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Faraway, so close!

Functions of Endoplasmic reticulum – Endosome contacts

Thomas Di Mattia¹²³⁴, Catherine Tomasetto^{1234*} and Fabien Alpy^{1234*}

- 1 Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Illkirch, France
- 2 Institut National de la Santé et de la Recherche Médicale (INSERM), U1258, Illkirch, France
- 3 Centre National de la Recherche Scientifique (CNRS), UMR7104, Illkirch, France
- 4 Université de Strasbourg, Illkirch, France

* : correspondence to Catherine Tomasetto, IGBMC, 1 rue Laurent Fries, 67400 Illkirch, France; Tel: +33 3 88 65 34 24; Fax: +33 3 88 65 32 01; email: Catherine-Laure.Tomasetto@igbmc.fr or Fabien Alpy, IGBMC, 1 rue Laurent Fries, 67400 Illkirch, France; Tel: +33 3 88 65 35 19; Fax: +33 3 88 65 32 01; email: Fabien.Alpy@igbmc.fr

1 **Abstract**

2
3 Eukaryotic cells are partitioned into functionally distinct organelles. Long considered as
4 independent units in the cytosol, organelles are actually in constant and direct interaction with each
5 other, mostly through the establishment of physical connections named membrane contact sites.
6 Membrane contact sites constitute specific active regions involved in organelle dynamics, inter-
7 organelle exchanges and communications. The endoplasmic reticulum (ER), which spreads throughout
8 the cytosol, forms an extensive network that has many connections with the other organelles of the
9 cell. Ample connections between the ER and endocytic organelles are observed in many cell types,
10 highlighting their prominent physiological roles. Even though morphologically similar - a contact is a
11 contact -, the identity of ER-Endosome contacts is defined by their specific molecular composition,
12 which in turn determines the function of the contact. Here, we review the molecular mechanisms of
13 ER-Endosome contact site formation and their associated cellular functions.
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29 **Introduction**

30 **The ER and endosomes come close**

31 **Components of ER-Endosome contact sites**

32 **ER-Endosome contacts regulate endosome dynamics**

33 *ER-Endosome contacts in endosome tubulation and fission*

34 *ER-Endosome contacts in endosome positioning*

35 **Lipid transfer across ER-endosome contacts**

36 **Membrane contact sites along membrane receptor endocytic route**

37 **Calcium signaling at ER-Endosome contact sites**

38 **Endosomes too contact other organelles**

39 **Conclusion**

40

41

1 Introduction

2 The cell interior is in constant exchange with the extracellular space. Endocytosis allows the
3 displacement of material from the plasma membrane (PM) and the exterior of the cell to the inside of
4 the cell. Endocytosis uses a complex machinery made of cytosolic proteins and a pleiomorphic
5 membrane system composed of tubes and vesicles [1,2]. After the inward budding of a membrane
6 vesicle from the PM, endosomes gradually mature into intermediate compartments named early
7 endosomes (EE) and late endosomes (LE), and ultimately generate the degradative compartment
8 named lysosome (LY) (Fig. 1). This one-way route was challenged by observations uncovering a number
9 of alternative paths along endocytosis [3]. As such, endosomes are also sorting machines that allow
10 either the recycling of a subset of membrane receptors towards the cell surface, or the targeting of
11 others towards lysosomes for degradation (Fig. 1). Furthermore, endosomes mediate the retrograde
12 transport of proteins to the Golgi, or the release of inner luminal vesicles (ILVs) after fusion with the
13 PM, the so-called exosomes [4–6]. In the last decade, new insights have explained how the variety of
14 events occurring within the endocytic compartment can be integrated with the rest of the cell. Indeed,
15 it was shown that endosomes physically contact other cellular organelles and that these contacts in
16 turn regulate the endocytic machinery [7–9]. As endosomes contact more predominantly the
17 endoplasmic reticulum (ER), in this review, we will discuss the mode of formation and the different
18 functions that were described for ER-Endosome contacts (Fig. 1).

19 20 21 The ER and endosomes come close

22 The endolysosomal network is made of membrane-bound organelles functionally inter-connected.
23 The physical barrier created by membranes around organelles is crucial to create favorable
24 environments allowing specific metabolic activities, and to give each compartment its identity
25 [1,2,10,11]. In accordance with this idea, organelles were for a long time considered as independent
26 entities, being more or less permeable to cytosolic compounds, and/or being linked to the rest of the
27 cell through vesicular trafficking. Interestingly, early observations in yeast showed that two organelles,
28 the nuclear envelope which is composed of ER sheets, and the vacuole, the organelle equivalent to the
29 lysosome, were physically associated [12]. This structure called the nucleus–vacuole junction was later
30 functionally characterized and shown to be generated, at least in part, by the interaction between two
31 proteins from the ER (Nvj1p) and the vacuole (Vac8) [13,14]. In mammalian cells, the identification of
32 contacts between endosomes and the ER is more recent. The first clues that physical contacts occurred
33 between an endocytic compartment and the ER came from time-lapse observations of live cells
34 expressing the Niemann Pick C1 (NPC1) protein fused to a fluorescent protein. These data showed that
35 NPC1-positive LE move in association with ER tubes [15]. Later, electron microscopy observations of a
36 specific form of endocytosis named phagocytosis, during which a specialized cell of the immune system
37 engulfs a large foreign particle such as a pathogen, led to the description of a physical association
38 between this endocytic compartment and the ER [16]. The role of these phagosome-ER contacts was
39 thoroughly discussed in a recent review [17].

40 Besides these observations, a general definition of inter-organelle contacts was later given. Inter-
41 organelle contacts, or membrane contact sites, are defined as subcellular regions where the
42 membranes of distinct organelles come into close apposition, with the two heterologous membranes
43 being separated by less than 30 nm [18,19]. At the molecular level, the building of contacts requires
44 protein-protein and/or protein-membrane interactions. Electron and fluorescent microscopy studies
45 showed that endosomes and the ER form extended contacts [20–23] along the endocytic pathway (Fig.
46 2A). Intriguingly, during their maturation from early to LEs/LYs, the association with the ER becomes
47 more and more pronounced, ranging from around 60-80% of EE being associated to the ER, to around
48 90% of LEs/LYs in contact with the ER in Cos-7 cells [22,24]. Of note, in resting cells, around 3-5 % of

1 the surface of the endocytic machinery is in direct contact with the ER [20,22,25]. As a matter of
2 comparison, the surface of mitochondria in contact with the ER is slightly higher, ranging from 5 to
3 15% [25–29]. At the region of contact, the ER and endosome membranes can be separated by less than
4 10 nm [25], and ribosomes are excluded from the ER membrane touching the endosome membrane,
5 while the other side of the ER can be either covered with ribosomes or naked [22,25]. This suggests
6 that ribosomes are excluded from the contact area because of the tight association of the membranes
7 of the two organelles created by protein complexes functioning in these contacts.

8 Thus, ER-Endosome contacts appear to be general structures of the cell. However, the extent of the
9 organelle surface involved in contacts, and the very nature of these contacts (i.e. the proteins/lipids
10 present in the contact) most likely vary according to the cell type, and their physiological state.

13 **Components of ER-Endosome contact sites**

14 The establishment and the support of contacts between the membranes of two organelles require
15 the presence of scaffold proteins defined as tethers [30]. A tether can be a protein or a complex made
16 of two or more proteins (tethering complex) bridging two membranes, and enabling contact
17 maintenance. Three major receptors present on the surface of the ER act as molecular hooks by
18 interacting promiscuously with proteins at the surface of other organelles. These proteins named
19 vesicle-associated membrane protein-associated proteins (VAP) A and B, and motile sperm domain-
20 containing protein 2 (MOSPD2), belong to the major sperm protein (MSP) domain-containing family
21 (Table I) [26,31,32]. VAP-A, VAP-B and MOSPD2 are anchored in the ER membrane by a carboxyl-
22 terminal transmembrane domain, and their cytoplasmic MSP domain is able to interact with proteins
23 that possess a small linear motif named two phenylalanines (FF) in an acidic tract (AT) (FFAT) (Table I)
24 [31,33,34]. Through these interactions, VAP-A/B/MOSPD2 and their organelle-bound FFAT-containing
25 partners assemble into tethering complexes allowing the building of contact sites. Actually, a number
26 of distinct proteins having a FFAT motif are at the surface of endosomes and allow the formation of
27 contact sites with the ER (Table I).

28 Most of the different tethering complexes present in ER-Endosome contacts involve VAP-A, VAP-B
29 and MOSPD2 (Table I and II). The other tethering complexes consist of an ER-associated protein
30 interacting with an endosomal protein or lipid. For instance, the ER-associated S100-A11 protein
31 interacts with its endosomal protein partner Annexin A1 in a calcium-dependent manner (Table II) [35].
32 Furthermore, lipids and lipid-binding proteins can be part of organelle tethering complexes. For
33 example, Protrudin, an ER-anchored protein, binds to phosphatidylinositol 3-phosphate [PI(3)P] at the
34 surface of endosomes to form an ER-Endosome contact (Table II) [36].

35 Even if all ER-Endosome contacts look quite similar, each tethering complex has its own mode of
36 formation and function (Table I and II). The current knowledge on the roles and makeup of the different
37 tethering complexes involved in ER-Endosome contacts will be described throughout this review.

40 **ER-Endosome contacts regulate endosome dynamics**

41 Endo/lysosomes are highly motile organelles which traffic between the cell center and periphery.
42 These long range movements are mediated by molecular motors attached to the organelle by specific
43 adaptor proteins, and sliding along microtubules (Fig. 1). Microtubules are polarized polymers of
44 tubulin, and most motors from the kinesin family drive organelle transport towards the microtubule
45 plus-end, while motors from the dynein family drive transport in the opposite direction [37,38]. Thus,
46 one aspect of the dynamics of endolysosomal structures lies in their regulated movements within the
47 cytosol. Another aspect comes from the mosaic morphology of endolysosomal organelles, ranging
48 from large round vesicles to thin elongated tubules. Intriguingly, endosome positioning and tubulation

1 are both controlled by the ER, and involve ER-Endosome contacts. In this section, we will describe the
2 involvement of the ER in endosome positioning, and then in endosome tubulation and fission.

3 4 *ER-Endosome contacts in endosome positioning*

5 Endosomes come in multiple shapes from large vesicles to thin extended tubules which both move
6 dynamically inside the cytosol. These movements are regulated by the recruitment of adaptor proteins
7 which link endosomes and microtubule motors. Interestingly, adaptor proteins recruitment onto
8 endosomes was shown to be controlled in part by ER-Endosome contacts.

9 Cholesterol is the major non-polar lipid of mammalian biological membranes [39]. Cholesterol
10 content and transport in the endosomal system is linked to LEs/LYs trafficking, as illustrated by the
11 NPC disease [40]. NPC is an autosomal recessive neurodegenerative disorder associated with
12 mutations in NPC1 and NPC2 genes. NPC1 and NPC2 mutations result in cholesterol accumulation in
13 LEs/LYs which is linked to the blockage of these organelles at the microtubule minus-end, in the
14 perinuclear region [15,41,42]. One mechanism regulating the recruitment of motor proteins on
15 endosomes relies on the sensing of cholesterol levels at their limiting membrane by the oxysterol
16 binding protein (OSBP)-related protein 1L (ORP1L). This protein belongs to the OSBP family, a family
17 composed of twelve lipid transport protein (LTP) genes in mammals [43]. LTPs are involved in the
18 monomeric transport of lipid molecules between membranes. ORP1L is one of the two major isoforms
19 produced from the OSBPL1A gene, together with ORP1S [44]. ORP1L possesses three amino-terminal
20 ankyrin repeats (AR), a pleckstrin homology (PH) domain, an FFAT motif, and a carboxyl-terminal OSBP-
21 related ligand-binding domain (ORD) (Tables I and II) [45]. This protein organization explains the ability
22 of ORP1L to connect two distinct organelles. Indeed, ORP1L can associate with the ER thanks to its
23 FFAT motif which interacts with VAPs and MOSPD2, and with LEs/LYs by interacting with the active
24 form of the guanosine triphosphate (GTP)ase Rab7, thanks to its AR [23,26,46]. Comparatively, ORP1S
25 is a cytosolic protein lacking the AR and PH domains, as well as the FFAT motif. Thus, unlike ORP1L,
26 ORP1S is not involved in ER-Endosome tethering. In 2005, Olkkonen and collaborators showed that the
27 overexpression of ORP1L induces the perinuclear clustering of LEs/LYs [46]. A model based on
28 cholesterol-dependent protein conformational changes was later proposed to explain the molecular
29 mechanism of endosome positioning controlled by ORP1L. ORP1L possesses two alternative
30 conformations [23]: i) under high endosomal cholesterol levels, ORP1L harbors a closed conformation
31 compatible with the recruitment of the molecular motor dynein onto endosomes, thereby favoring
32 minus end microtubule transport; this recruitment involves a complex composed of the small GTPase
33 Rab7, the Rab-interacting lysosomal protein (RILP), and dynactin; ii) alternatively, under low
34 endosomal cholesterol levels, ORP1L is in an open conformation freeing its FFAT motif, and thus
35 allowing its interaction with VAP-A. ORP1L-VAP-A interaction promotes ER-Endosome contact
36 formation, and impedes minus end transport [23]. Along the same line, ORP1L has been shown to
37 localize to late autophagosomes, controlling their transport and fusion with the endocytic pathway
38 [47]. Accordingly, in ORP1L-depleted cells, a lower fraction of structures positive for the autophagic
39 marker microtubule associated protein 1 light chain 3 (LC3) is found at the cell periphery. Moreover,
40 the expression of an ORD-depleted form of ORP1L increases the percentage of scattered
41 autophagosomes. This phenotype is linked to the increased association of this ORP1L mutant with VAP-
42 A, which induces ER-autophagosome contact formation. In this context, ORP1L in contact with the ER
43 prevents both the fusion of autophagosomes with LEs, and their minus end transport [47].

44 The transport of LEs/LYs towards the periphery of the cells is also controlled by ER-Endosome
45 contacts. Protrudin is a transmembrane protein localized in the ER membrane. Structurally, this
46 protein is composed of several regions including a FFAT motif, a low complexity region (LCR) and a
47 FYVE [named after Fab 1, YOTB, Vac1, and EEA1] domain. The LCR and FYVE domains are able to bind
48 the endosomal protein Rab7 and the endosomal lipid PI(3)P, respectively (Table II) [36,48]. As a result,

1 since Protrudin is at the same time anchored to the ER membrane and able to interact with
2 endosomes, this protein allows the building of ER-Endosome contacts [36]. Surprisingly, Protrudin
3 expression also causes LE accumulation at the periphery of the cell in a FYVE and coiled-coil domain
4 containing 1 (FYCO1)-dependent manner. FYCO1 is another Rab7/PI(3)P-interacting protein found in
5 the same complex as Protrudin. Furthermore, FYCO1 and Protrudin are both kinesin interacting
6 proteins. Hence, it has been proposed that, at the site of Protrudin-mediated ER-Endosome contacts,
7 Protrudin promotes the loading of the motor protein kinesin on FYCO1, which in turn mediates LE plus
8 end transport. At the end of their journey, LEs fuse with the PM in a synaptotagmin-7 (SYT7)-
9 dependent manner and promote outgrowth of protrusions and neurites [36]. Intriguingly, Protrudin
10 also contains an FFAT motif that allows its interaction with VAP-A inside the membrane of the ER
11 [34,49]. Functionally, this interaction is required to promote Protrudin-mediated neurite extension
12 [49]. However, the role played by Protrudin/VAP-A association in LE translocation towards the PM
13 remains elusive. Furthermore, in a more general context, the meaning of the interaction of VAP
14 proteins with other ER proteins via an FFAT motif remains to be characterized. It is tempting to
15 speculate that such interactions could be involved in ER-ER contacts, and therefore in ER dynamics.

16 The positioning of LEs/LYs is influenced by nutrient availability. Starvation induces the relocation of
17 LEs/LYs in the perinuclear region, while nutrient abundance causes LEs/LYs dispersion [50]. This
18 regulated positioning of LEs/LYs coordinates the cellular nutrient response, and in particular the
19 activation of the major metabolism regulator mechanistic Target Of Rapamycin Complex 1 (mTORC1).
20 In response to serum and amino acid starvation, mTORC1 signaling is turned off and the retrograde
21 transport of LEs/LYs mediated by the dynein-dynactin motor and microtubules is activated, which leads
22 to the perinuclear accumulation of LEs/LYs [51]. Conversely, when nutrients and growth factors are
23 present, anterograde transport of LEs/LYs is promoted and mTORC1 activated. In this later condition,
24 ER-Endosome contacts involving Protrudin favor the plus end transport of mTORC1 bearing endosomal
25 vesicles towards the cell periphery [52]. Amino acids activate PI(3)P production on LEs/LYs, which is
26 mediated by the class III phosphatidylinositol 3-kinase VPS34/PIK3C3, and thus allow the recruitment
27 of Protrudin and the formation of contact sites with the ER. Similarly to the mechanism described
28 above, Protrudin then promotes the loading of Kinesin on FYCO1, which in turn mediates LE plus end
29 transport [52]. This transport of mTORC1-positive LEs/LYs to the cell periphery is crucial for mTORC1
30 activation. Indeed, in cells silenced for Protrudin or FYCO1, mTORC1 activation is reduced, which
31 suggests that mTORC1 needs to be brought in the vicinity of the PM to be properly activated by the
32 growth factor signaling cascades. Conversely, in starved condition, the inactivation of mTORC1
33 signaling is accompanied by the induction of autophagy, which fuels the cellular metabolism [50].
34 LEs/LYs are both transported towards the center of the cell, and actively retained in this area of the
35 cell.

36 Endosome motility varies according to their position in the cell: perinuclear endosomes are mostly
37 static, while peripheral endosomes are highly mobile [53–55]. A recent study has demonstrated that
38 the ubiquitin ligase RING finger protein 26 (RNF26; Table II) controls the spatiotemporal organization
39 of endosomes by trapping them onto the perinuclear ER [54]. Accordingly, in cells devoid of RNF26,
40 both early endosomes and LEs/LYs become more peripherally localized. RNF26 is an ER-anchored
41 protein localized predominantly in the perinuclear ER where it interacts and ubiquitinates its substrate
42 the sequestosome 1 (SQSTM1) protein, also known as ubiquitin-binding protein p62 (Table II) [54]. In
43 turn, ubiquitinated SQSTM1 recruits several ubiquitin-binding domain (UBD)-containing proteins
44 associated with endocytic organelles, such as the EE protein epidermal growth factor receptor
45 substrate 15 (EPS15; Table II), and the LE protein Toll interacting protein (TOLLIP; Table II). These
46 molecular assemblies, by bridging the ER and endosomes, restrain endosomes in the perinuclear
47 region. The release of endosomes from the ER, which favors their anterograde transport, is mediated
48 by the de-ubiquitination of SQSTM1 by the ubiquitin carboxyl-terminal hydrolase 15 (USP15).

1
2 ER-Endosome contacts in endosome tubulation and fission

3 A startling function of ER-organelle contacts was discovered by Voeltz and collaborators in the
4 mitochondria. They showed that ER tubules come in contact with mitochondria and mark the site
5 where organelle fission occurs afterwards [56]. The same laboratory later showed that endosome
6 fission also occurs at sites of ER contact [57].

7 During its journey, the endocytosed cargo is sorted along the endocytic compartment. Cargo
8 segregation depends on its destination, the PM, the Golgi, or LYs which correspond to recycling,
9 retrograde, and degradation routes, respectively. Besides, this mechanism is accompanied by the
10 segregation of Rab GTPases into distinct domains in the endosomes [5,11,58]. The recycling and
11 retrograde transport routes involve endosomal tubulation, a mechanism which requires the actin and
12 microtubule cytoskeletons, and their regulatory components, i.e microtubule motor proteins, Wiskott-
13 Aldrich syndrome protein and scar homolog (WASH)-mediated branched actin network, retromer and
14 sorting nexins (SNXs) [58,59] (Fig. 1 and 3).

15 Intriguingly, the ER is directly involved in these endosomal membrane dynamics (Fig. 3). Indeed, ER
16 tubules are recruited to tubulating early and late endosomes, and fission takes place at the site of
17 contact marked by the retromer-associated protein FAM21 [57]. A direct physical link between the ER
18 and the retromer complex ensues from the interaction between the ER resident VAP proteins and the
19 retromer complex component SNX2 (Table I) [60]. Retromer is a multiprotein complex which controls
20 cargo sorting and mediates endosome tubulation; this complex was primarily involved in endosome to
21 Golgi transport of cargos such as the cation-independent mannose-6-phosphate receptor (CI-MPR), a
22 protein which shuttles from the Golgi to endosomes to transport acid hydrolases [61].

23 In order to better visualize the relationship between ER tubes and endosome fission, Rowland et
24 al. generated elongated endosomes by inhibiting the fission machinery, either by treating the cells with
25 the dynamin inhibitor dynasore, or by depleting Dynamin-2 (Dyn2). They observed that more than 80%
26 and 100% of EE and LE fission sites were crossed by ER tubules, respectively. Thus, the authors
27 proposed a model in which endosomal fission is regulated in time and space by ER contact sites [57].
28 More recently, proteins acting as tethers at ER-mediated endosome fission sites were identified [62].
29 By using a proximity-based labelling strategy, the authors uncovered the presence of the ER-resident
30 protein Transmembrane and Coiled-Coil domains protein 1 (TMCC1) (Table II) at the level of FAM21-
31 positive endosome fission sites. TMCC1 specifically drives the ER to the budding region where the
32 endosomal protein Coronin 1C (CORO1C) is present (Fig. 3; Table II). Depletion of CORO1C or TMCC1
33 prevents the recruitment of the ER to the endosome bud, and consequently decreases bud fission. This
34 loss of function strategy showed that TMCC1-CORO1C association mediates the formation of ER-
35 Endosome contacts involved in endosomes fission [62]. Interestingly, depletion of TMCC1 or CORO1C
36 does not completely suppress endosome tube fission, suggesting that other unidentified complexes
37 involved in this process exist. Furthermore, because budding/fission events are associated with
38 membrane elongation and curvature, which need lipids to build up the membrane, it is tempting to
39 speculate that LTPs could be recruited at ER-Endosome tube contacts, to provide membrane lipids to
40 the endosome in order to favor membrane expansion in the growing tube.

41 At the point of contact with the ER, endosome tubules are constricted, and then they undergo
42 fission [57,62]. In mitochondria, ER-associated constriction sites are marked by mitochondrial fission
43 factors mediating the recruitment of the fission machinery, namely the dynamin-related protein 1
44 GTPase (Drp-1), and later in the process Dyn2 [56,63,64]. At the level of endosomes, the fission
45 machinery is not completely identified. One promising complex candidate, localized at ER-Endosome
46 contacts, is composed of Spastin (a microtubule-severing ATPase), and IST1 (an endosomal sorting
47 complex required for transport (ESCRT)-III -associated protein) (Table II) [65]. At the subcellular level,
48 Spastin associates with the ER by interacting with several ER-shaping proteins from the receptor

1 expression-enhancing protein (REEP), reticulon, and atlastin families [66]. In cells lacking Spastin, the
2 life-time of endosome tubules is increased concomitantly with the number of ER-Endosome contacts,
3 showing that Spastin is needed to promote endosome tube fission, but not for the formation of ER-
4 Endosome contacts [65,67]. The same phenotype is observed in mouse embryonic fibroblasts
5 expressing an ATPase-defective Spastin, supporting the idea that Spastin-mediated endosomal fission
6 requires a functional ATPase activity. Furthermore, depletion of the Spastin-binding partner IST1 is also
7 associated with an increased tubulation life-time, from less than 10 seconds (s) in control cells to
8 around 15 s in IST1-depleted cells. Moreover, the re-expression in IST1-depleted cells of a mutant IST1
9 protein unable to interact with Spastin failed to rescue the normal life-time of endosome tubules,
10 indicating that Spastin-IST1 interaction is necessary for efficient endosomal fission at ER contacts [65].
11 Whether the Spastin-IST1 complex works in synergy with the TMCC1-CORO1C tethering complex at
12 ER-Endosome contacts remains an open question.

13 Interestingly, Spastin (also known as spastic gait 4 - SPG4) is mutated in hereditary spastic
14 paraplegia (HSP) disease, a condition characterized by axon degeneration [68]. Fibroblasts from
15 Spastin-HSP patients display lysosomal abnormalities, namely an increased proportion of enlarged
16 lysosomes with altered morphology. These phenotypes are also observed in cells lacking other HSP-
17 associated proteins, strumpellin and REEP1 [65]. These data directly link defects of ER-dependent
18 endosomal fission and axonopathy [65].

21 **Lipid transfer across ER-Endosome contacts**

22 In eukaryotic cells, lipids are unevenly distributed between organelles [39]. For instance, cholesterol
23 is highly present in the PM, intermediately in endosomes, and is only scarce in the ER. This
24 characteristic distribution of lipids is caused by selective and directional lipid movements. Since lipids
25 are highly insoluble in water, their transport in between organelles occurs by vesicular or non-vesicular
26 pathways. Non-vesicular transport usually involves LTPs [69–71]. Structural and biochemical analyses
27 have shown that most LTPs contain a hydrophobic lipid-binding tunnel which accommodates one lipid
28 molecule. The mode of action of LTPs requires the protein to take one lipid molecule from the outer
29 leaflet of a donor membrane, to travel across the hydrophilic environment in between the two
30 membranes (for instance the cytosol), and to deliver its cargo to the outer leaflet of an acceptor
31 membrane [18,71]. Thus, LTPs can be considered as devices equilibrating lipid concentration between
32 membranes. Several associated mechanisms explain how such a simple function ensures a selective
33 and directional transport of lipids. For instance, each LTP usually binds one, or a restricted number of
34 lipid species, which accounts for specificity [69]. Directional transport is mostly achieved through lipid
35 trapping, and heterotypic lipid exchange [70]. For instance, lipid trapping in the acceptor compartment
36 can be mediated by the enzymatic conversion of this lipid into another lipid species, preventing its
37 transport in the opposite direction, and thus allowing a unidirectional transport. This mechanism can
38 be exemplified with ceramide transfer from the ER to the trans-Golgi, which is carried out by the LTP
39 StAR related lipid transfer domain-11 (STARD11); indeed, once in the trans-Golgi, ceramide is
40 converted into sphingomyelin [72]. The heterotypic exchange of lipids consists in the transfer of two
41 different lipid species (lipid A and B) by an LTP; the trapping of lipid A fuels the counterflow transport
42 of lipid B [73]. For instance, the OSBP-related protein Osh4p counter-transport sterol and PI(4)P
43 between the ER and the Golgi; PI(4)P consumption in the ER by the phosphatase Sac1 allows the
44 anterograde transport of sterol against its concentration gradient [73,74].

45 The main parameters affecting the efficiency of LTP-mediated lipid transport are the energy barrier
46 of lipid desorption from the membrane, and the distance between membranes [18,75]. Hence, some
47 LTPs act specifically in contact sites between organelles [71,76,77]. Contact sites formation spatially

1 restricts the action of LTPs, and facilitates its regulation [75]. To date, cholesterol transport is the most
2 documented lipid transport in ER-Endosome contacts.

3 Cells can acquire cholesterol by two means, biosynthesis which occurs in the ER, and endocytosis
4 of lipoproteins [78]. Thus, ER-Endosome contacts are at the crossroad of cholesterol homeostasis. Two
5 LTPs, STARD3 belonging to the Steroidogenic Acute Regulatory protein related lipid transfer (START)
6 family, and ORP1L belonging to the OSBP family, were shown to mediate cholesterol transport in ER-
7 Endosome contacts (Fig. 4A; Table I).

8 STARD3 (START domain-3) is a transmembrane protein localized in LEs (Fig. 2) [79]. This protein is
9 composed by two distinct domains, an amino-terminal MENTAL transmembrane domain which
10 anchors the protein in the membrane of LEs, and a cytosolic carboxyl-terminal START domain (Table I)
11 [80]. STARD3 is one of the 15 human START proteins, and it belongs to the START protein sub-family
12 involved in cholesterol transport [81,82]. At the boundary between the MENTAL and the START domain
13 of STARD3 lies an FFAT-like motif allowing its interaction with VAPs and MOSPD2 [25,26].
14 Ultrastructural observations of STARD3 expressing cells showed that LEs positive for STARD3 are
15 wrapped by ER membranes [25]. Indeed, the interaction between STARD3, anchored onto the
16 endosome, and VAP-A, VAP-B or MOSPD2, anchored onto the ER, induces the formation of ER-
17 Endosome contacts (Fig. 2 B-D).

18 The presence of STARD3 at the interface between endosomes and the ER suggested that it could
19 exchange cholesterol between these two organelles. Cholesterol labeling using fluorescent probes
20 indeed showed that STARD3 expression mediates cholesterol transport towards endosomes
21 [77,79,83]. Moreover, structure-function studies demonstrated that both a functional START domain
22 binding cholesterol, and the ability of the protein to mediate ER-Endosome contact formation were
23 required for cholesterol transport [77] (Fig. 4A). In agreement with these data, *in vitro* reconstitution
24 of ER-Endosome contact sites using liposomes harboring STARD3 (Endosome-like liposome) and VAP-
25 A (ER-like liposome) showed that the STARD3-VAP-A tethering complex is a highly efficient machine to
26 build a contact and transfer cholesterol between membranes [77]. The importance of STARD3 in
27 cholesterol transport is supported by a genome-wide association study which linked the STARD3 gene
28 and high density lipoprotein (HDL) cholesterol concentration [84]. However, STARD3-deficient mice
29 showed only modest cholesterol alterations, suggesting that intracellular cholesterol transport
30 pathways are highly redundant [85].

31 Intriguingly, another human protein possesses a MENTAL domain. This protein, named STARD3NL
32 (STARD3 N-terminal like) is not a LTP, as it does not possess a lipid-binding domain, but similarly to
33 STARD3, it is anchored in LE membranes, and it harbors an FFAT-like motif (Table I) [25,86]. Indeed,
34 STARD3NL interacts with VAPs and MOSPD2 to mediate the formation of ER-endosome contacts
35 [25,26]. However, the function of this protein within ER-Endosome contacts remains elusive. It was
36 shown that STARD3NL interacts with STARD3 as well as cholesterol, and therefore we can hypothesize
37 that STARD3NL regulates the function of STARD3 [80,87].

38 As described above, ORP1L is involved in ER-Endosome contact formation under low cholesterol
39 conditions [23]. The ability of ORP1L to tether two organelles, and to transport cholesterol with its
40 ORD domain, suggested that this protein could mediate cholesterol transport within ER-Endosome
41 contacts [23,88,89] (Fig. 4A). Besides, the involvement of ORP1L in cholesterol transport is further
42 supported by the identification of an ORP1L heterozygous truncating mutation associated with low
43 circulating HDL levels in human [90].

44 In *Caenorhabditis elegans*, inactivation of ORP genes induced defects in the formation and function
45 of multivesicular endosomes, associated with a reduced cholesterol content in LEs/LYs [91]. Moreover,
46 this function is conserved in human since ORP1L knockdown resulted in a similar phenotype with the
47 formation of enlarged endosomes containing less ILVs. Besides, this role of ORP1L was recently linked
48 with the ability of the protein to fuel cholesterol from the ER into endosomes, in low cholesterol
49 conditions, to favor membrane, and specifically ILV formation [35]. Accordingly, depletion of ORP1L or

1 VAPs reduces both ILV formation and cholesterol content in Epidermal Growth Factor Receptor (EGFR)-
2 endosomes. Interestingly, the LTP STARD3 is possibly regulating ILV formation by transporting
3 cholesterol within ER-Endosome contacts [77]. Indeed, cells expressing STARD3 exhibited endosomes
4 with more internal membranes. Structure/function studies revealed that this phenotype required the
5 ability of STARD3 to scaffold ER-endosome contacts, and the START domain of STARD3 [77]. Thus
6 ORP1L and STARD3 are involved in the transport of ER-derived cholesterol to provide building blocks
7 for membrane formation in endosomes. However, VAP/ORP1L and VAP/STARD3 do not operate in the
8 same endosome population, VAP/ORP1L and VAP-STARD3 being associated with EGFR and non-EGFR
9 endosomes, respectively [35,77].

10 Interestingly, ORP1L-mediated cholesterol transport is not unidirectional as ORP1L-deficient cells
11 display endosome-to-ER cholesterol transfer defects [92]. Actually, ORP1L-knock-out cells display the
12 features of defective endosome-to-ER LDL cholesterol export. When cultivated in LDL containing
13 medium, these cells exhibited Acyl-CoA: cholesterol acyltransferase (ACAT)-mediated esterification
14 defects, this enzyme being an ER-resident enzyme which esterifies the excess of cholesterol when it
15 reaches the ER. In a similar way, sterol-regulated genes were increased, which was accompanied with
16 an increase in sterol synthesis, and finally cholesterol accumulated in endosomes. Structure/function
17 studies showed that the ability of ORP1L to interact with VAPs, and to bind cholesterol and PIPs in its
18 ORD domain were both required for endosome cholesterol egress, further supporting the ability of the
19 protein to promote cholesterol transport in ER-Endosome contacts [92].

20 Recent *in vitro* analysis showed that PIPs, and more specifically PI(4,5)P₂ and PI(3,4)P₂, enhance
21 cholesterol transport by an allosteric mechanism facilitating ORP1 membrane targeting and
22 cholesterol extraction [93]. This role of phosphoinositides is different from what was shown for other
23 OSBP-related proteins for which PIPs, and in particular PI(4)P, is counter-transported by the protein.
24 Indeed, Drin and collaborators have shown that the transport of lipids, such as cholesterol or
25 phosphatidylserine, from the ER to their target compartment (Golgi, PM) is coupled to the backward
26 transport of PI(4)P [73,94]. PI(4)P synthesis in the target organelle, and its degradation by the
27 phosphatase Sac1 in the ER, fuels energy in the lipid transport cycle, and permits the creation of a lipid
28 gradient [74,95].

29 OSBP is the founding member of the OSBP protein family (Table I). This protein counter-exchanges
30 cholesterol and PI(4)P in ER-Golgi contacts [76]. In addition to being present at the level of ER-Golgi
31 contacts, OSBP is also found in ER-Endosome contacts built by the interaction between VAP proteins
32 and SNX2 [60]. Indeed, OSBP is implicated in endosome-to-Golgi traffic by regulating actin nucleation
33 and retromer function through PI(4)P transport in ER-Endosome contacts mediated by VAP/SNX2
34 interaction. In the absence of OSBP, PI(4)P accumulates on endosomes and induces WASH recruitment,
35 and actin nucleation. Given that OSBP counter-exchanges cholesterol and PI(4)P between the ER and
36 Golgi, it most probably regulates PI(4)P levels in the endosome by counter-transporting cholesterol
37 from the ER. The role of this presumed cholesterol flux during endosome-to-Golgi traffic remains to be
38 determined (Fig. 4B).

39 Cholesterol transport at ER-endosome contacts is not a unique one-way process. Several protein
40 complexes are involved, and cholesterol transport can be bi-directional. This supports the idea that
41 different pools of endosomal cholesterol exist and that its transport must be adapted to the biological
42 context thanks to specialized endosome populations and the presence of discrete functional domains.
43
44

45 **Membrane contact sites along membrane receptor endocytic route**

46 Endosomes play a crucial role in the spatio-temporal regulation of receptor tyrosine kinase (RTK)
47 activity [96,97]. EGFR represents the archetype of endocytosed RTK destined for degradation (Table II)
48 [97]. Upon ligand binding, EGFR dimerizes and dimers cross-phosphorylate their cytoplasmic tail; the
49 phosphorylated tyrosines act as docking sites for the recruitment and activation of downstream

1 signaling targets. After its activation, EGFR is rapidly endocytosed, which is the first step of signal
2 termination. Although signaling from EGFR takes place predominantly at the PM, the receptor is still
3 active within the endosomal system where it is able to recruit and activate its cytoplasmic substrates
4 [98]. Finally, the receptor is dephosphorylated by specific phosphatases, and is subjected to
5 internalization into ILVs, which suppress EGFR signaling. In the end, EGFR reaches the lysosome where
6 it is degraded [99]. Thus, activation and inactivation of EGFR occur at different subcellular areas.
7 Contacts between EGFR-endosomes and the ER were shown to be involved in signal termination.
8 Indeed, EGFR interacts with the ER-anchored protein tyrosine phosphatase PTP1B within ER-Endosome
9 contacts, which allows its dephosphorylation [20,100] (Fig. 5A; Table II). Moreover, ER-Endosome
10 contacts are involved in the formation of ILVs and the sequestration of EGFR in these structures
11 [20,35,101].

12 To identify the tethering complex involved in the formation of ER-Endosome contacts allowing EGFR
13 dephosphorylation by PTP1B, Eden and collaborators analyzed ER-Endosome contacts in cells treated
14 with EGF in the absence of selected candidate genes [35]. Despite the notion that most ER-Endosome
15 contacts involve VAP proteins, the number of contacts between EGFR-containing endosomes and the
16 ER is not perturbed in VAP-depleted cells stimulated with EGF. Rather, depletion of Annexin A1 (Table
17 II), an endosome-localized EGFR substrate, specifically decreases ER/EGFR-Endosome contacts which
18 is accompanied by a prolonged EGFR phosphorylation [35,102]. Furthermore, electron microscopy
19 observations revealed that Annexin A1, EGFR and PTP1B co-localize in ER-Endosome contacts,
20 reinforcing the idea that PTP1B-mediated EGFR dephosphorylation occurs in contacts built by Annexin
21 A1. The quest for a tethering partner revealed that to build contacts, Annexin A1 needs to interact with
22 the ER-localized Protein S100-A11 in a calcium-dependent manner (Fig. 5B; Table II) [35,103]. Thus,
23 Annexin A1 and S100-A11 build ER-Endosome contacts that are specifically populated by EGFR and
24 PTP1B in the endosome and ER membrane, respectively, and constitute a dephosphorylation
25 compartment for EGFR en route for degradation in the lysosome (Fig. 5B).

26 Importantly, Annexin A1/S100-A11-regulated ER-Endosome contacts are not only involved in EGFR
27 dephosphorylation, but also in the formation of ILVs. Indeed, the absence of either Annexin A1 or S100-
28 A11 disrupts ILV formation, in addition to decreasing contacts [35]. The formation of ILVs is mediated
29 by the ESCRT machinery, which is made up of cytosolic protein complexes named ESCRT-0, ESCRT-I,
30 ESCRT-II and ESCRT-III [104]. Hepatocyte growth factor-regulated tyrosine kinase substrate (HRS) and
31 signal-transducing adaptor molecule (STAM) are ESCRT-0 components which are phosphorylated in
32 response to growth factor stimulation, such as EGF. Interestingly, besides acting on EGFR, PTP1B also
33 dephosphorylates HRS and STAM, likely at ER-Endosome contacts made by Annexin A1/S100-A11
34 [20,35,105]. Although the role of HRS and STAM phosphorylation in regulating ESCRT-0 activity is
35 unclear, PTP1B-mediated dephosphorylation of HRS and STAM seems to be required for ILV formation
36 [20,105]. Accordingly, the disruption of ILV formation associated with the loss of Annexin A1 or S100-
37 A11 is likely due, at least in part, to the absence of dephosphorylation by PTP1B of the ESCRT-0
38 components HRS and STAM. Interestingly, although EGFR sorting onto ILVs actively depends on the
39 ESCRT machinery, ILV assembly is also supported by cholesterol transport by the LTP ORP1L and VAP
40 from the ER towards the endosome, when low density lipoprotein (LDL)-derived cholesterol is low (Fig.
41 5B) [35,106].

42 Thus, along the endocytic route, ER-Endosome contacts control EGFR signal termination. These
43 specialized ER-Endosome contacts are built by the Annexin A1 / S100-A11 complex, and recruit several
44 proteins, allowing a division of labor: PTP1B is involved in the dephosphorylation of EGFR, and
45 ORP1L/VAP in the transport of cholesterol towards the endosome to build ILVs. Besides EGFR, other
46 RTKs such as the platelet-derived growth factor receptor- β (PDGFR) and the granulocyte colony-
47 stimulating factor receptor (G-CSFR) are also subjected to PTP1B-dependent dephosphorylation in ER-

1 endosome contacts, thus providing an example of specialized contacts implicated in RTK signal
2 attenuation along the endocytic route [100,107].
3
4

5 **Calcium signaling at ER-Endosome contact sites**

6 Besides lipids, calcium is also exchanged at the interface between the ER and endosomes. Calcium
7 ion (Ca^{2+}) is an ubiquitous second messenger involved in a large variety of physiological events such as
8 apoptosis, neurotransmitter release, muscle contraction, fertilization... [108]. Therefore, intracellular
9 Ca^{2+} concentration is tightly regulated. When it is required, localized increase in Ca^{2+} concentration is
10 mediated by Ca^{2+} influx from the extracellular space through Ca^{2+} channels present at the PM, but also
11 by Ca^{2+} efflux from storage organelles such as the ER [108]. The release of Ca^{2+} from ER stores is
12 triggered by the second messenger inositol 1,4,5-trisphosphate (IP3) synthesized by phospholipase C
13 (PLC) at the PM. On the ER surface, IP3 binds to calcium channels named IP3 receptors (IP3Rs; Table
14 II), and Ca^{2+} is released in the cytosol [109]. Although the ER represents the main Ca^{2+} storage organelle,
15 endo/lysosomes also sequester Ca^{2+} , with a similar concentration of around $500\mu\text{M}$ [110].
16 Furthermore, endo/lysosomes express several channels allowing Ca^{2+} release, including two-pore
17 channels (TPC1 and TPC2), which can be stimulated by nicotinic acid adenine dinucleotide phosphate
18 (NAADP), a powerful activator of Ca^{2+} release [111–117]. Thus, besides the ER, endo/lysosomes are
19 key Ca^{2+} storage organelles which can be mobilized for localized Ca^{2+} delivery.

20 Consistent with the idea that ER-endosomes contacts are regulated by Ca^{2+} , the TPC1 channel was
21 localized in ER-Endosome contact sites (Table II) [118]. Upon TPC1 depletion, the frequency of ER-
22 endosome contact is decreased. A similar phenotype is observed by a pharmacological inhibition of
23 the TPC channels through an NAADP antagonist. These results indicate that some ER-Endosome
24 contacts might be regulated by local Ca^{2+} ions derived from endosomes. Because TPC1 does not
25 interact directly with the ER, it is tempting to speculate that TPC1-dependent ER-Endosome contacts
26 are linked to the tethering complex Annexin A1/S100-A11, which depends on Ca^{2+} for interaction [103].
27 Furthermore, in line with the notion that ER-endosomes contacts functions in signal attenuation,
28 inhibition of TPC1 in EGF-stimulated cells enhances and prolongs EGFR tyrosine phosphorylation which
29 in turn, perturbs downstream signaling by Mitogen-activated protein (MAP) kinase and phospholipase
30 C [118]. Thus, some ER-Endosome contacts appear to be Ca^{2+} dependent signaling platform.

31 More recently, another study reported that ER and endo/lysosome Ca^{2+} stores are functionally
32 linked. Indeed, lysosomes are able to selectively sequester Ca^{2+} released from the ER [119,120]. This
33 Ca^{2+} exchange was shown to occur at sites of contact between the ER and lysosomes, marked by the
34 presence of IP3Rs [121]. Thus, lysosomes selectively sequester Ca^{2+} released by IP3Rs in ER/Lys
35 contacts [121]. Furthermore, the close association of the ER and LYs is partially disrupted in cells
36 treated with pharmacological components inhibiting the vacuolar H^+ -ATPase (V-ATPase), a pump
37 responsible for the acidification of lysosomes. These data suggest that a low lysosome pH is required
38 to maintain ER-IP3R/Lysosome connection [121]. Importantly, IP3Rs are not required for ER-LE/LY
39 contact formation, since the association between these organelles was unchanged in cells devoid of
40 IP3Rs. The tethering machinery implicated in IP3R-mediated Ca^{2+} delivering from the ER to LEs/LYs
41 remains to be identified.
42
43

44 **Nucleoplasmic reticulum-Endosome contacts**

45 The ER, which is the largest organelle in eukaryotic cells, extends throughout the cytosol and
46 forms a continuum with the nuclear envelope (NE). The nuclear envelope makes invaginations going
47 deep inside the nucleoplasm, which are named nucleoplasmic reticulum (NR). NRs are divided into two
48 classes: type I NR, involving inner nuclear membrane invaginations into the nucleoplasm (INM), and

1 type II NR, corresponding to the invagination of both the INM and the outer nuclear membrane (ONM)
2 [122]. Consequently, type II NR, which enclose a portion of cytoplasm, can contain cytoplasmic
3 organelles. Electron and light microscopy revealed the presence of Rab7-positive LE into NE
4 invaginations [123,124]. The ER protein VAP-A is present in type II NRs. Interestingly, less endosomes
5 are present in NE invaginations in VAP-A-depleted cells (but not in VAP-B-depleted cells), which
6 revealed a requirement for VAP-A in the recruitment of endosomes into these structures. Moreover,
7 ORP3, a protein belonging to the OSBP family (Table II), and a VAP-A partner, was also present in
8 endosomes enclosed into the NR [124,125]. Indeed, VAP-A, ORP3 and Rab7 were present in a tripartite
9 complex specifically in NE invaginations, but not in the cytoplasm [124]. These data indicate that the
10 entry and/or the maintenance of LE in NE invaginations is mediated by the formation of a VAP-
11 A/ORP3/Rab7 complex, which is likely present in contacts between these LE and the nuclear envelope.
12 How this Rab7/ORP3 endosomal subpopulation is targeted to the NE remains poorly understood. An
13 intact microtubule network seems to be required (Table II) [124], but the mechanism regulating the
14 association with microtubules, and the identity of the motor proteins involved is still unknown. Also,
15 the biological function of endosomes present in NE invaginations is elusive. One tempting hypothesis
16 is that these structures could be involved in the transport of endocytosed exosomes en route to the
17 nucleus to deliver their content, i.e. proteins and nucleic acids [124].
18
19

20 **Endosomes also contact other organelles**

21 Membrane contacts exist between virtually every pair of organelles in eukaryotic cells [126–128].
22 Hence, besides the ER, endocytic vesicles were shown to physically associate with peroxisomes,
23 mitochondria, lipid droplets, the Golgi, and the PM [24,129]. Up to now, the function of these contacts
24 remains ill-defined.

25 LY–Peroxisome contacts were shown to be involved in cholesterol egress from the endocytic
26 pathway [130]. Indeed, peroxisomes transiently and dynamically contact lysosomes. Most of these
27 contacts are tethered by Synaptotagmin-7, which is on lysosomes, and interacts with PI(4,5)P₂ on the
28 peroxisome surface with its two C2 domains. In accordance with this result, the reduction of PI(4,5)P₂
29 levels at the surface of peroxisomes, induced by the silencing of phosphatidylinositol 5-phosphate 4-
30 kinase type-2 α (PIP4K2A) which generates this lipid from PI(5)P, reduces LY–Peroxisome contacts
31 [131]. This in turn induces an accumulation of cholesterol in lysosomes. Most importantly, the
32 pathological relevance of cholesterol transport in lysosome–peroxisome contacts was shown in
33 fibroblasts of patients with a peroxisomal disorder. Indeed, fibroblasts of adrenoleukodystrophy,
34 Infantile Refsum disease and Zellweger syndrome patients were shown to accumulate cholesterol,
35 further supporting a role for peroxisomes, and in particular LY–peroxisome contacts, in intracellular
36 cholesterol transport [130].

37 Besides contacting peroxisomes, lysosomes also connect mitochondria to regulate mitochondrial
38 fission, a crucial process to maintain a proper cellular distribution of mitochondria [132–134]. By using
39 live imaging, authors showed that contact formation and duration are increased in cells expressing a
40 constitutively active form of the RAB7 protein. This suggests that formation and stabilization of
41 Lysosome-Mitochondria contacts are promoted by GTP-bound RAB7. In fact, mitochondrial fission
42 events are observed at sites of contact with lysosomes. Remarkably, the expression of the RAB7 active
43 form severely decreases the rate of mitochondrial fission. On the mitochondrial side, the fission factor
44 FIS1 is able to recruit the Rab7/ GTPase-activating protein (GAP) TBC1 domain family member 15
45 (TBC1D15) [135]. As a result, the expression of the TBC1D15 GAP mutant - unable to hydrolyze RAB7 -
46 markedly increases mitochondria-lysosome contact duration concomitantly to a decrease of
47 mitochondrial fission events. Together, these results indicate that mitochondrial fission can be
48 regulated at the level of contacts with lysosomes, through TBC1D15-mediated RAB7 hydrolysis [133].

1 As discussed above, nutrient availability influences the positioning of LEs/LYs. Interestingly, this
2 process does not only involve ER-dependent contacts. Indeed, in response to serum and amino acid
3 starvation, the formation of LY-Golgi contacts is promoted [136]. These contacts actively retain LYs in
4 the center of the cell. The machinery involved is composed of the renal tumor suppressor folliculin
5 (FLCN) and Rab34. FLCN is recruited onto LYs and promotes the association of Rab-34 with its effector,
6 the dynactin protein complex-interacting protein RILP [136,137]. The association of FLCN on LYs, and
7 Rab34 primarily associated with Golgi membranes, drives the formation of contact sites which restrain
8 LYs in the perinuclear area [136].

9 A specialized endocytic network, composed of membrane-bound structures named lysosome-
10 related organelles (LROs), is present in specific cell types, such as melanosomes in pigment cells
11 [138,139]. Electron microscopy analyses revealed the presence of Melanosome-Mitochondria contacts
12 in melanocyte cell lines and human melanoma cells [140]. The dynamin-like GTPase mitofusin 2 (Mfn2)
13 is localized to, and required for Melanosome-Mitochondria contacts, since Mfn2 depletion reduces
14 connections between these organelles. Functionally, Mfn2-mediated contacts seem to be involved in
15 melanosome biogenesis [140].

16 Interestingly, endosomes are also able to contact other endosomes. For instance, we recently
17 showed that the depletion of the ER-tether protein MOSPD2 significantly increases Endosome-
18 Endosome contacts, suggesting that MOSPD2 plays a role in the regulation of the endosomal system
19 dynamics [26]. Endosome-endosome contact formation seems to be linked to the clustering of these
20 organelles in the perinuclear region, and most likely involves specific tether complexes [141,142].
21
22

23 **Conclusion**

24 To conclude, endosomes and the ER have a parallel life with many direct encounters - mediated
25 through the establishment of physical contacts - that regulate endosome compartment dynamics and
26 function. These physical contacts are mediated by the building of tethering complexes at the
27 organelles' interface. A number of ER-Endosome tethering complexes have been identified and, most
28 probably, many more will be discovered in the near future. While all these complexes build
29 morphologically similar ER-Endosome contact sites – a contact is a contact –, they are associated with
30 a variety of distinct functions such as lipid or Ca²⁺ transport, signaling, molecular motor recruitment...
31 VAP-A, VAP-B and MOSPD2 proteins play a central role in the formation of contacts between the ER
32 and other organelles, and more particularly endosomes. How the cell orchestrates the formation and
33 function of these tethering complexes remains an open question. Noteworthy, recent observations
34 showed that the fission of ER tubules can occur at sites of contact with LE [143]. Although the molecular
35 identity of these contacts is not known, these data highlight the possibility of a bidirectional functional
36 regulation between the ER and endosomes.
37
38
39

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Figure legends

Figure 1: The endocytic organelles are physically connected to the ER network

Schematic representation of the different step of clathrin-mediated endocytosis, from the uptake of exogenous biological material to lysosomal degradation. Clathrin coated pits are formed by inward budding of the plasma membrane. The newly formed clathrin vesicles containing endocytosed cargoes gradually mature into early endosomes, late endosomes and lysosomes. Endocytosed cargoes can be recycled by recycling endosomes. Endosomal positioning and tubulation need an active microtubule network. All along their life, endocytic organelles contact ER tubules (highlighted by purple circles). These ER-endosome membrane contacts regulate the dynamics of the endocytic compartment.

Figure 2: ER-Endosome contacts observed by light and electron microscopy

A. Example of an extended ER-endosome contact visualized by electron microscopy (EM). Detailed view of STARD3-expressing HeLa cells showing a contact between an ER sheet and an endosome. False colors were assigned to the ER (green), endosome (magenta), mitochondria (yellow), and nucleus (blue). An interpretation scheme of the EM image is shown on the right.

B-D. VAP-A (B), VAP-B (C) and MOSPD2 (D) labelled in green have a typical ER localization when they are expressed alone (left panels). Note the reticulated localization extending throughout the cytoplasm with a perinuclear enrichment. When co-expressed with STARD3 (right panels), VAP-A, VAP-B and MOSPD2 are enriched in ER subregions around STARD3 positive endosomes corresponding to membrane contacts. The subpanels on the right display higher magnification images (3.5X) of regions boxed in white. The Overlay panel shows merged green and magenta images. The Coloc. panel shows a colocalization mask highlighting pixels which overlap between the green and magenta channels in white. Scale bar: 10 μm .

Figure 3: Model of ER-mediated endosome fission

A. Schematic representation of an ER-Endosome contact not involved in endosome fission.

B-C. ER mediated endosome fission. **B.** Rab7-positive endosomes contain EGFR. At the budding region of the endosome, several proteins accumulate such as FAM21 and CORO1C, while the neighboring ER tube becomes enriched in TMCC1.

C. The ER-resident TMCC1 protein drives the ER to the endosome bud, through its interaction with CORO1C. This ER-Endosome contact is built before fission occurs.

D. Rapidly after the contact (<10 s), the endosome tubule is severed from the vesicle; the contact is maintained between the ER and the endosome tubule.

Figure 4: Lipid transport at ER-Endosome contacts

A. STARD3 and ORP1L are two endosome tethers. STARD3 is an integral protein, whereas ORP1L is associated to LEs by binding with PI(4)P (via its pleckstrin homology (PH) domain) and Rab7 (via its ankyrin repeat (AR) domain). These 2 proteins have an FFAT motif enabling the interaction with the MSP domain of the ER protein VAP. At the contact, the START domain of STARD3 transports cholesterol from the ER to the LE. ORP1L transports cholesterol between the ER and LEs in both directions.

B. SNX2 and OSBP are found in early endosomes (EE). Their association with EE is mediated by phosphoinositides; SNX2 interacts with PI(3)P through its PX domain, and OSBP interacts with PI(4)P with its PH domain. SNX2 and OSBP both bind VAPs. At the interface of the ER-EE contact, the ORD domain of OSBP transports PI(4)P from the EE to the ER, making PI(4)P accessible to the phosphatase Sac1. This mechanism regulates the PI(4)P cycle on endosomes.

Figure 5: Regulation of EGFR sorting at Annexin A1-mediated ER-Endosome contacts

1 This model illustrates EGFR endocytosis after EGF-binding, and in the absence of low density
2 lipoprotein (LDL)-derived cholesterol.

3 **A.** EGFR binds its ligand, which results in dimerization, autophosphorylation, and receptor activation.
4 The activated receptor is then endocytosed. In multi-vesicular bodies (MVB), EGFR mediates Annexin
5 A1 phosphorylation. EGFR-MVB/ER contact is formed by Annexin A1-S100-A11 interaction. This allows
6 the phosphatase PTP1B sitting on the ER to interact with EGFR, and the formation of a protein complex
7 composed of VAP and ORP1L. At the interface of the contact, EGFR is dephosphorylated by PTP1B, and
8 internalized in intra luminal vesicles (ILVs) formed by the endosomal sorting complexes required for
9 transport (ESCRT) machinery. Within the contact, cholesterol is transferred to the endosome by ORP1L.

10 **B.** Close-up of the EGFR-MVB/ER contact. Through its amino-terminal part, Annexin A1 interacts with
11 S100-A11 in a calcium-dependent manner. PTP1B interacts with EGFR and the ESCRT-0 component
12 HRS, and mediates their dephosphorylation. The ubiquitinated EGFR is recognized by HRS via its
13 ubiquitin-interacting motif. The VAP-ORP1L complex is formed by the interaction of the MSP domain
14 of VAP with the FFAT motif of ORP1L.

15 16 **Table I: Molecular architecture and associated functions of ER-Endosome contacts involving proteins 17 from the MSP-domain family**

18 The proteins in rows are partners. On the left are shown the ER-receptors VAP-A, VAP-B and MOSPD2
19 belonging to the MSP family, and on the middle their endosomal partners, all having a FFAT motif. On
20 the right, functions associated with each tethering complex are indicated.

21 Abbreviations: Proteins: **STARD3**: StAR related lipid transfer protein 3; **STARD3NL**: STARD3 N-terminal
22 like; **ORPs**: oxysterol binding protein (OSBP)-related proteins; **SNX2**: sorting nexin 2. Protein regions:
23 **TM**: transmembrane domain; **CC**: coiled-coil domain; **MSP**: major sperm protein domain; **CRAL-TRIO**:
24 cellular retinaldehyde-binding protein and triple functional domain protein domain [144]; **MENTAL**:
25 Metastatic Lymph Node 64 (MLN64/STARD3) N-terminal domain; **START**: steroidogenic Acute
26 Regulatory protein related lipid transfer domain; **AR**: ankyrin repeat; **PH**: pleckstrin homology domain;
27 **ORD**: oxysterol binding protein (OSBP)-related ligand-binding domain; **PX**: phox domain; **BAR**: bin-
28 Amphiphysin-Rvs domain. Main source: Uniprot [145].

29 30 **Table II: Molecular architecture of ER-Endosome contacts excluding the MSP-containing family, and 31 associated functions**

32 The proteins in rows are partners. The ER tether is on the right, the endosome partner on the middle
33 and the associated function on the right. Note that Vacuolar protein sorting-associated protein 13-C
34 (VPS13-C) protein builds ER-Endosome contacts; the molecular mechanism of formation of this contact
35 is not known [146]. Inositol 1,4,5-trisphosphate (IP3) receptor (IP3R) acts at ER-Endosome contacts
36 built by another yet unidentified machinery. Protein regions mediating the interaction within the
37 complex are labeled with an asterisk.

38 Abbreviations: Proteins: **TMCC1**: transmembrane and coiled-coil domains protein 1; **RNF26**: Ring finger
39 protein 26; **PTP1B**: protein tyrosine phosphatase 1B; **CORO1C**: coronin 1C; **SQSTM1**: sequestosome 1;
40 **TOLLIP**: toll-interacting protein; **EPS15**: Epidermal growth factor receptor substrate 15; **HRS**:
41 hepatocyte growth factor-regulated tyrosine kinase substrate; **TPC1**: two-pore channel 1; **EGFR**:
42 epidermal growth factor (EGF) receptor. Protein regions: **TM**: transmembrane domain; **CC**: coiled-coil
43 domain; **HD**: hydrophobic domain; **MIT**: microtubule interacting and trafficking domain; **Microtubule-**
44 **BD** : Microtubule-binding domain [65]; **Rab11-BD** : Rab11-binding domain ; **LCR** : low complexity
45 region ; **KIF5-BD**: kinesin family member 5B-binding domain; **FYVE**: domain named after Fab 1, YOTB,
46 Vac1, and EEA1 [36,49]; **TPP** : tyrosine protein phosphatase domain; **WD40**: WD40 repeats; **DH-like**:
47 Dbl homology-like domain; **PH**; pleckstrin homology domain; **IP3-BD**: inositol 1,4,5-trisphosphate-
48 binding region; **MIR** : motif named after Mannosyltransferase, IP3R and Ryanodine receptor (RyR);

1 **ELYC** domain [147]; **PI(3)P**: phosphatidylinositol 3-phosphate; **RING**: really interesting new gene
2 domain; **PB1**: Phox and Bem1 domain; **ZZ**: zinc finger ZZ-type domain; **LIR**: LC3-interacting region; **UBA**:
3 ubiquitin-associated domain; **TBD**: Tbk1/Ikki binding domain; **CUE**: coupling of ubiquitin conjugation
4 to endoplasmic reticulum (ER) degradation domain; **EH**: Eps15 homology domain; **UIM**: ubiquitin-
5 interacting motif; **S100-A11-BR**: S100-A11-binding region [103]; **PK**: protein kinase domain; **Ligand-**
6 **BD**: ligand-binding region; **VHS**: domain derived from its occurrence in VPS-27, Hrs and STAM. Main
7 source: Uniprot [145].
8

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2

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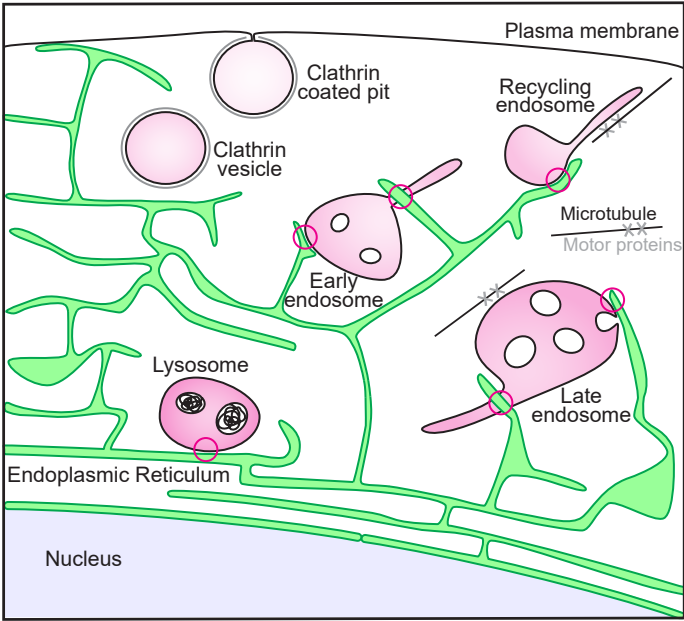


Figure 1

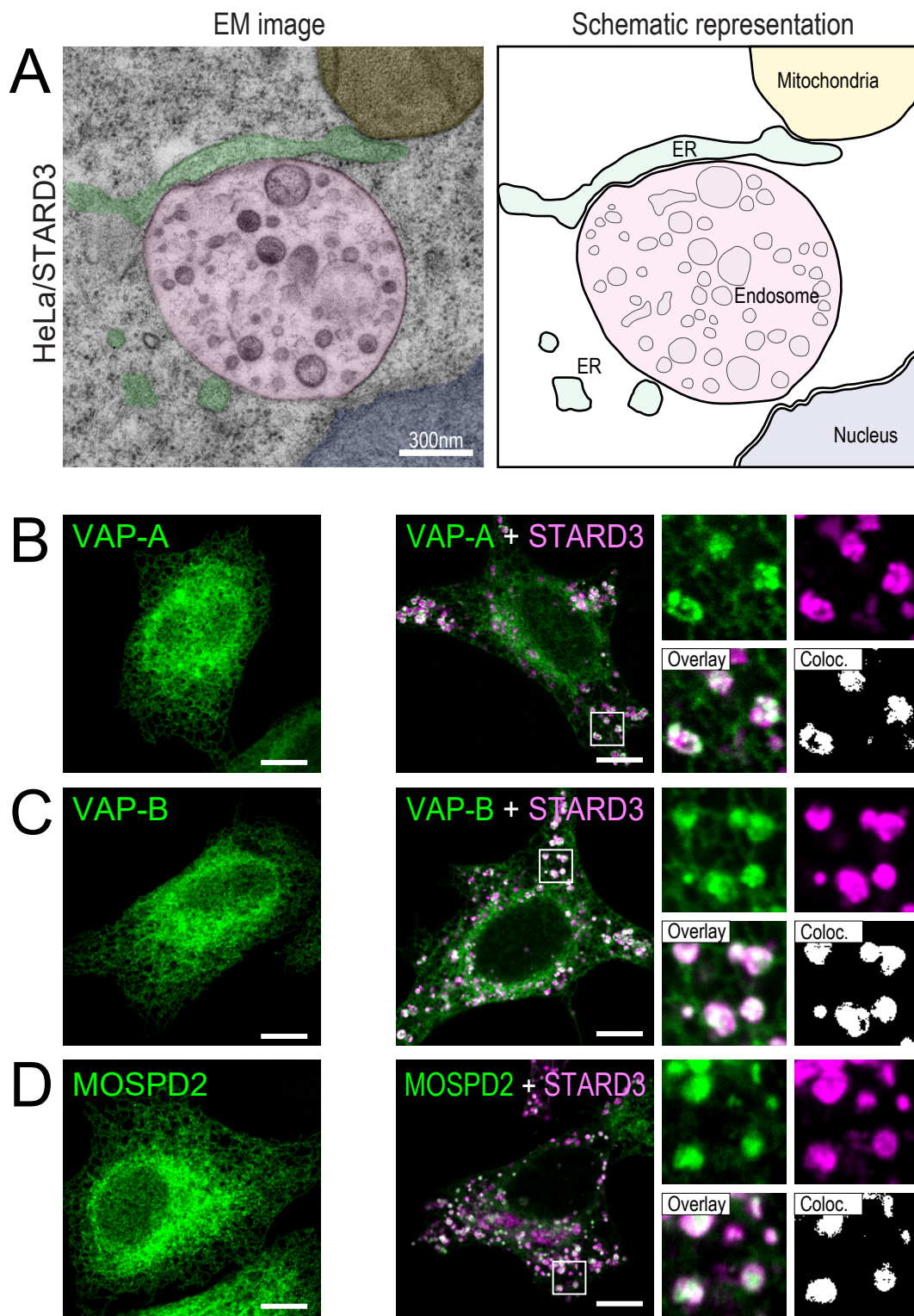


Figure 2

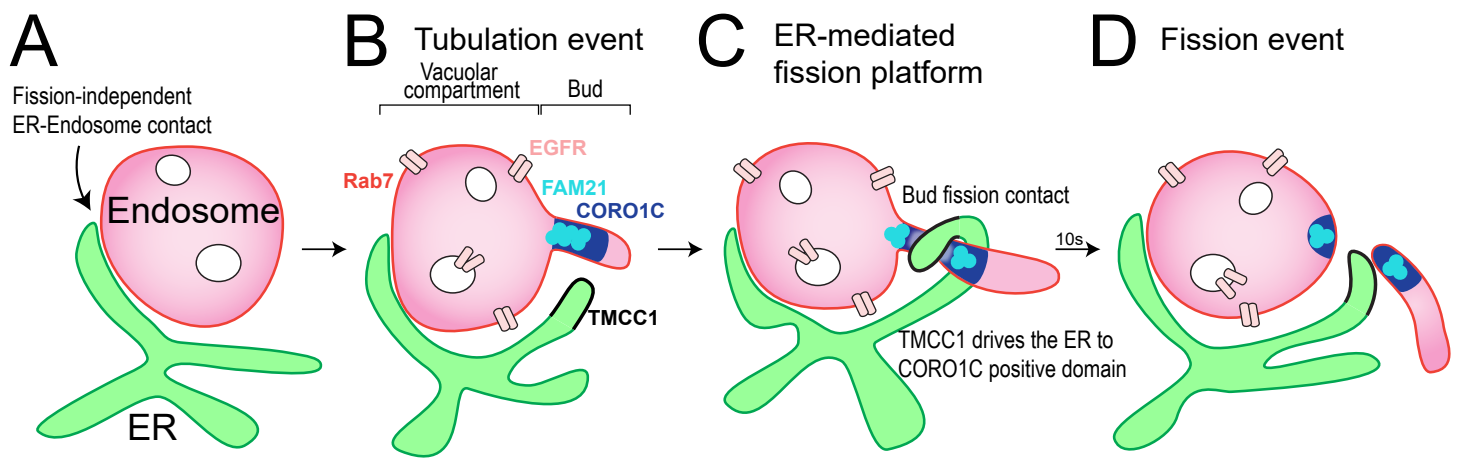


Figure 3

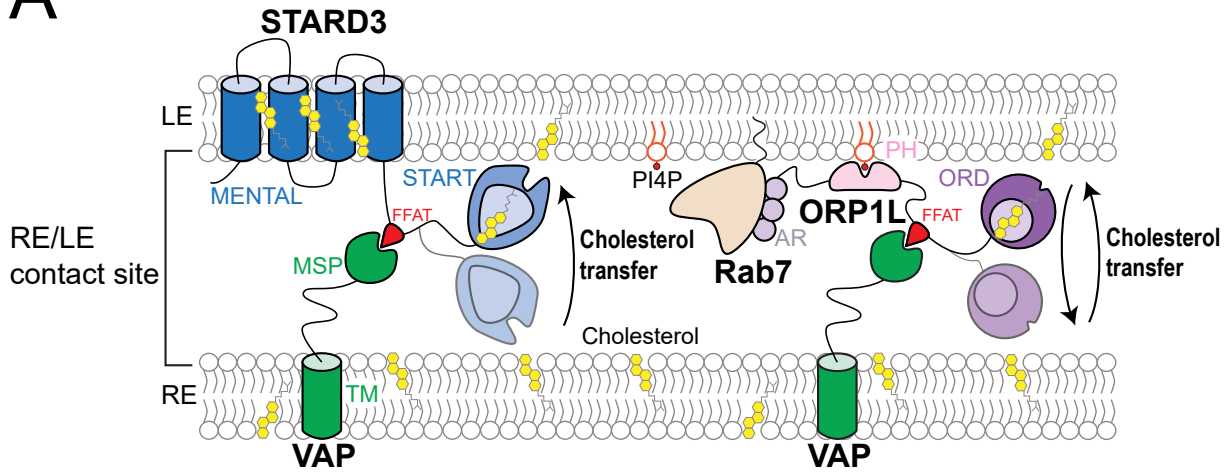
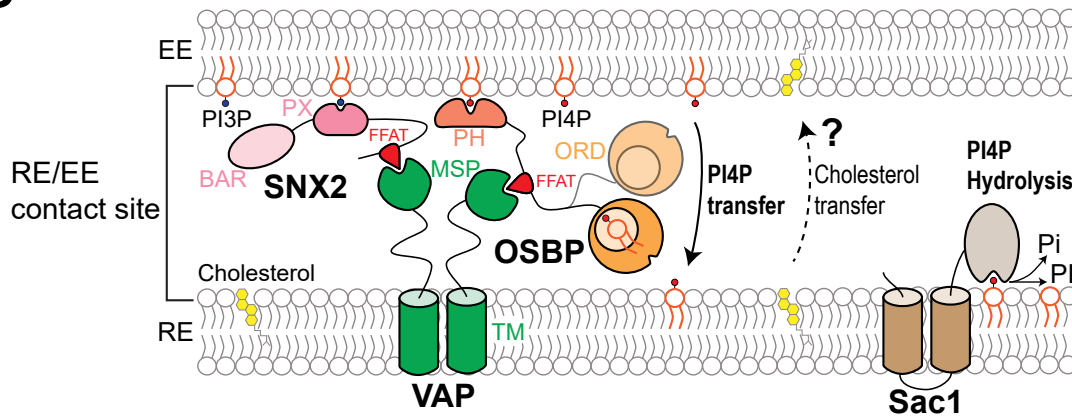
A**B**

Figure 4

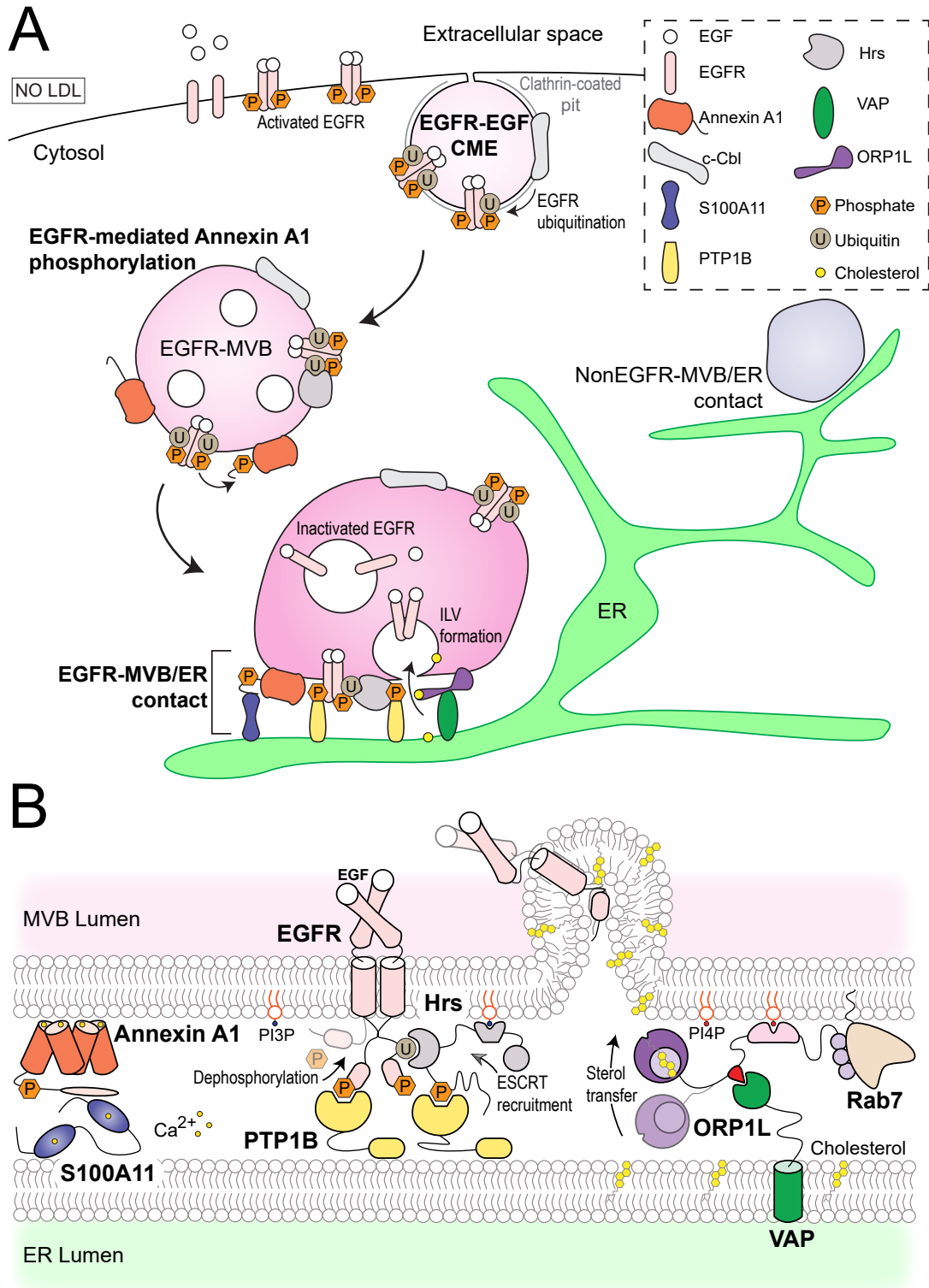


Figure 5

Protein complexes

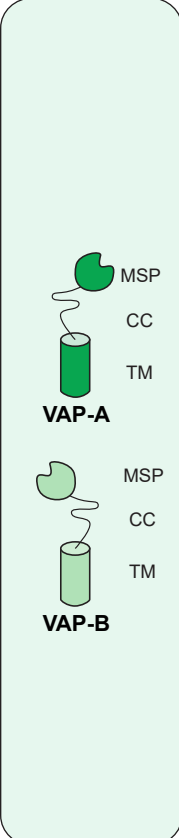
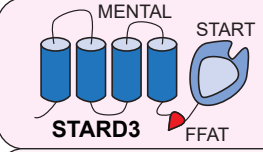
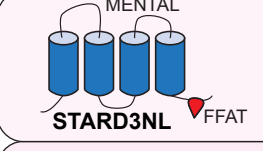
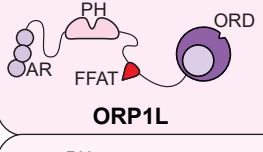
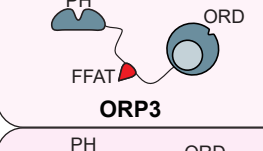
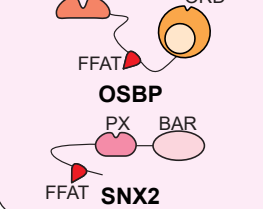
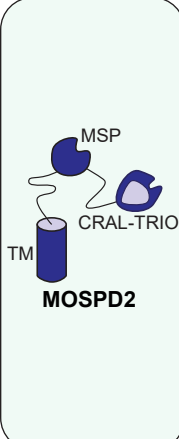
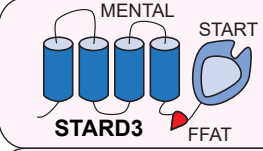
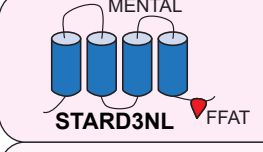
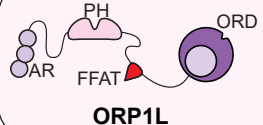
ER-protein (with an MSP domain)	Endosomal protein (with an FFAT motif)	Associated function
 <p>VAP-A</p> <p>VAP-B</p>	 <p>STARD3</p>	<p>Cholesterol transport from the ER to late endosomes mediated by the START domain of STARD3 (see Fig. 4A).</p> <p>Alpy et al., 2013; Wilhelm et al., 2017</p>
	 <p>STARD3NL</p>	<p>Control of endosomal vesicle-to-tubule transitions.</p> <p>Alpy et al., 2013</p>
	 <p>ORP1L</p>	<ul style="list-style-type: none"> - Regulation of endosome positioning. - Cholesterol transport between late endosomes and the ER, mediated by the ORD domain of ORP1L (see Fig. 4A). <p>Rocha et al., 2009; Eden et al., 2016; Zhao and Ridgway 2017</p>
	 <p>ORP3</p>	<p>Mediates late endosomes and nucleoplasmic reticulum contact formation.</p> <p>Santos et al., 2018</p>
	 <p>OSBP</p> <p>SNX2</p>	<p>PI4P transport from endosomes to the ER mediated by the ORD domain of OSBP (see Fig. 4B). This VAP-dependent regulation of PI4P level affects actin nucleation and retromer function.</p> <p>Dong et al., 2016</p>
	 <p>MOSPD2</p>	 <p>STARD3</p>
 <p>STARD3NL</p>		<p>Mediates ER-Endosome tethering.</p> <p>Di Mattia et al., 2018</p>
 <p>ORP1L</p>		<p>Mediates ER-Endosome tethering.</p> <p>Di Mattia et al., 2018</p>

Table I

Protein complexes

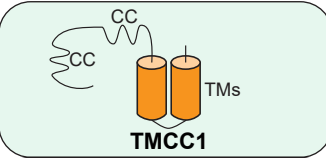
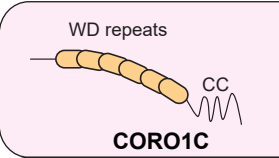
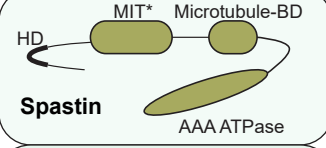
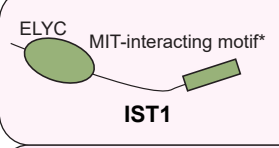
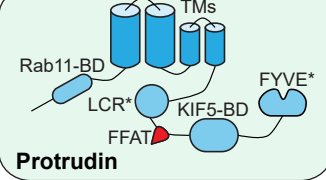
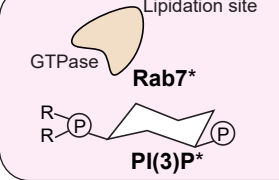
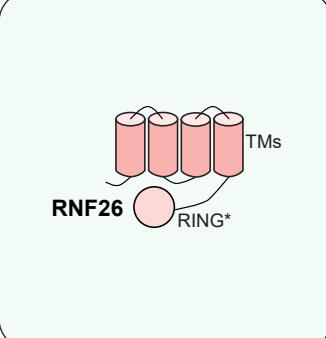
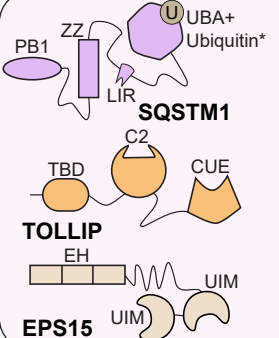

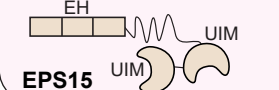
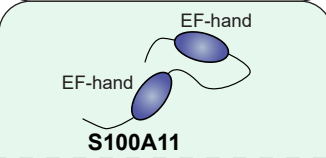
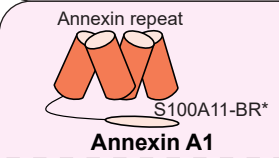
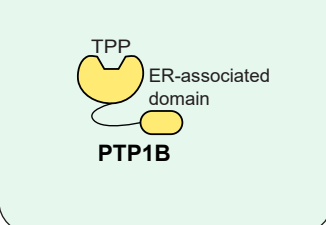


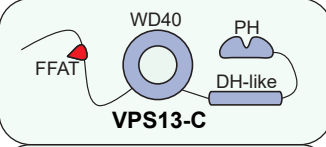
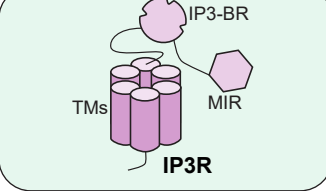
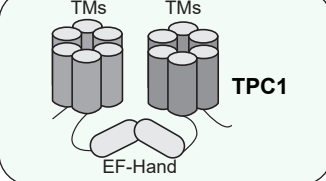
ER-protein	Endosomal protein/lipid	Associated function
 <p>TMCC1</p>	 <p>CORO1C</p>	<p>TMCC1 drives the ER to the endosomal CORO1C positive domains which regulates endosomal fission (see Fig. 3).</p> <p>Rowland et al., 2014; Hoyer et al., 2018</p>
 <p>Spastin</p>	 <p>IST1</p>	<p>Regulation of endosomal fission, depending on a functional ATPase activity.</p> <p>Allison et al., 2017</p>
 <p>Protrudin</p>	 <p>PI(3)P*</p>	<p>Regulation of endosome translocation and neurite outgrowth.</p> <p>Raiborg et al., 2015</p>
 <p>RNF26</p>	 <p>SQSTM1</p>  <p>TOLLIP</p>  <p>EPS15</p>	<p>Regulation of the dynamics and positioning of endosomal vesicles.</p> <p>Jongsma et al., 2016</p>
 <p>S100A11</p>	 <p>Annexin A1</p>	<p>Dephosphorylation of endocytosed EGFR by the phosphatase PTP1B in Annexin A1-dependent contact sites. PTP1B also dephosphorylates the ESCRT0 components HRS and STAM (not represented here). Furthermore, in the contact, ORP1L transports cholesterol from the ER to the EGFR/MVB; this transport of cholesterol supports the formation of intra luminal vesicles (see Fig. 5).</p> <p>Haj et al., 2002; Stuible et al., 2010; Eden et al., 2016</p>
 <p>PTP1B</p>	 <p>EGFR</p>  <p>HRS</p>	
 <p>VPS13-C</p>		<p>Glycerolipid transport between ER-Endosome membranes mediated by the amino-terminal part of the VPS13 protein.</p> <p>Kumar et al., 2018</p>
 <p>IP3R</p>		<p>Calcium delivery from the ER to lysosomes.</p> <p>Atakpa et al., 2018</p>
 <p>TPC1</p>		<p>Regulation of EGFR signaling.</p> <p>Kilpatrick et al., 2017</p>

Table II