H_{abc} domain), which acts in auto-inhibition by inducing a “closed” SNARE conformation, and is also a target of regulation by key factors, such as the SM (Sec1/Munc18-like) proteins [65]. Tethers have also been shown to regulate SNARE function. The GARP complex has been observed to interact with and promote a SNARE complex that consists of syntaxin 6, syntaxin 16 and VAMP4 [58]. GCC185 has

been observed to bind and regulate syntaxin 16 [45]. Other examples in the endocytic pathways include EEA1 binding to syntaxin 6 [66], the exocyst binding to the t-SNARE Sec9p in yeast, as well as, the t-SNARE interacting proteins, such as Snapin and Sec1 [52,67,68]. Thus, regulation of SNARE function is now appreciated to be increasingly complex, occurring at multiple mechanistic layers that involve different classes of transport factors.

Defining pathways and compartments through Rab small GTPases

The identification of different classes of key transport factors acting in pathways involving the RE has led to an appreciation for the complexity of these pathways. For retrograde transport from the RE to the TGN, suggestion for such complexity has already been discussed above, in the context of multiple coat components that have been identified to act in this pathway. For recycling from the RE to the PM, suggestion for complexity has come mainly from studies on Rab small GTPases. Whereas Rab11 is involved in the recycling of many cargoes from the RE [69], Rabs 8, 10, 13 and 22a have also been implicated in endocytic recycling [70–73]. In some cases, these Rabs appear to act independent of Rab11, while in other cases, they appear to coordinate with Rab11. Moreover, it is notable that different circumstances can lead to differential relationship between Rab11 and other Rabs. In polarized cells, Rabs 8 and 11 can act in distinct transport pathways. Whereas Rab11 acts in apical recycling [73,74], Rab8 has a role in basolateral transport between the TGN and the RE [75,76]. In contrast, a recent study on cilium formation shows that these two Rabs can act in a more intimately coupled manner, with Rab11 activating Rab8 through a GEF to organize transport at the base of the cilium [77]. Such differential mechanistic relationship among Rabs in endocytic pathways suggests that complexities in these pathways will unlikely be delineated solely by one class of transport factors. Indeed, as different classes of transport factors are now appreciated to assemble in transient complexes in linking the sequential mechanistic steps of transport, a future goal will be to understand how these transient assemblies are regulated in spatial and temporal ways, and thereby providing further insight into how complexities in endocytic pathways are achieved.

Such understanding will also help to delineate with better molecular precision organelle identity, such as the early recycling compartments. Early morphologic studies that tracked recycling proteins initially led to the concept that the early endosome is divided into a sorting compartment and a recycling compartment [1]. The subsequent identification of Rab small GTPases that act at these compartments has not only helped to identify new pathways involving these compartments, but also begun to provide a molecular description of compartmental entity. In particular, the SE has been suggested to possess a Rab5 domain that receives cargoes into this compartment from the PM, and a Rab4 domain that is involved in transport from this compartment to either the PM or the RE [78]. Similarly, the RE can also be further subdivided into a Rab4 domain that receives cargoes from the SE, and either Rab4 or Rab11 domains that are involved in transport from this compartment to the PM [78]. In light of additional transport factors that have now been identified (as listed in the above sections), a future goal will be to take advantage of this advancement in further delineating the different domains of the RE, and also the relationship of this compartment to others that operate in the early endosomal pathways.

Conclusions

Significant progress has been made in the mechanistic understanding of transport pathways that transit through the RE. This advancement has come from the identification of key transport factors that act in different mechanistic steps of transport in achieving a round of transport for a particular pathway. These studies have enlightened our appreciation for the

complexity in transport pathways involving the RE, and also insights into how such complexities are achieved. Recent results have also raised new questions and goals for future investigation, for which we have attempted to highlight throughout this review. In particular, although multiple types of coats, tethers and SNAREs have been identified for pathways involving the RE, less clear has been the precise sequential combinations of these different classes of transport factors in describing a particular transport pathway. We also note that the RE is beginning to be defined in molecular terms, similar to other endosomal compartments such as the SE and the late endosome. Further progress in refining these molecular definitions will also contribute to a better understanding of how key transport factors act in pathways that link these various endosomal compartments.

Acknowledgments

We apologize to our colleagues for not being able to cite all work related to the RE, due to the focused nature of this review and its requirement for brevity. Work in our laboratories has been funded by the National Institute of Health, DK064380 (RP) and GM073016 (VWH), and also the Susan G. Komens Breast Cancer Research Foundation (RP) and the DOD Breast Cancer Research Program (VWH).

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Figure 1. Endocytic pathways. Arrows associated with red lines indicate pathways that are the focus of the review. Arrows associated with black lines indicate other endocytic pathways.

Table 1

Coats acting in the major pathways involving the recycling endosome.

Coat Components	Pathways*	Function
Clathrin	Both pathways	coat complex
CHC22 (in humans)	?Both pathways	coat complex
Retromer	Retrograde to TGN	coat complex
AP1	Retrograde to TGN	adaptor
EpsinR	Retrograde to TGN	adaptor
PACS-1	Retrograde to TGN	adaptor
GGA3	Retrograde to TGN	adaptor
ACAP1	Recycling to PM	adaptor
AP1B (polarized cells)	Recycling to PM	adaptor
EHD1	Recycling to PM	fission
Amphiphysin	Recycling to PM	fission
WASH	Both pathways	fission
SNX18	Retrograde to TGN	fission

* PM (plasma membrane), TGN (trans-Golgi network)

Table 2

Summary of mammalian endocytic tethers and their binding proteins

Thether	Target Organelle	Binding Proteins
Multiprotein tethers		
Exocyst complex (Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, Exo84)	Plasma mebrane	Rab11a (Sec15)
		Arf6 (Sec10)
		TC10 (Exo10)
		Ral (Sec5, Exo84)
		Snapin (Exo70)
		Arp2/3 (Exo70)
		PSD-95 (Sec8)
		Centriolin (Sec15)
Centralspindlin complex (Cyk4, MKLP1)	Midbody	Rab11-FIP3 (Cyk4)
		ECT2 (Cyk4)
		Anillin (Cyk4)
		Cep55 (MKLP1)
GARP complex (Vps52, Vps53, Vps54)	TGN	Syntaxin6, Syntaxin16, VAMP4 (Vps53, Vps54)
Coiled-coil tethers		
Golgin-97	TGN	Arl1
Golgin-245	TGN	Arl1
GCC88	TGN	?
GCC185	TGN	Arl1
		Rab6
		Syntaxin16
		Rab9
		Rab1,2,15,27B,30,33B,35,36
EEA1	Early/Sorting Endosomes	Rab5
		PI3 kinase
		Syntaxin6
		Rab22a