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REVIEW

The enigmatic endosome – sorting the ins and outs of endocytic trafficking

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ABSTRACT

The early endosome (EE), also known as the sorting endosome (SE) is a crucial station for the sorting of cargoes, such as receptors and lipids, through the endocytic pathways. The term endosome relates to the receptacle-like nature of this organelle, to which endocytosed cargoes are funneled upon internalization from the plasma membrane. Having been delivered by the fusion of internalized vesicles with the EE or SE, cargo molecules are then sorted to a variety of endocytic pathways, including the endo-lysosomal pathway for degradation, direct or rapid recycling to the plasma membrane, and to a slower recycling pathway that involves a specialized form of endosome known as a recycling endosome (RE), often localized to the perinuclear endocytic recycling compartment (ERC). It is striking that 'the endosome', which plays such essential cellular roles, has managed to avoid a precise description, and its characteristics remain ambiguous and heterogeneous. Moreover, despite the rapid advances in scientific methodologies, including breakthroughs in light microscopy, overall, the endosome remains poorly defined. This Review will attempt to collate key characteristics of the different types of endosomes and provide a platform for discussion of this unique and fascinating collection of organelles. Moreover, under-developed, poorly understood and important open questions will be discussed.

KEY WORDS: Budding, Early endosome, Fission, Late endosome, Recycling endosome, Sorting endosome

Introduction

The early endosome (EE), also known as the sorting endosome (SE) (hereafter EE), is the initial destination for material internalized from the plasma membrane (reviewed in Jovic et al., 2010) (Fig. 1). The unique membrane-bound EE compartment is a major cellular sorting station from which cargo molecules can either be trafficked to the late endosomes (LE) and lysosome for degradation, or be returned to the plasma membrane by various routes. Some receptors are recycled to the plasma membrane directly from the EE via a rapid recycling pathway, whereas other receptors are transported to more specialized recycling endosomes (REs), often clustered in the perinuclear-localized endocytic recycling compartment (ERC) adjacent to the microtubule-organizing center (MTOC).

Despite the vast number of studies that refer to the various types and subtypes of endosomes, our understanding of the enigmatic endosome remains limited, and fundamental questions about its nature remain unanswered. Even very basic questions regarding the characterization of endosomes have yet to be satisfactorily resolved (see Box 1). For example, are EEs a heterogeneous population of endosomes, each marked by an overlapping but different array of proteins? If so, do these different EEs carry out distinct functions, or are they a progressive series of endosomal structures along a pathway whereby the EE eventually 'evolves' into a more mature organelle?

Additional key questions remain about the fundamental ways that endosomes function. For example, a wealth of evidence supports the notion that EEs function by sorting cargo to distinct endosomal membrane domains that subsequently undergo budding and fission and give rise to an array of tubulo-vesicular structures that transport cargo along microtubules to the perinuclear ERC (Grant and Donaldson, 2009; Jovic et al., 2010), which in itself is a poorly understood concept (Fig. 2A). However, it is also possible that EEs are mobile, and can themselves undergo microtubule-dependent directional transport to the ERC (endosome relocation; Fig. 2B) similar to the way peripheral lysosomes move to the cell center (Khatter et al., 2015), raising the possibility that, at least under certain conditions, not all slow recycling cargo reaches the ERC via classic vesicular transport (budding, fission, transport and fusion). Moreover, the ERC is a dense, crowded region of the cell that also includes the MTOC (Maxfield and McGraw, 2004). Accordingly, the complex organization of the ERC, and how its multitude of tubular and vesicular membrane-bound structures coordinate recycling, is only beginning to be understood.

The purpose of this Review is to provide an up-to-date understanding of the enigmatic endosome, with a focus on the EE, because this organelle represents the major crossroads for endosomal activity. Less emphasis will be placed on the LE, because many excellent reviews have recently addressed this organelle and the subsequent degradation pathways (Scott et al., 2014; Frankel and Audhya, 2017). Important characteristics of EE, RE and LE will be outlined first, including a table of key proteins that localize to endosomes, which includes their proposed functions (Table 1). We will then highlight recent findings that address the array of protein sorting complexes that associate with EEs and direct trafficking events, including the classical retromer, sorting nexins, Wiskott–Aldrich syndrome protein and SCAR homolog (WASH), retriever and CCC complexes. We will also showcase exciting recent studies that overturn the long-held assumption that endocytic recycling is primarily a default pathway, whereby proteins, in the absence of selective signals to target them to degradation, are typically recycled.

Early or sorting endosomes

The EE is considered to be the first subcellular organelle with which internalized vesicles fuse, and we will discuss below important characteristics that define EEs, the sorting and trafficking pathways that originate from EEs, mechanisms for fusion of endosomes or vesicles with endosomes, and the role of key endocytic protein

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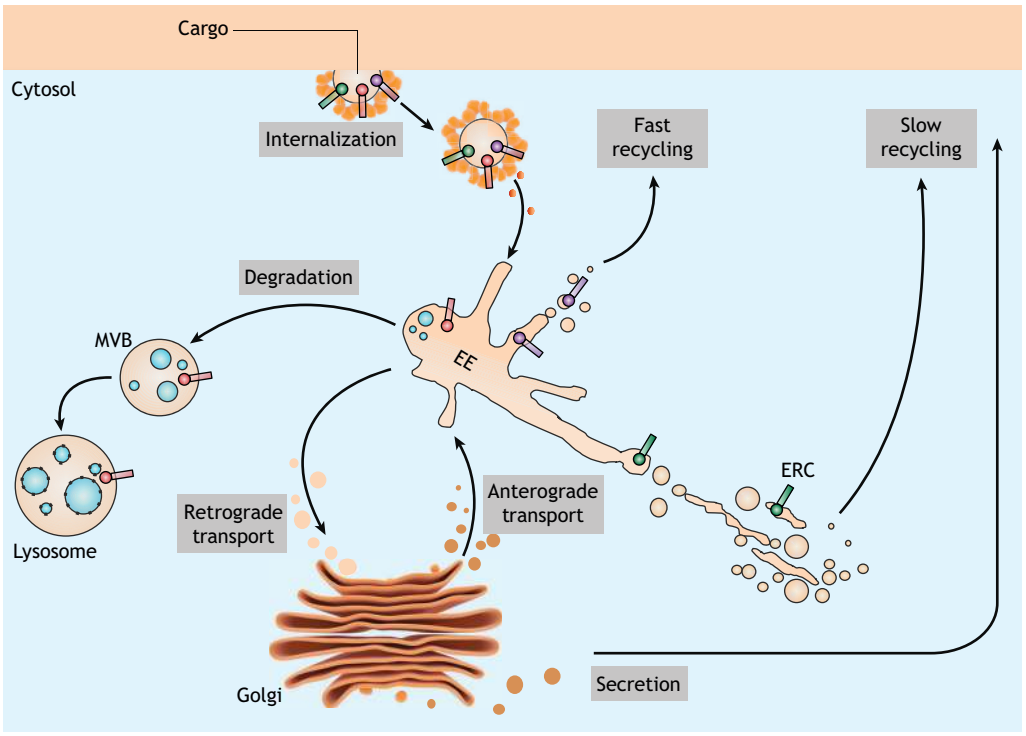

Fig. 1. Overview of endocytic pathways. Once internalized from the plasma membrane, membrane-bound vesicles that carry receptors from the cell surface fuse with the EEs. The EE serves as a sorting station from which either tubulo-vesicular carriers deliver cargo to the endo-lysosomal system for degradation, or cargoes are recycled directly or indirectly to the plasma membrane via the endocytic recycling compartment.

complexes, including the retromer and affiliated complexes, in the regulation of endocytic trafficking.

Characteristics of the EE

A body of work has established several important characteristics of EEs. First, EEs have a limiting membrane bilayer that is enriched in the phosphoinositide phosphatidylinositol 3-phosphate (PI3P), often generated by the lipid kinase PI3P kinase (reviewed in Corvera et al., 1999; Kobayashi et al., 1998a). Recent studies suggest that phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2] and some phosphatidylinositol 4-monophosphate (PI4P) reside on segregated membranes of endosomes in addition to PI3P (Giridharan et al., 2013; Yoshida et al., 2017). Second, EEs are dynamic in movement and frequently undergo fusion with one another and with incoming endocytic vesicles (Diaz et al., 1988). Furthermore, EEs have a lumenal pH of ∼6.2, and their lumen is acidified through the function of a V-ATPase proton pump (Murphy et al., 1984). In addition, key proteins associated with EEs often contain the FYVE (called after several protein containing the domain, namely Fab1, YOTB, Vac1 and EEA1) PI3P-binding domain (Gaullier et al., 1998; Kutateladze and Overduin, 2001; Stenmark et al., 2002). Among the mammalian FYVE-domain-containing proteins localized to EE are EEA1 and rabenosyn-5 (see Table 1). Finally, a number of small GTP-binding Rab proteins typically associate with EE in their GTP-bound state. These include members of the Rab4, Rab5, Rab10, Rab14, Rab21 and Rab22 family and others (Delevoye and Goud, 2015).

Box 1. A challenge – the complex characterization of endosomal compartments

One of the most daunting challenges, which represents a serious gap in the understanding of endosomes, is the difficulty in characterizing these organelles. On the one hand, as noted for LEs and lysosomes, there are not sufficiently distinctive membrane markers that allow researchers to distinguish between these organelles by light microscopy (Scott et al., 2014). This necessitates either electron microscopy for visual determination, or density gradients for enrichment and biochemical analysis. On the other hand, it is often possible to distinguish between various types of endosomes based on either the proteins associated with the outer bilayer of their surrounding membrane, or the selective phospholipids that are enriched in these membranes. For example, EE typically have a high concentration of the lipid PI3P (Corvera et al., 1999; Kobayashi et al., 1998a), as well as Rab5 (Gorvel et al., 1991) and various Rab5 effectors and proteins that interact with PI3P (Stenmark et al., 2002). Paradoxically, the Rab proteins are likely more than simply 'markers' of endosomes, because Rab5, when associated with an endosome, recruits PI3P kinase, which subsequently generates PI3P and facilitates the recruitment of FYVE domain-containing proteins (Gaullier et al., 1998; Kutateladze and Overduin, 2001; Stenmark et al., 2002), resulting in a characteristic EE. However, the mechanisms by which specific Rabs are selectively recruited to the various types of endosomal structures remain largely unknown.

The EE gets the cargo

The EE is the first endocytic structure that accepts the many types of receptors, lipid membranes and extracellular fluids that are internalized by small vesicles through a variety of internalization modes at the plasma membrane (Gruenberg et al., 1989). Essentially, the EE is a major sorting station and crossroad for internalized receptors and membranes (reviewed in Jovic et al., 2010). At the EE, rapid sorting occurs, and receptors are either transported to LEs and the endo-lysosomal pathway for degradation, or they are recycled back to the plasma membrane (Fig. 1). The recycling routes have been grossly classified as into the 'fast recycling pathway' or the 'slow recycling pathway'. For fast recycling, also known as the 'short loop' (Jones et al., 2006), vesicles containing the receptors to be recycled bud off from the EE membrane and undergo transport directly to the plasma membrane (Maxfield and McGraw, 2004). By contrast, slow recycling is considered to be a budding and fission process that is followed

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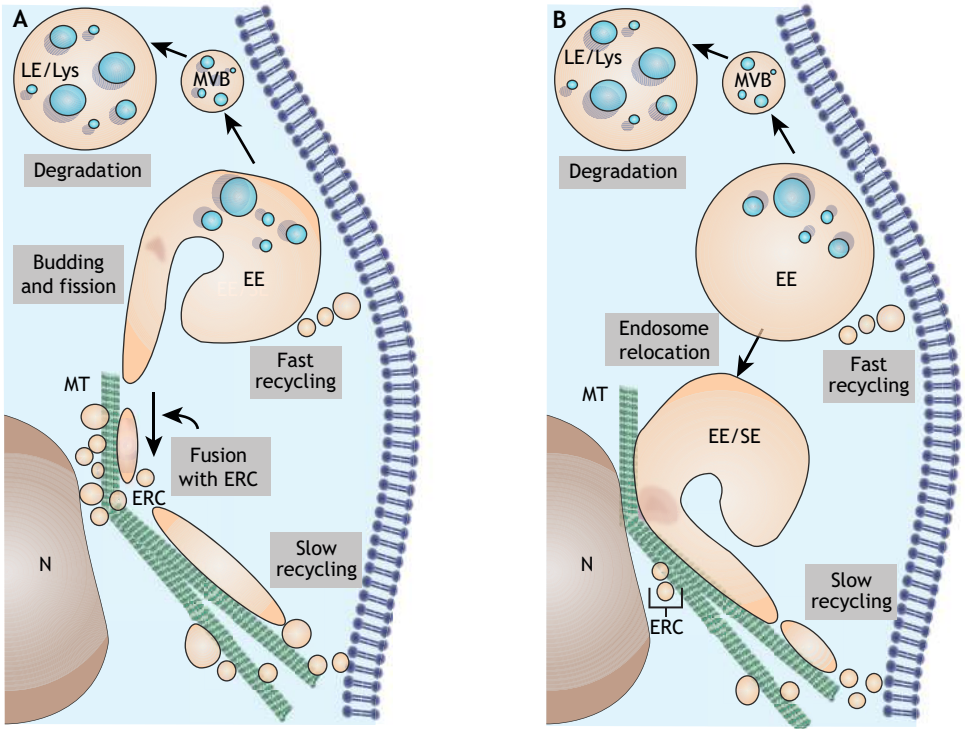

Fig. 2. Possible models for the slow recycling pathway. EEs sort cargo toward degradation in the endo-lysosomal pathway, or to the recycling pathways, either directly from EEs or through a transitory ERC. (A) Budding and fission model. Here, vesicles and tubules bud and undergo fission at the EE and carry cargo in transport carriers to the perinuclear region of the cell, where they likely undergo fusion with recycling endosomes. REs at the ERC are dynamic, fuse with one another, and eventually vesicles pinch off in a budding process leading to fission and the generation of transport carriers that transport recycling receptors along microtubules to the plasma membrane. (B) Endosome relocation model. In this model, recycling occurs from largely intact EEs that do not have any intralumenal vesicles and that are repositioned and transported along microtubules to the ERC region. Eventually, tubules and vesicles undergo budding and fission from the EEs that have relocalized to the ERC to recycle cargo receptors along microtubules to the plasma membrane. MT, microtubules; N, nucleus.

by transport of the tubular and/or vesicular structures to a perinuclear-localized tubulo-vesicular RE (Grant and Donaldson, 2009). Having reached the RE or the ERC and undergone fusion, a process of budding and fission is hypothesized to recur, with transport of the released vesicles to the plasma membrane proceeding along microtubule tracks (Maxfield and McGraw, 2004).

Although the rapid sorting of receptors within the EE is a hallmark of the function of this organelle, very few studies have directly addressed the mechanisms for such sorting. A landmark paper by the Zerial group, published before the advent of super-resolution light microscopy, provided evidence that different Rab proteins on EEs localize to distinct membrane subdomains; for example, Rab11, Rab5 and Rab4, all common Rab proteins that associate with the EE, segregate to distinct EE microdomains (Sönnichsen et al., 2000). Nonetheless, the mechanisms by which proteins are effectively sorted into these domains and thus recycled, are only beginning to be understood.

Setting a SNARE or global endosome relocation?

The soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE)-based vesicle fusion system facilitates the fusion of internalized vesicles with the EE, thereby supplying this compartment with cargo receptors to be sorted (Bennett, 1995; Söllner, 1995). Once cargo has been sorted into EE microdomains, these membrane regions bud into tubulo-vesicular structures that subsequently undergo fission and are transported along microtubule tracks to their target organelle (e.g. the RE or plasma membrane). This is followed by SNARE-based fusion and cargo delivery to this organelle.

The interaction of appropriate sets of SNAREs with one another leads to the spontaneous formation of highly stable helical core complexes that link vesicles/organelles (Chen and Scheller, 2001). Such a functional complex, which promotes membrane fusion, typically requires four SNAREs of the Qa-, Qb-, Qc and R-SNARE subfamilies (Fasshauer, 2003; Fasshauer et al., 1998; Hanson et al., 1997; Lin and Scheller, 1997).

Despite strong support for SNARE-mediated fusion between EEs and REs, including the identification of many EE SNAREs involved in homotypic and heterotypic EE fusion (Hanson and Whiteheart, 2005; McBride et al., 1999; Simonsen et al., 1999, 1998), including syntaxin 13 (Prekeris et al., 1998), Vt1b (Kreykenbohm et al., 2002) and Vamp4 (Tran et al., 2007; Zeng et al., 2003), there is the possibility of an alternative mechanism for cargo transfer from the EE to the RE, in that ‘intact’, peripheral EEs may relocate from the cell periphery to the perinuclear region of the cell, where they then acquire characteristics of REs (depicted in Fig. 2B). Indeed, our data based on depletion of the endocytic regulatory protein EHD1 have demonstrated that peripheral endosomes with characteristic markers of EE coalesce at the centralized ERC region, potentially by linkage to dynein motors, where they acquire RE markers including Rab11 and its effectors (Carson et al., 2013; Pasqualato et al., 2004; Ren et al., 1998; Zeng et al., 1999), and begin to function in recycling (Caplan et al., 2002; Naslavsky et al., 2004; Naslavsky and Caplan, 2005, 2011; Rahajeng et al., 2010). However, whether endosome relocation represents a major mechanism for cargo transport to the ERC remains to be determined.

Retromer-based generation of tubulo-vesicular carriers and trafficking

One protein complex at the core of sorting and trafficking from EEs is the retromer complex (Fig. 3). Highly conserved throughout evolution, and initially identified in yeast (Seaman et al., 1998), the retromer was first characterized as a complex that returns receptors (such as the mammalian mannose-6-phosphate receptor) from peripheral endosomes to the Golgi (Arighi et al., 2004; Seaman et al., 1998), although it is now known that the retromer also plays an essential role in endosome-to-plasma membrane transport (Follett et al., 2017).

The retromer can be divided into two subcomplexes: (1) a trimer consisting of Vps35, Vps26 (Vps26a and Vps26b isoforms) and Vps29, known as the cargo selection complex (CSC), and (2) a dimer comprising the sorting nexins Snx1 or Snx2, and Snx5, Snx6

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Table 1. Key mammalian endosomal proteins and their functions

Protein	Function	References
Key GTP-binding proteins (reviewed in Pfeffer, 2017)		
Rab4	Fast recycling	Van Der Sluijs et al., 1991
Rab5	Sorting, fusion, endosome maturation	Gorvel et al., 1991
Rab7	Conversion of EE to LE, recruitment of retromer to endosomes	Harrison et al., 2014; Rink et al., 2005
Rab10	Recycling	Babbey et al., 2006
Rab11	Slow recycling	Ullrich et al., 1996
Rab14	Recycling	Jagath et al., 2004
Rab15	EE sorting and trafficking	Zuk and Elferink, 1999, 2000
Rab21	Recycling	Simpson et al., 2004
Rab22	Recycling	Kauppi et al., 2002; Magadan et al., 2006; Mesa et al., 2001; Weigert et al., 2004
Rab35	Recycling	Allaire et al., 2013; Rahajeng et al., 2012
Arf6	Recycling	Donaldson, 2003
Key Rab effectors (reviewed in Grosshans et al., 2006)		
EEA1	Rab5 effector, FYVE domain, fusion, tether	Simonsen et al., 1998
Rabenosyn-5	Rab4/Rab5 dual effector, FYVE domain, NPF motifs, tether, recycling, interacts with EHD1	Naslavsky et al., 2004; Nielsen et al., 2000
APPL1/2	Rab5 effectors	Miaczynska et al., 2004
PI3-kinase	Rab5 effector	Clague et al., 1995; Jones and Clague, 1995; Li et al., 1995
Rabankyrin-5	Rab5 effector, interacts with EHD1	Schnatwinkel et al., 2004; Zhang et al., 2012b
HAP40	Rab5 effector	Pal et al., 2006
Rabaptin-5	Maintains and stabilizes GTP-Rab5 on EE	Stenmark et al., 1995
Rabex-5	Maintains and stabilizes GTP-Rab5 on EE	Lippe et al., 2001
SNAREs (reviewed in Wang et al., 2017)		
Syntaxin13	SNARE-based fusion at tubulo-vesicular RE	Prekeris et al., 1998
Vamp4	SNARE-based fusion at RE and Golgi	Tran et al., 2007; Zeng et al., 2003
Vti1b	SNARE-based fusion at EE	Kreykenbohm et al., 2002
Tethering factors (reviewed in Balderhaar and Ungermann, 2013)		
Vps11	HOPS/CORVET complex subunit	Robinson et al., 1991
Vps16	HOPS/CORVET complex subunit	Horazdovsky and Emr, 1993
Vps18	HOPS/CORVET complex subunit	Robinson et al., 1991
Vps33a/b	HOPS/CORVET complex subunit	Banta et al., 1990
Vam6p/Vps39p	HOPS complex subunit	Caplan et al., 2001; Nakamura et al., 1997
Vps41	HOPS complex subunit	Nakamura et al., 1997
TRAP1	HOPS complex subunit	Lachmann et al., 2014
Vps8	CORVET complex subunit	Chen and Stevens, 1996
Membrane remodeling (reviewed in Christ et al., 2017; Frankel and Audhya, 2017)		
AnnexinA2	Binds lipids, nucleates actin on EE	Morel et al., 2009
Actin	Endosome membrane remodeling	Seaman et al., 2013
Hrs	Part of ESCRT-0, has a FYVE domain	Komada et al., 1997
STAM1/2	Part of ESCRT-0 on EE	Bache et al., 2003
Tsg101	Part of ESCRT-1	Bishop and Woodman, 2001
Vps28	Part of ESCRT-1	Bishop and Woodman, 2001
Vps37	Part of ESCRT-1	Bilodeau et al., 2003; Piper et al., 1995
UBAP1	Part of ESCRT-1	Stefani et al., 2011
MVB12	Part of ESCRT-1	Oestreich et al., 2007
Eap45 (yeast Vps36p)	Part of ESCRT-2	Babst et al., 2002
Eap20 (yeast Vps25p)	Part of ESCRT-2	Babst et al., 2002
Eap30 (yeast Vps22p)	Part of ESCRT-2	Babst et al., 2002
CHMP1-7	Part of ESCRT-3	Stuchell-Brereton et al., 2007
Ist1	Regulates assembly of ESCRT-3	Frankel et al., 2017
Alix	Accessory protein for ESCRT function	Matsuo et al., 2004
Vps4	Accessory protein for ESCRT function	Babst et al., 1997
Key motor proteins (reviewed in Kardon and Vale, 2009)		
Dynein/dynactin	Microtubule-based minus-end motors for EE	Gibbons and Rowe, 1965
Kinesin-1	EE and LE/LYS plus-end motor	Loubery et al., 2008
KIF5	Microtubule-based plus-end motor for EE and RE	Schmidt et al., 2009
Kinesin-2	Microtubule-based plus-end motor for RE	Schonteich et al., 2008
Kif3A/B	Microtubule-based plus-end motor for EE	Noda et al., 1995

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Table 1. Continued

Protein	Function	References
KIF1Bβ	Microtubule-based plus-end motor for LE/LYS	Matsushita et al., 2004
KIF13A	Microtubule-based plus-end motor for EE and RE	Delevoye et al., 2014
KIF16B	Microtubule-based plus-end motor for EE and RE	Hoepfner et al., 2005
KIF2β	Microtubule-based plus-end motor for LE/LYS	Santama et al., 1998
KIFC1/2	Microtubule-based minus-end motors for EE	Bananis et al., 2003
Myosin 1b	Actin-based motor for EE	Salas-Cortes et al., 2005
Myosin Va	Actin-based motor for endosomal vesicles	Holt et al., 2007
Myosin Vb	Actin-based motor for RE	Hales et al., 2002
Myosin VI	Actin-based motor to deliver EE to ERC region	Chibalina et al., 2007
Endocytic regulatory proteins		
EHD proteins (reviewed in Naslavsky and Caplan, 2011)		
EHD1	Slow recycling, tubule/vesicle fission, interaction with retromer	Cai et al., 2012, 2014; Caplan et al., 2002; Gokool et al., 2007; Grant et al., 2001; McKenzie et al., 2012; Zhang et al., 2012b
EHD3	Slow recycling, tubule/vesicle stability, interaction with retromer	Bahl et al., 2016; Lu et al., 2015; Naslavsky et al., 2006
EHD4	Regulation of EE function	Sharma et al., 2008
MICAL-L1	Tubular recycling endosome protein and EHD interaction partner	Sharma et al., 2009
EHBP1	Interacts with Rab10 to regulate recycling	Shi et al., 2010
Syndapin2	Generates tubular recycling endosomes with MICAL-L1	Giridharan et al., 2013
Retromer (reviewed in Bonifacino and Hurley, 2008)		
Vps35	Retromer cargo selection complex (CSC)	Arighi et al., 2004
Vps26	Retromer cargo selection complex (CSC)	Arighi et al., 2004
Vps29	Retromer cargo selection complex (CSC) and retriever complex that functions with Snx17 in recycling	Arighi et al., 2004; McNally et al., 2017
Sorting nexins (reviewed in Burd and Cullen, 2014)		
Snx1	Sorting nexin complexes with retromer CSC	Haft et al., 1998; Kurten et al., 1996
Snx2	Sorting nexin complexes with retromer CSC	Haft et al., 1998
Snx3	Sorting nexin that binds PI3P, interacts with retromer CSC, controls EE function, involved in ILV generation	Haft et al., 1998; Pons et al., 2008; Xu et al., 2001
Snx4	Sorting nexin regulates transport to ERC	Traer et al., 2007
Snx5	Sorting nexin complexes with retromer CSC	Otsuki et al., 1999; Teasdale et al., 2001
Snx6	Sorting nexin complexes with retromer CSC	Parks et al., 2001; Teasdale et al., 2001
Snx8	Sorting nexin involved in endosome to Golgi transport	Dyve et al., 2009
Snx12	Sorting nexin involved in ILV formation	Pons et al., 2012
Snx15	Sorting nexin involved in recycling from EE	Phillips et al., 2001
Snx16	Sorting nexin primarily on LE	Hanson and Hong, 2003
Snx17	Sorting nexin involved in recycling from EE	Florian et al., 2001; Stockinger et al., 2002
Snx27	Sorting nexin complexes with retromer CSC	Joubert et al., 2004; Rincon et al., 2007
Retriever (reviewed in Wang et al., 2018)		
DSCR3/Vps26C	Retriever complex that functions with Snx17 in recycling	McNally et al., 2017
C16ORF62/Vps35L	Retriever complex that functions with Snx17 in recycling	McNally et al., 2017
Vps29	Retriever complex that functions with Snx17 in recycling	McNally et al., 2017
WASH complex (reviewed in Seaman et al., 2013)		
Fam21	WASH complex protein involved in actin nucleation at EE and interacts with Vps35	Gomez and Billadeau, 2009
WASH1	WASH complex protein involved in actin nucleation at EE	Gomez and Billadeau, 2009
Strumpellin	WASH complex protein involved in actin nucleation at EE	Gomez and Billadeau, 2009
CCDC53	WASH complex protein involved in actin nucleation at EE	Gomez and Billadeau, 2009
KIAA1033/SWIP	WASH complex protein involved in actin nucleation at EE	Gomez and Billadeau, 2009
CCC complex (reviewed in Wang et al., 2018)		
CCDC22	CCC complex protein at the EE involved in recycling, interacts with Fam21	Phillips-Krawczak et al., 2015
CCDC93	CCC complex protein at the EE involved in recycling	Phillips-Krawczak et al., 2015
COMMD proteins	Part of CCC complex at the EE involved in recycling, CMMD1 interacts with C16ORF62/Vps35L of the retriever complex	Phillips-Krawczak et al., 2015

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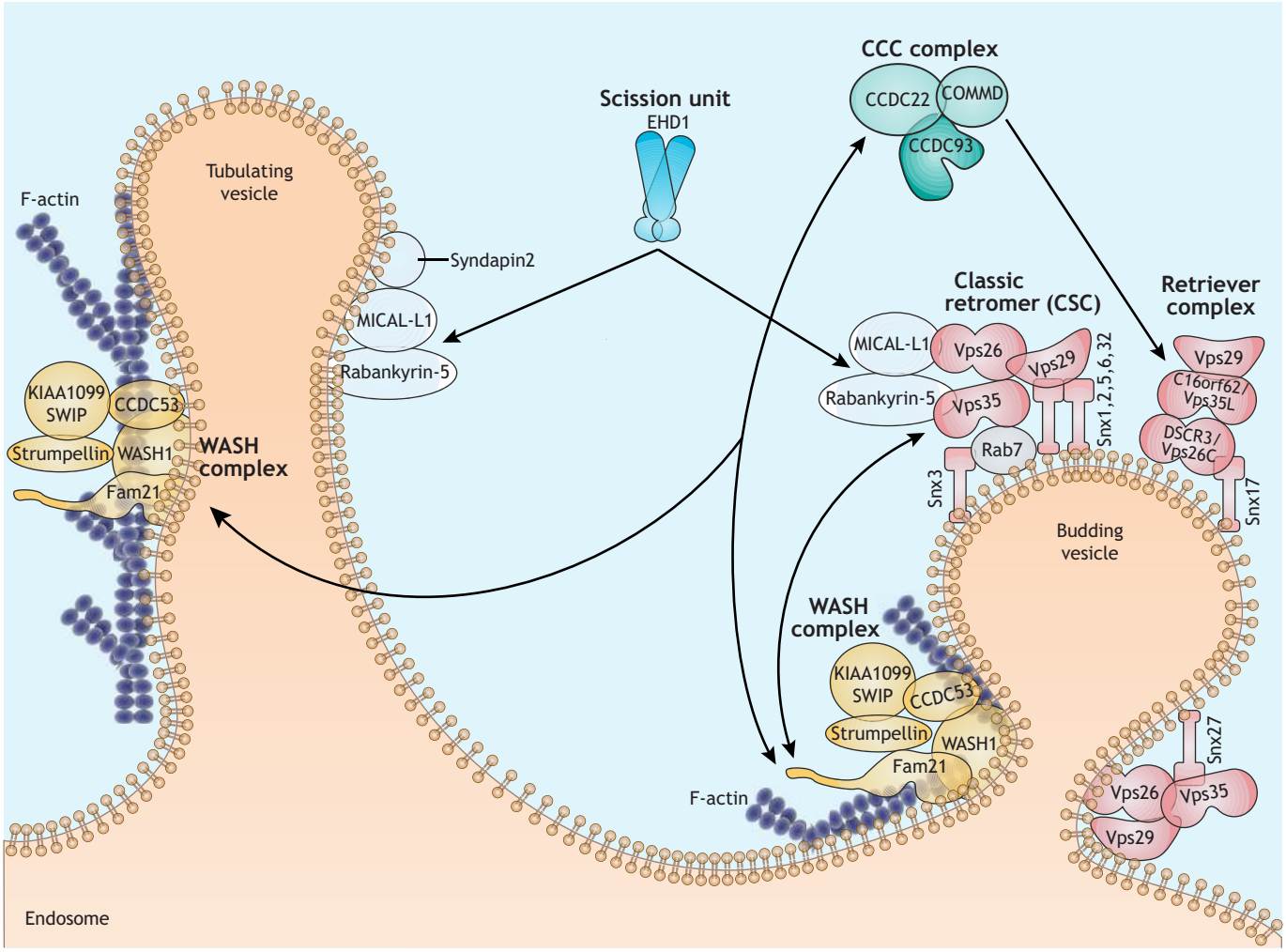

Fig. 3. Endocytic regulatory complexes at the EE. EEs recruit multiple endocytic complexes for the sorting of cargo and the subsequent budding and fission of transport carriers. The classic retromer (CSC), comprising Vps35, Vps26 and Vps29, is recruited to EE through interactions with Snx3, Rab7 and/or SNX BAR proteins (Snx1, Snx2, Snx5, Snx6 and Snx32), or Snx27. Additional interactions occur between the retromer and WASH complex that are mediated by the WASH subunit Fam21 and the retromer component Vps35. The WASH complex binds to phospholipids through its Fam21 subunit, and nucleates actin at the EE, potentially providing a force for constriction of budding vesicles. The WASH complex also interacts with the CCC complex through the binding of Fam21 to the CCDC93 subunit of the CCC complex, and regulates endosome to plasma membrane recycling through an as-yet-uncharacterized mechanism. The CCC complex is also responsible for recruitment of the retriever complex to the EE, where it interacts with Snx17 at the EE membrane and selects cargo such as β1 integrins to budding vesicles for recycling. Tubular carriers are generated by complexes that include MICAL-L1 and syndapin 2, a BAR-domain protein that inserts itself into bilayers and bends membranes (Dharmalingam et al., 2009; Giridharan et al., 2013). EHD1 interacts with syndapin 2, MICAL-L1, rabankyrin-5 and the retromer, leading to speculation that EHD1 could serve as a general fission factor not only for MICAL-L1-containing tubular carriers, but also for tubulovesicular structures that contain the retromer and affiliated cargo.

or Snx32 (van Weering and Cullen, 2014). While the sorting nexins contain PX domains and interact with PI3P (Seaman and Williams, 2002), the CSC was initially considered to be essential for interaction with and sorting of specific cargo (Arighi et al., 2004; Seaman, 2004). Indeed, the retromer CSC requires an interaction with Snx3 and Rab7 (Rab7a or Rab7b isoforms) for optimal recruitment to endosomal membranes (Harrison et al., 2014). However, recent studies with Snx27 and Snx17 are now challenging the exclusive role of the retromer CSC in cargo selection, by demonstrating that sorting nexins themselves are also involved in the cargo selection process (Clairfeuille et al., 2016; Fárfan et al., 2013; Steinberg et al., 2012; van Kerkhof et al., 2005).

Intriguingly, the retromer has enabled researchers who study endocytic membrane trafficking to make an unexpected and important mechanistic connection with mitochondria. A number of papers have implicated Vps35 and the retromer in Parkinson's disease (Deutschlander et al., 2017; Vilariño-Güell et al., 2011; Zimprich et al., 2011), and new evidence suggests that mitochondrial dysfunction is a key factor in Parkinson's disease pathophysiology (Park et al., 2018). Moreover, Vps35-mediated transport of the mitochondrial fission GTPase dynamin-related protein 1 (Drp1, also known as DNM1L), is essential for mitochondrial membrane homeostasis (Braschi et al., 2010; Farmer et al., 2017; Tang et al., 2015; Wang et al., 2016), although it remains possible that Vps35 might influence mitochondrial fission independently of membrane trafficking. In addition to this novel retromer–mitochondria connection, a new study has demonstrated that Rab5 translocates to the mitochondrial membrane upon oxidative stress (Hsu et al., 2018). Moreover, dynamin 2, a key endocytic fission protein, has recently been implicated in mitochondrial fission, together with Drp1 (Lee et al., 2016a). The discoveries that endocytic regulatory proteins,

including dynamin 2, the retromer and EHD1, all regulate mitochondrial homeostasis suggest a novel and previously unappreciated relationship between endocytic pathways and mitochondrial function. In particular, the crosstalk between endosomal protein regulatory complexes including the retromer, mitochondrial function and Parkinson's disease have wide-ranging implications for the significance of the endosome.

The retromer CSC has been established as a hub at EE as it interacts with multiple proteins and protein complexes, in addition to Snx1/2 and Snx5/6. For example, retromer interacts with the tail of the Fam21 (also known as WASHC2) protein, a member of the Wiskott–Aldrich syndrome protein and SCAR homolog (WASH) complex (Gomez and Billadeau, 2009; Harbour et al., 2012) (Fig. 3). The WASH complex comprises WASH1 (also known as WASHC1), Strumpellin (WASHC5), CCDC53 (WASHC3), KIAA1033/SWIP (WASHC4) and Fam21, and induces the nucleation of filamentous actin on EEs (Jia et al., 2012, 2010), potentially to provide the force required for vesiculation, tubulation and fission of the EE membranes. Although it is not known whether a GTPase (i.e. dynamin) or ATPase (i.e. EHD1) participates in the fission of retromer-containing tubules and vesicles at EEs, the demonstration that C-terminal Eps15 homology domain-containing (EHD) proteins and several of their well-characterized endocytic interaction partners, such as MICAL-L1 and rabankyrin-5, form a complex with the retromer at budding vesicles (in addition to their involvement in tubule generation; Giridharan et al., 2013) hints at their involvement in the fission of retromer-containing vesicles at EEs (Gokool et al., 2007; McKenzie et al., 2012; Zhang et al., 2012a,b). It is therefore tempting to speculate that EHD1 might be involved in the formation of a 'scission complex' that is recruited to either the budding vesicles or tubules through its interaction partners [MICAL-L1, syndapin 2 (also known as PACSIN2) and others] to induce fission at multiple budding sites on endosomes, including those that contain retromer (Fig. 3). In such a model, the prediction is that, in addition to the actin nucleation initiated by the WASH complex and a potential motor protein pulling on the forming bud, the ATPase hydrolysis activity of EHD1 might induce fission in a similar manner to that mediated by its GTPase homolog dynamin 2.

Compounding the involvement of the WASH complex at the EE is evidence for an additional complex that interacts with the WASH complex (via Fam21), known as the COMMD complex or CCC complex (Phillips-Krawczak et al., 2015) (Fig. 3). The CCC complex comprises at least three additional proteins, CCDC22 (which also interacts with Fam21; Harbour et al., 2012), CCDC93 and a member of the COMMD family (COMMD1–COMMD9) (Phillips-Krawczak et al., 2015). COMMD1 interacts with CCDC22, CCDC93 and a protein called Vps35L (also known as C16ORF62), a member of another endosomal complex, termed the retriever complex (see below) (McNally et al., 2017; Phillips-Krawczak et al., 2015). Although its function is poorly understood, it has been suggested that the CCC complex mediates the trafficking of low-density-lipoprotein receptor, and CCC mutations that affect formation of the complex cause hypercholesteremia in mice, dogs and humans (Bartuzi et al., 2016). Overall, the retromer and its affiliated complexes play significant roles in modulating classical endocytic trafficking, in addition to their more recently discovered role in the regulation of mitochondrial homeostasis (Farmer et al., 2018).

Get active – emerging mechanisms for receptor recycling

In addition to the sorting nexins that comprise the classical retromer (Snx1, Snx2, Snx5, Snx6 and Snx32), recent studies have described the involvement of other sorting nexin proteins and their interaction partners in trafficking at the EE (Fig. 3). For example, Snx27, a sorting nexin that regulates trafficking of β2 adrenergic receptor (Lauffer et al., 2010; Temkin et al., 2011), interacts with Fam21 of the WASH complex, thus linking it to the retromer (Lee et al., 2016b). Snx27 interacts with multiple cargoes through its PSD95-Dlg-ZO1 (PDZ) domain, binding to proteins with either classic PDZ-binding motifs or with phosphorylated amino acids that substitute for acidic residues (Clairfeuille et al., 2016; Ghai et al., 2013; Steinberg et al., 2013). Snx3 interacts with PI3P on the EE through its PX domain (Xu et al., 2001) and is involved in the recycling of receptors, such as the transferrin receptor (Chen et al., 2013), and in multivesicular body formation (Pons et al., 2008), similar to its more weakly expressed homolog Snx12 (Pons et al., 2012).

The recently described retriever complex also functions at the EE (McNally et al., 2017) in coordination with Snx17 (Donoso et al., 2009; McNally et al., 2017; Stockinger et al., 2002) (Fig. 3). As noted above, the retriever consists of Vps35 homolog Vps35L, which also interacts with the COMMD protein of the CCC complex, the Vps26 homolog DSCR3/Vps26C and Vps29, with the latter also being a subunit of the retromer CSC, further highlighting the complexities of the interwoven networks formed by these endosomal proteins. Retriever has been suggested to function with Snx17 to regulate the recycling of α5β1 integrin and over 120 additional receptors that interact with the Snx17 FERM domain (McNally et al., 2017). The presence of Vps29 as a subunit in both retromer and retriever, and the additional structural and functional similarities between the two complexes suggest a possible evolutionary duplication to broaden the capabilities for regulating recycling at the EE in higher organisms.

The field of endocytic recycling has undergone a quiet revolution over the past few years. Until very recently, most researchers viewed recycling as a passive or default event that occurred when a receptor failed to be actively sorted toward the degradation pathway (Hsu et al., 2012). While this model may still be relevant in many cases, several new lines of evidence now support the idea that there is a more active sorting of receptors into various recycling pathways.

In a seminal study, and one of the first that challenged the model of default recycling, Hsu and co-workers showed that the protein ARF GTPase-activating protein with coiled-coil ankyrin repeat and PH domain-containing protein 1 (ACAP1) recognizes previously uncharacterized sequences within the cytoplasmic tails of the transferrin receptor (TfR), GLUT4 and integrins to direct these proteins to recycling pathways (Dai et al., 2004). In several additional recent studies, it has been shown that members of the sorting nexin family, specifically Snx27 and Snx17, interact directly with cargo receptors and facilitate their recycling to the plasma membrane (Clairfeuille et al., 2016; Gallon et al., 2014; McNally et al., 2017) – overturning, to some degree, the long-held notion that retromer-mediated cargo selection is carried out by its three Vps subunits (Vps35, Vps26 and Vps29) rather than the sorting nexins. Snx27 was originally found to interact with the G protein-coupled receptor β2 adrenergic receptor (Lauffer et al., 2010; Temkin et al., 2011), and, remarkably, hundreds of cargo receptors that interact with the FERM domain of Snx27 protein have since been identified (Clairfeuille et al., 2016). Snx17 has been shown to interact with the cytoplasmic tail of the lipoprotein receptor-related protein 1 (Fárfan et al., 2013; van Kerkhof et al., 2005), as well as integrins (Steinberg et al., 2012). Although the precise mechanisms by which these sorting nexins package receptor cargo and induce the budding and fission of endosomal microdomains remains to be elucidated,

the recent identification of additional sorting nexin-associated complexes, including retriever and the CCC and COMMD complexes (Bartuzi et al., 2016; McNally et al., 2017) have shed new light on the complexities of endocytic recycling pathways.

In recent years, the identification and characterization of key new complexes localized to the EE have dramatically altered our views of these heterogeneous organelles. Not only have we come to a realization that these structures are significantly more complex than originally estimated, but that they function to actively and selectively recruit proteins for recycling. In the following section, endosomes that are expressly involved in the recycling of proteins to the plasma membrane will be discussed.

Recycling endosomes

While it is widely accepted that receptors can be directly recycled back to the plasma membrane from EEs in a pathway that is dependent on the function of Rab4, Rab35, AP-1, and/or Snx17 and Snx27, many cargoes are thought to recycle from an endosomal structure known as the RE. However, the relationship between the EE-involving recycling pathways and those that are regulated by RE at the ERC is not well understood.

Characteristics of REs

RE exhibit several unique characteristics. In a number of cell types, REs are localized to the perinuclear region of the cell known as the ERC, adjacent to the MTOC, and are less abundant in the cell periphery (Grant and Donaldson, 2009), although in many cells including neurons, the ERC is more dispersed (Joensuu et al., 2017). RE are often found to have a tubular shape (Maxfield and McGraw, 2004), and can form a complex network of tubulo-vesicular endosomes that are clustered together in the MTOC, known as the ERC (Maxfield and McGraw, 2004). REs are typically enriched in Rab11 and Rab8 family proteins, Rab22a, Arf6, EHD1 and/or MICAL-L1 (Naslavsky and Caplan, 2005, 2011).

Copernicus revisited – the ERC as the center of the recycling universe

Most receptors, whether they are endocytosed via clathrin-coated pits or in a clathrin-independent manner, are internalized in vesicles that lose their coat, converge, and fuse with the EE membrane in a mechanism that involves the small GTP-binding protein Arf6 (Naslavsky et al., 2003). While there are clearly mechanisms for the direct and rapid recycling of receptors from EE (as noted above), the ‘slower’ recycling of many receptors that first traffic to a perinuclear ERC station has also been well documented (Grant and Donaldson, 2009). Although vesicular transport of receptors from EEs to Rab11- and/or EHD1-containing endosomes at the ERC is a major recycling pathway (Fig. 1; slow recycling), it is also possible that EEs are translocated from the cell periphery to the ERC (Fig. 2). Intriguingly, a recycling pathway for the transferrin receptor has also been identified from the trans-Golgi network (Schindler et al., 2015), suggesting potential functional overlap between these two closely apposed organelles. Thus, while the ERC remains a ‘center’ for regulation of endocytic recycling, additional pathways that bypass the ERC also play significant roles in the retrieval of receptors to the plasma membrane.

C-terminal EHD proteins and their interaction partners in receptor trafficking to the ERC

A number of proteins have been implicated in the control of receptor transport from classic EEs to the ERC. One family of proteins involved in these processes is the EHDs. Unlike other proteins with Eps15 homology (EH) domains, EHDs have their EH domains localized to the C-terminal region of the protein (Naslavsky and Caplan, 2005, 2011) (see Fig. 4). For example, we have shown that depletion of the EHD3 in HeLa cells leads to an increase in the size of peripheral EE and failure of receptors such as TfR to reach the ERC, as well as a measurably faster recycling, presumably directly from EE (Naslavsky et al., 2006). Depletion of the EHD3 paralog EHD4 has a similar effect on the EEs, but it may also regulate the trafficking to LEs rather than the ERC (Sharma et al., 2008). However, it has also been shown that EHD3 associates with and stabilizes ERC-affiliated tubular recycling endosomes (TREs) through the interaction of its EH domain with MICAL-L1 and syndapin 2 (also known as PACSIN2) (Giridharan et al., 2013; Rahajeng et al., 2012; Sharma et al., 2009). Despite a growing consensus for a role for EHD1 in the fission of TREs at the ERC as well as potentially at EEs (Daumke et al., 2007; Giridharan et al., 2013; Jakobsson et al., 2011; Rahajeng et al., 2012; Sharma et al., 2009), evidence also suggests that EHD1 cooperates with its interaction partners MICAL-L1 and CRMP2 (also known as DPYSL2) to control dynein-mediated motor transport of TfR-containing endosomes and/or vesicles from the periphery to the ERC (Rahajeng et al., 2010). In addition, various Rab effectors provide direct links between Rabs involved in recycling and EHD proteins (see Fig. 4). For example, rabenosyn-5 is an EE protein that serves as a dual effector for both Rab4 and Rab5 (Navaroli et al., 2012; Nielsen et al., 2000) and interacts with EHD1 and EHD3 (Bahl et al., 2016; Kieken et al., 2007, 2009, 2010; Naslavsky et al., 2004). Indeed, depletion of rabenosyn-5 causes swelling of EE and prevents TfR from reaching the ERC (Naslavsky et al., 2004), suggesting that this protein coordinates vesicular transport of TfR and possibly other receptor cargo to the ERC. Rab11FIP2, an effector for the RE protein Rab11, also binds to EHD1 and EHD3 through its asparagine-proline-phenylalanine (NPF) motifs, providing yet another link between Rab and EHD proteins, and its interaction with EHD3 appears to be necessary for transport of receptors from EE to the ERC (Naslavsky et al., 2006). MICAL-L1 also facilitates recycling by mediating the generation of tubular carriers via its interaction with syndapin 2, a BAR-domain protein that inserts itself into bilayers and bends membranes (Dharmalingam et al., 2009; Giridharan et al., 2013). MICAL-L1 interacts with both EHD1 and EHD3 through its multiple NPF motifs (Sharma et al., 2010, 2009), with the syndapin 2 SH3 domain through its proline-rich motifs (Giridharan et al., 2013), and with Rab8 on TREs (Roland et al., 2007).

Do all roads lead to or from the ERC?

As noted above, in many non-polarized cells, the ERC tends to represent a juxtanuclear physical region adjacent to the MTOC (Maxfield and McGraw, 2004). Early studies using electron microscopy argued for a cluster of membrane-bound vesicles that may be interconnected via an elaborate network of tubular membranes (Hopkins, 1983), but their dynamics and the degree to which these membranes are connected in steady-state has remained relatively unknown. Indeed, it was largely unknown whether cargoes that are internalized by distinct mechanisms remain segregated at the ERC following their sorting at EEs. A recent study has used advanced single-molecule super-resolution microscopy to provide evidence that cargoes internalized by clathrin-dependent and -independent pathways remain segregated once they reach the ERC (Xie et al., 2015). Moreover, this study further suggests that TREs, which remain largely distinct from spherical REs at the ERC, preferentially traffic cargo from clathrin-independent pathways that regulate receptors, such as CD59 and β1

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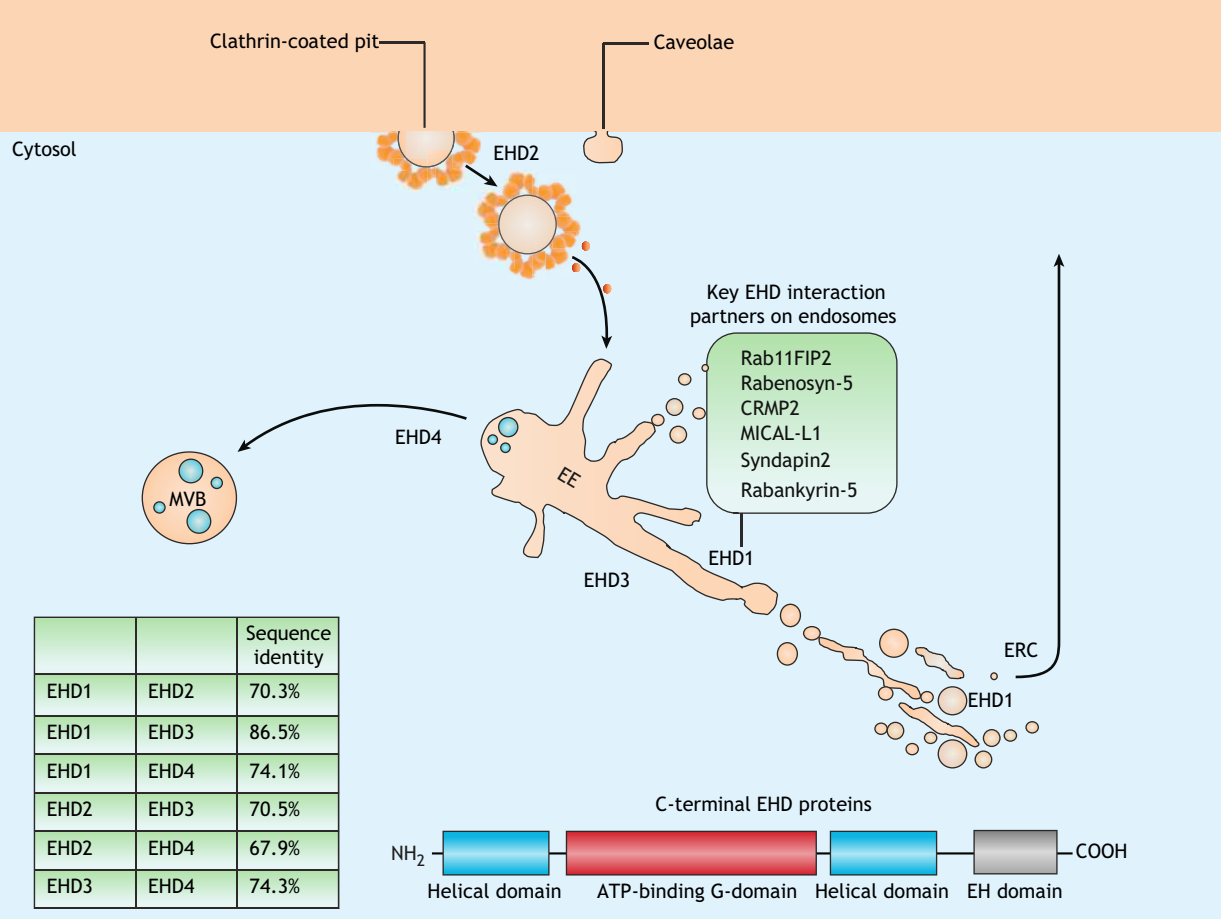

		Sequence identity
EHD1	EHD2	70.3%
EHD1	EHD3	86.5%
EHD1	EHD4	74.1%
EHD2	EHD3	70.5%
EHD2	EHD4	67.9%
EHD3	EHD4	74.3%

Fig. 4. Role of EHD proteins in membrane trafficking. The four EHD proteins display considerable sequence identity, from ~68–87%, and have been implicated in membrane remodeling (table inset). EHD1, EHD3 and EHD4 have been characterized in the regulation of endosomal transport, primarily at the EE, with EHD1 additionally involved in the regulation of recycling from the ERC. EHD2, the most divergent of the EHD proteins, controls caveolar mobility and may influence internalization at the plasma membrane. For further details on the EHD protein family see Naslavsky and Caplan (2011).

integrins (Xie et al., 2015). Although once considered a 'staging ground' for the recycling of most cellular receptors to the plasma membrane, the recent advances in understanding EEs point to considerable recycling from this endosome. Unlike EEs and REs, as discussed below, LEs primarily target receptors for endolysosomal degradation.

Late endosomes

Despite studies that establish the conversion of Rab5-containing EEs into Rab7-containing LEs (Poteryaev et al., 2010), LEs are often viewed as more closely related to (and in a continuum with) lysosomes, rather than other endosomes (Scott et al., 2014). Indeed this degradation pathway is often referred to as the endosomal-lysosomal system (Hu et al., 2015). Moreover, distinguishing between LEs and lysosomes at the molecular level is challenging, because very few proteins selectively mark one compartment over the other (Scott et al., 2014). However, it is possible to discriminate between LEs and lysosomes based on their morphology and buoyant density; initially, LEs were termed 'light lysosomes' as opposed to the heavier 'secondary lysosomes' (Brotherus and Renkonen, 1977; de Duve, 2005; Kobayashi et al., 1998b). Obviously, the limited ability to differentiate between these organelles at the light microscopy level has been a serious drawback for researchers in the field. A number of excellent recent reviews have addressed aspects of LEs that relate to degradation, autophagy and lysosomes (Frankel and Audhya, 2017; Nakamura and Yoshimori, 2017; Pu et al., 2016; Scott et al., 2014; Szymanska et al., 2017), and, LEs will thus be addressed only briefly here, focusing on their relationship to other endosomes. Although it remains incompletely resolved whether endosomes actually 'mature' from an EE into a LE, or whether vesicles bud from EE and then transport cargo to a more static LE, there are a number of well-established differences between EEs and LEs.

LEs generally have a lower pH than EEs (Griffiths, 1989) and are typically enriched in the lipids PI(3,5)P2 (Shaw et al., 2003) and 2′ 2-dioleoyl lysobisphosphatidic acid (LBPA) (Matsuo et al., 2004; Frederick et al., 2009; Hullin-Matsuda et al., 2007). LE are more likely to exclude Rab4 and Rab5, and to instead contain Rab7 (Poteryaev et al., 2010), although it is worth noting that Rab7 is also present on EEs and there helps to recruit the retromer (Liu et al., 2012). Like all endosomes, LEs are a heterogenous group of organelles, but they are considered to be more closely related to lysosomes rather than to EEs (Scott et al., 2014). Furthemore, LEs, like lysosomes, sense nutrients through the mTOR pathway (Flinn et al., 2010), as well as sense and transport cholesterol to other organelles, primarily the ER (Bissig and Gruenberg, 2013).

The growing consensus in the field is that LEs are generated from endosomal carrier vesicle (ECVs) and/or multivesicular bodies (MVBs), both structures that are derived from EEs (Scott et al., 2014). Whereas internalized membrane-bound receptors are sorted

within EEs to distinct tubular membrane buds, any soluble internalized cargo is relegated to intralumenal vesicles (ILVs) that form within the EE through a process of inward invagination. Vesicles containing these ILVs eventually separate themselves from tubular EE regions (that then bud off into separate vesicles) and become ECVs and/or MVBs, which both recruit the HOPS and CORVET complexes typical of LEs. They also undergo the so-called Rab5-to-Rab7 conversion, which is a hallmark of LEs, acquire SNAREs that are specific to LEs, and undergo fusion with existing LE and lysosomes (Frankel and Audhya, 2017). In summary, despite their EE origin, intriguingly, LEs are functionally closer to lysosomes than to other endosomes.

Conclusions, perspectives and open questions

Although there is a clear consensus in the field regarding some aspects of endosomes as highlighted in this Review, key points of contention also remain, which underscore an incomplete understanding of the heterogeneous membrane organelles that are collectively known as endosomes. In some cases, the inability to unambiguously classify endosomes also reveals a fundamental lack of understanding of the biogenesis of these organelles. For example, the literature contains significant discrepancies regarding LEs. Are they, as often suggested, virtually indistinguishable from lysosomes, and best classified as a subpopulation of lysosomes that are constantly undergoing fusion and fission with the latter? Or are LEs a more-evolved form of sorting endosome, essentially an endosomal way-station on route to the degradation pathway? In the latter scenario, LEs would be an alternative destination for cargo that has been sorted from EEs and are not destined for REs. The challenge in defining LEs comes, at least in part, from the disparate models of endosome biogenesis: are LEs generated by evolution of dynamic EEs that shed some markers and acquire others as they perhaps generate ILVs, detach as ECVs/MVBs and slowly become more LE-like in nature? Or are the EEs and LEs more static, with budding, fission and fusion of vesicles from earlier less-specialized endosomes being delivered to a later endosomal compartment? These questions largely remain, despite significant advances in recent years, but answering them is essential for a complete understanding of the endosomal compartment.

Another crucial question deals with the heterogeneity of EEs, and whether there is a gradient of different endosome subtypes with partially distinct markers in the periphery of the cell. Further complicating these issues is whether different EEs exist for the processing of different types of receptors. One early study suggested that despite internalization through different mechanisms (i.e. clathrin-dependent versus clathrin-independent), all internalized cargo end up in a ‘common’ EE (Naslavsky et al., 2003). However, it remains possible that there are earlier stages, in which internalized cargoes first enter distinctive ‘pre-EEs’, prior to accumulating in a common compartment.

The complex role of sorting nexins, the retromer and its affiliated complexes remains another issue of intense investigation, with rapid discoveries driving our current understanding forward. Nonetheless, important questions remain unresolved. For example, although it is clear that Rab7 and Snx3 serve as anchors to recruit the retromer CSC to endosomes, it is not known whether this occurs only on LEs, or whether Rab7 also serves in this capacity in select populations of EE that contain this Rab. It is possible that recruitment to LE serves the classic function of the retromer in retrieval of mannose-6-phosphate receptor from LEs back to the Golgi. At the same time, retromer recruitment to EEs by additional mechanisms (that include other Snx proteins, such as Snx1, Snx2, Snx5, Snx6 and Snx27, among others), may allow the retromer CSC to carry out different roles in regulating membrane trafficking. As noted above, in some cases answering these questions may first require a deeper understanding of endosome biogenesis and characterization.

REs, too, remain enigmatic. In some scenarios, where receptors are rapidly recycled in a ‘short loop’, REs are essentially a form of EE localized to the cell periphery. Indeed, EEs were initially defined as having at least three Rab markers (Rab4, Rab5 and Rab11, albeit in distinct microdomains) – suggesting that Rab11 (the recycling Rab) functions at EEs (Sönnichsen et al., 2000). However, it is also clear that the highly dense perinuclear region is heavily enriched in vesicular and tubular shaped REs (positive for a number of key markers, including Rab11, EHD1 and MICAL-L1), which regulate the receptors that take the ‘longer loop’ for their recycling. Although recent evidence supports the notion that the tubular and vesicular organelles situated at the ERC are likely mostly distinct structures at steady state (Xie et al., 2015), their dynamics are unknown and the relationship between the tubular and vesicular REs have yet to be fully clarified.

Finally, a key open question is whether EEs and/or REs serve as biological cell sensors, much in the way that LEs and lysosomes do. Do EEs sense ligation of receptors and undergo biogenesis as a result? For example, the increased endocytosis and pinching off of vesicles from the plasma might stimulate the generation of additional EEs that serve as receptacles for incoming vesicles. At the same time, one might envision that feedback mechanisms allow REs to sense the presence of ligands in the extracellular milieu, and thus regulate receptor recycling in synchronization with biological feedback. For example, receptor ligation might induce intracellular signals that spur RE biogenesis and recycling. While we recognize that many of these open questions remain difficult to answer, the hope is that acknowledging the great unknowns of the enigmatic endosome will spur investigators to tackle these intriguing questions.

Competing interests

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