Organelle biogenesis in the endoplasmic reticulum

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Understanding organelle biogenesis is a central focus of cell biology. Whereas some are generated from existing organelles, others can be generated *de novo*. Most *de novo* organelle biogenesis occurs in the endoplasmic reticulum (ER). Here, we review the role of the ER in the generation of peroxisomes, lipid droplets, and omegasomes, which are platforms for autophagosome production, and discuss how ER subdomains with specific protein and lipid composition form and promote organelle biogenesis.

Organelle biogenesis mechanisms can be divided into two categories. In one, existing organelles grow and divide to generate new organelles, a process analogous to cell division. Mitochondria, for example, are only generated from existing mitochondria[1]. Similarly, newly formed endoplasmic reticulum (ER) is only derived from existing ER[2]. Some organelles, however, are not formed by the growth and division of existing organelles but instead form *de novo*. Because all cellular membranes are derived from existing membranes, organelles generated *de novo* are not made from unassembled lipids and proteins, but from existing membranous structures.

This type of *de novo* biogenesis of a number of organelles begins in the ER, which plays a significant and perhaps unsurprising role in this process given that it is a major hub of biosynthesis in cells. Most proteins destined for intercellular compartments are synthesized at the ER membrane where they are inserted into or across the membrane. Once in the ER, proteins are often modified and assembled into complexes. The ER is also the site of most lipid synthesis[3,4]. Because the ER is one continuous structure, organelle biogenesis requires specialized subdomains within the ER. Here, we focus on how such subdomains form and facilitate the biogenesis of peroxisomes, lipid droplets (LDs), and omegasomes, which mediate autophagosome formation. As organelle biogenesis in the ER is comparable to vesicular trafficking from the ER, which occurs at specialized regions called ER exit sites (ERESs)[5–8], we begin by discussing these specialized ER domains.

ER exit site formation

Vesicular trafficking from the ER to the Golgi complex is required for the generation of the numerous compartments of the secretory and endocytic pathways. Most transport vesicles departing from the ER are formed by coat protein complex II (COPII) proteins[9], which, together with the GTPase Sar1, are sufficient to reconstitute COPII-mediated budding *in vitro* from synthetic liposomes[10]. While the mechanism of COPII-vesicle biogenesis is relatively well understood[11–17], less is known about how proteins are sorted into or excluded from these vesicles[18].

COPII vesicles bud from the ER at subdomains known as ERESs or transitional ER, which is devoid of ribosomes and forms cup-like structures approximately 400 nm in diameter[5,16]. Cells can have several to a few hundred ERESs, depending on the cell type[19]. ERES number and size are regulated in response to increasing load on ER-to-Golgi transport[20,21]. ERESs are long-lived and can bud numerous COPII vesicles. They are mobile within the ER and seem to fuse and, after fusion, grow smaller until they are about the same size as other ERESs, suggesting ERES size is regulated[8,22–24]. There is also evidence that different kinds of ERES exist depending on the types of cargo exported[25,26].

ERES biogenesis is not well understood, but the protein Sec16 may play a role. Sec16 is a conserved, peripheral ER membrane protein necessary for COPII-vesicle trafficking *in vivo*, though not for COPII-vesicle budding *in vitro*. Sec16 oligomerizes and physically associates with most COPII components[27–30]. Thus, Sec16 could help establish ERESs, perhaps by acting as a scaffold linking nascent COPII coats. However, the idea that Sec16 functions as a scaffold or that any scaffold-like protein is necessary for ERES biogenesis has been questioned[19]. Instead, ERES formation may be promoted by ER tethering to early Golgi membrane compartments, given that these portions of the Golgi are closely apposed to the ER. The identity of these tethers is unknown.

Lipids may also play a role in generating ERESs. Sar1, which regulates COPII coat assembly at ERESs, also activates phospholipase D. The product of this enzyme, phosphatidic acid, is enriched at ERESs in a Sar1-dependent manner[31]. Phosphatidylinositol-4-phosphate (PtdIns(4)P) is also enriched at these sites and may function in COPII-vesicle budding and ERES generation, as knockdown of PtdIns(4)KIIIα, a kinase that produces PtdIns(4)P in the ER, decreases ERES number[20].

ERES biogenesis may be similar to organelle biogenesis in the ER. ERES formation requires protein and lipid enrichment at these sites. This might be driven in either of two ways. One possibility is that scaffolding proteins

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or the interactions of proteins (and lipids) that regulate COPII-vesicle formation and cargo loading drive ERES formation. Alternatively, the enrichment (or exclusion) of ER proteins and lipids in ERESs may be driven by the association of ER regions with early Golgi compartments or other structures outside the ER. Similar mechanisms may drive ER subdomain formation where *de novo* organelle biogenesis occurs.

De novo peroxisome biogenesis

Peroxisomes are single membrane-bound organelles ubiquitously present in all eukaryotes. They rapidly increase in size and number in response to cellular metabolic needs[32]. In animal cells, peroxisomes are essential for metabolism of cholesterol, bile acids, polyamines, D-amino acids and β-oxidation of very long chain fatty acids[33]. Defects in peroxisome biogenesis lead to a spectrum of human disorders[34].

Ever since the discovery of peroxisomes, their biogenesis has been debated. Electron microscopy (EM) suggested that peroxisomes bud from terminal ends of specialized ER regions[35]. However, it was subsequently found that peroxisome matrix proteins are post-translationally imported into peroxisomes after being synthesized on free ribosomes, not associated with the ER[36]. This led to the idea that peroxisomes, similar to mitochondria and chloroplasts, are semi-autonomous organelles only derived from the growth and division of existing peroxisomes[37]. However, this model was questioned as mutant cells with defects in peroxisome biogenesis were isolated and it was found that cells lacking some proteins needed for peroxisome biogenesis (Pex3, Pex19 or Pex16 in mammals; Pex3 or Pex19 in yeasts) were devoid of peroxisomes and non-functional peroxisome remnant vesicles called ghosts[38,39]. Remarkably, re-expression of the missing proteins caused the mutant cells to generate peroxisomes, indicating that peroxisome biogenesis could occur *de novo*. Later, Hoepfner *et. al.* showed that *de novo* peroxisome biogenesis is initiated at the ER in *Saccharomyces cerevisiae*[40]. It is currently thought that peroxisomes arise both from the growth and division of existing peroxisomes and *de novo* from the ER[41,42], though recent evidence in mammalian cells suggests that mitochondria may also play a role in *de novo* peroxisome biogenesis[43]. Here, we discuss what is known about *de novo* biogenesis in the ER.

The ER may also play a role in later steps of peroxisome biogenesis by forming close contacts with peroxisomes. Lipid transport to peroxisomes may occur at ER contact sites[44]. Recently, two groups identified a complex that tethers the ER and peroxisomes, which may be required for peroxisome proliferation[45,46]. The ER protein Pex30 may be enriched in portions of the ER in contact with peroxisomes[47].

Pre-peroxisomal vesicle generation in the ER. Pre-peroxisomal vesicles (PPVs) are vesicles that can mature into functional peroxisomes (Fig. 1). During *de novo* peroxisome biogenesis, PPVs are thought to bud from the ER and acquire additional proteins and lipids as they mature to form functional peroxisomes. A recent study suggests that, in mammalian cells, PPVs are also generated on mitochondria[43]. The nature of PPVs is not well understood but they may be the same vesicles known to transport a subset of peroxisomal membrane proteins (PMPs), which are inserted into the ER membrane and exit the ER in vesicles[48,49]. Studies in human cells concluded that neither COPII nor another coat complex (coat protein complex I, COPI) are required for PMP trafficking to peroxisomes[50,51]. Using cell-free budding reactions, two independent studies showed that PMPs such as Pex3 and Pex15 in *S. cerevisiae* and Pex3 and Pex11 in *Pichia pastoris* bud from ER membranes in an ATP-dependent manner[52,53]. Budding requires Pex19 and other unknown cytosolic factors. Budded vesicles lack soluble peroxisome matrix proteins and most PMPs.

There may be more than one type of PPV (Fig. 1). Studies in *Yarrowia lipolytica* identified two PPV types that fuse and mature into functional peroxisomes[54]. This is consistent with findings in *S. cerevisiae* that also suggested there are two PPV types that bud from the ER[55]. This study investigated the assembly of the importomer complex, which translocates soluble proteins into peroxisomes. The Pex1–Pex6 complex is required for fusion of two distinct pools of PPVs. The importomer complex is assembled from two subcomplexes of PMPs: the docking complex (DC) (Pex13, Pex14, and Pex17) and the RING complex (RC) (Pex2, Pex10 and Pex12)[56]. The subcomplexes have been proposed to exit the ER in distinct PPVs that subsequently fuse, allowing importomer complex assembly. The idea that the subcomplexes may exit the ER in distinct PPVs is consistent with the finding that Pex3 is required for ER exit of the RING subcomplex but not the docking subcomplex[57]. However, the model describing that the two subcomplexes exit the ER in different vesicles has been questioned by two recent studies[58,59]. Using high-resolution EM in cells lacking Pex1 or Pex6, proteins from RCs and DCs were identified in the same PPVs[59]. Moreover, the importomer complex proteins appeared in the same vesicle after blocking pexophagy[58], indicating that the Pex1–Pex6 complex is not required for PPV fusion. Important questions concern where the DC and RC complexes assemble, and whether they assemble in the ER at the exit site or after PPV budding.

In mammalian cells lacking peroxisomes, Pex3 and Pex14 are targeted to mitochondria. These proteins appear to bud from the mitochondrial membrane in vesicles that fuse with ER-derived Pex16-containing vesicles to generate import-competent peroxisomes[43], suggesting that two PPV types exist in mammalian cells.

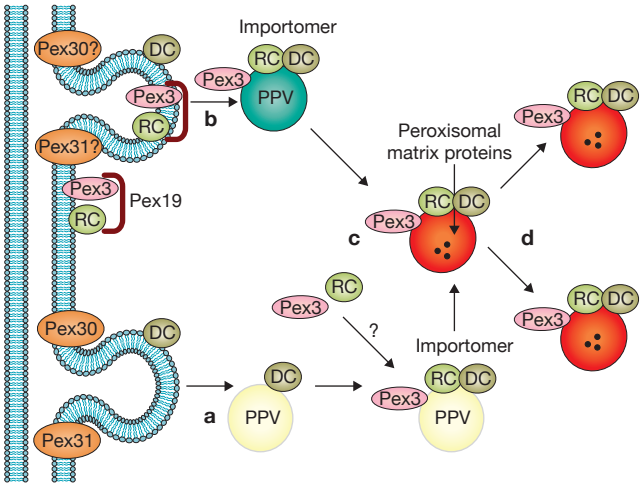

Figure 1 Model of *de novo* peroxisome biogenesis in yeast. (**a**,**b**) PPVs are generated by Pex3-independent (**a**) and Pex3-dependent (**b**) pathways at sites that probably contain Pex30 and Pex31. The docking complex (DC) can traffic from the ER in Pex3-independent vesicles. The ring complex (RC) protein exits the ER in Pex3-dependent PPVs. Pex3-independent PPVs may mature by fusion with a second type of PPV or may obtain the RC complex by an unknown mechanism. (**c**) After assembly of a functional importomer complex, the import of peroxisomal matrix proteins begins. (**d**) Mature peroxisomes proliferate by growth and division.

Understanding PPV biogenesis has been difficult as they are short-lived. In wild-type cells PPVs rapidly mature into, or fuse with, peroxisomes. In mutants devoid of peroxisomes (cells lacking Pex3 or Pex19) PPVs are degraded by autophagy[60,61]. Interestingly, in cells lacking either Pex3 or Pex19 as well as Atg1, which is required for autophagy, PPVs are longer-lived and can be studied[62]. These PPVs contain docking complex proteins such as Pex14 and Pex13. When Pex3 is re-expressed it is targeted to these PPVs, which then mature into functional peroxisomes. However, it is not known whether Pex3 is directly imported into the PPVs or traffics from the ER to the PPVs[62]. How other proteins that are required for PPV maturation reach PPVs and whether they are first inserted into the ER are important questions. Characterization of the protein and lipid composition of PPVs may reveal more about these vesicles and how they are generated at the ER.

PPV ER exit sites. Little is known about PPV exit sites, but it seems likely that, like ERESs, they are stable ER subdomains. An investigation of importomer complex trafficking found that soon after synthesis it is in the ER, in puncta that could be ER subdomains, where PPVs are generated[57]. Recently, two *S. cerevisiae* ER proteins, Pex30 and Pex31, were found to be enriched in ER subdomains that serve as PPV exit sites[63] (Fig. 1). These proteins, previously implicated in regulating peroxisome size and number, are part of a large family; there are three more in *S. cerevisiae* and most yeast contain multiple homologues[64,65]. Mammalian orthologues have not been described. Pex30 forms puncta in the ER, usually about 10–30 per cell, which are distinct from ERESs. Evidence that the Pex30-enriched subdomains are regions where PPVs originate from the ER came from microscopy of cells that lack Pex3, which renders them unable to make mature peroxisomes, and also lack Atg1, which is required for autophagy. PPVs are stabilized in these cells and Pex14 is found on the PPVs[62]. Newly synthesized Pex14 is first targeted to Pex30-containing ER subdomains and then moves away from the ER, possibly in PPVs[63]. Whether other PMPs exit the ER in PPVs generated at Pex30 subdomains remains to be investigated. Pex30 does not leave the ER but rather seems important for maintaining the ER subdomain where PPVs are generated, as loss of Pex30 results in a twofold increase in the size of PPVs and some PPVs form clusters near the ER membrane[63]. Interestingly, there are significantly more Pex30-enriched subdomains in the ER than PPVs, suggesting that the subdomains have functions in addition to PPV biogenesis.

Pex30 and Pex30 homologues are similar to ER-shaping proteins called reticulons, ER-resident proteins that localize to and stabilize ER tubules and ER sheet edges[63]. Pex30 subdomains have a similar localization and, interestingly, reticulons are excluded from Pex30 subdomains. Pex30 and similar proteins may shape ER subdomains where *de novo* peroxisome biogenesis occurs and perhaps help concentrate proteins necessary for this process.

LD biogenesis

LDs are found in virtually all eukaryotic cell types and play central roles in lipid metabolism and energy production[66–71]. LDs are lipid storehouses, and cells rapidly deposit or mobilize lipids from LDs in response to changes in metabolism. Interest in LD biogenesis has increased significantly as lipid metabolism disorders have grown more prevalent. LDs also function in protecting cells from lipotoxicity, in protein degradation, and in ER stress responses[72–76].

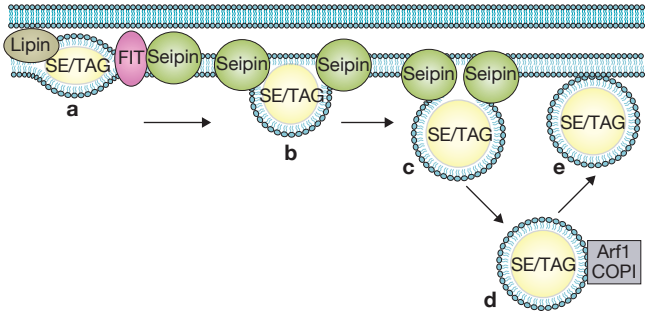

Figure 2 Lipid droplet biogenesis in the ER. (**a**) The neutral lipids steryl ester (SE) and triacylglycerol (TAG) are synthesized in the ER and accumulate within the bilayer, forming lenses within the membrane. These sites often contain the proteins lipin, seipin, and FIT. (**b**) As the lenses grow, they bud from the ER into the cytoplasm. (**c**,**d**) Mature LDs can remain attached to the ER (**c**) or separate completely (**d**). (**e**) The Arf1/COPI machinery may mediate the reattachment of mature LDs to the ER, which facilitates LD growth by allowing neutral lipids and neutral lipid-synthesizing enzymes to reach LDs.

LDs have a unique structure among organelles; they have a core of neutral lipids, steryl esters and triacylglycerols, surrounded by a phospholipid monolayer (Fig. 2). In most cell types, coat proteins stabilize LDs and may regulate the access of lipases and other enzymes to neutral lipids in LDs.

LD *de novo* biogenesis is driven by neutral lipid production in the ER and the behaviour of neutral lipids in membranes (Fig. 2). Phospholipid bilayers can only accommodate a small amount of neutral lipids, perhaps about 3 mol% (ref. 77). When neutral lipid concentration in a bilayer reaches a critical point, the neutral lipids coalesce to form lenses between the two leaflets of the membrane bilayer. Indeed, lenses of about 50 nm have been observed in the ER when nascent LD formation is induced in yeast[78]. As a nascent LD grows it will emerge (bud) from the ER and may separate from it. This process may not require proteins other than the enzymes that synthesize neutral lipids[79,80]. However, proteins do regulate LD biogenesis; two families have been identified: seipins and fat-storage-inducing transmembrane proteins (FITs).

Seipins are integral ER membrane proteins conserved from yeast to mammals. They were originally characterized when it was discovered that mutation in the gene encoding seipin causes Berardinelli–Seip congenital lipodystrophy[81]. Yeast cells lacking seipin have altered LD number and size, often containing either a few abnormally large ('supersized') LDs or numerous small LDs[82,83]. LDs are similarly affected in human cells deficient in seipin[84]. How seipins affect LD biogenesis remains unclear. In yeast, they are found at ER–LD contact sites, consistent with studies that find seipins directly affect LD biogenesis[83,85–88]. Others have found that seipins regulate lipid metabolism[82,84,89–94].

The FITs are another family of ER transmembrane proteins[95]. There are two FITs in humans: FIT1, primarily expressed in muscle, and FIT2, which is ubiquitously expressed. FIT2 overexpression increases LD size and number, whereas knockdown does the opposite. In mice, postnatal FIT2 knockdown causes the absence of cytosolic LDs in the intestine and fatal enteropathy[96]. In mouse adipose tissue, FIT knockdown causes lipodystrophy and insulin resistance[97]. The function of FITs is not known but FIT reduction or loss causes failure of nascent LDs to bud from the ER and they instead remain imbedded in the ER membrane, suggesting that FITs modulate LD budding from the ER[78]. They may not directly

mediate budding but instead may affect lipid homeostasis, perhaps at sites of LD biogenesis.

The ER plays an important role in nascent and mature LD growth. To grow, LDs may be re-connected to the ER by membrane bridges. These bridges allow lipids produced in the ER to reach LDs by diffusion, and also allow enzymes that participate in triacylglycerol production to migrate from the ER to LDs[98]. How are these bridges formed? In *S. cerevisiae*, there may be no need to form bridges given that LDs remain connected to the ER[99]. Mammalian cells seem to have a mechanism to re-attach LDs separated from the ER which uses the COPI machinery and ADP-ribosylation factor 1 (ref. 100). Details of the mechanism remain to be determined.

Sites of LD formation in the ER. There is evidence in both yeast and mammalian cells that LD formation occurs at specialized ER sites[101,102]. In some cases, proteins involved in triacylglycerol synthesis are enriched at sites within the ER, suggesting that neutral lipids could be synthesized at discrete zones, perhaps regions where LDs are generated[103]. However, many enzymes involved in neutral lipid synthesis are distributed throughout the ER, suggesting that some lipids may not be made at LD assembly sites. Alternatively, neutral lipid-synthesizing enzymes might be activated at LD biogenesis sites. The lipid synthesis regulator lipin, called Pah1 in yeast, may localize to LD biogenesis sites in the ER[90,104]. Both seipin and FIT2 have also been suggested to play roles at LD biogenesis sites[83,105]. How proteins might regulate LD biogenesis sites in the ER remains to be elucidated.

The omegasome

Autophagy is a cellular degradation process that requires the formation of autophagosomes, double-membrane structures that engulf portions of the cytoplasm and deliver it to lysosomes for degradation. Autophagosome formation involves a dynamic series of events, including the nucleation and initiation of a crescent-shaped membrane called the isolation membrane (IM, or phagophore) and its expansion and closure[106–108]. A set of autophagy proteins act at distinct steps of autophagosome formation. For example, two kinase complexes (ULK1–ATG13–FIP200 and VPS34–ATG14 PtdIns(3)P) are required for induction and nucleation of IMs, and two ubiquitin-conjugation systems (ATG8 conjugated to phosphatidylethanolamine (ATG8-PE) and ATG12–ATG5–ATG16 conjugates) are involved in IM expansion and closure[106,107]. How these ATG proteins act coordinately to mediate autophagosome formation remains largely unknown.

Unlike COPII vesicles, peroxisomes and LDs, autophagosomes do not bud directly from the ER. Nevertheless, the ER is intimately associated with autophagosome formation. Recent studies demonstrate that PtdIns(3)P-enriched ER subdomains, called omegasomes, act as cradles for autophagosome formation[109] (Fig. 3). Upon autophagy induction, the ER-associated PtdIns(3)P-binding protein DFCP1 redistributes from the ER strand to distinct punctate structures and ATG proteins are subsequently recruited for autophagosome formation[109]. ImmunoEM reveals that DFCP1 is mainly localized on clusters of tubules or vesicular elements adjacent to IM rims[110,111]. Thus, the structures linking the ER with the IM consist, at least in part, of the omegasome. The formation, shape and length of omegasomes appear to be determined by levels of PtdIns(3)P and its interacting proteins. Overexpression of DFCP1, which sequestrates functional PtdIns(3)P, causes the formation of abnormally long tubular omegasomes[112]. Simultaneously downregulating the myotubularin PtdIns(3)P phosphatase MTMR6 significantly inhibits tube formation[112].

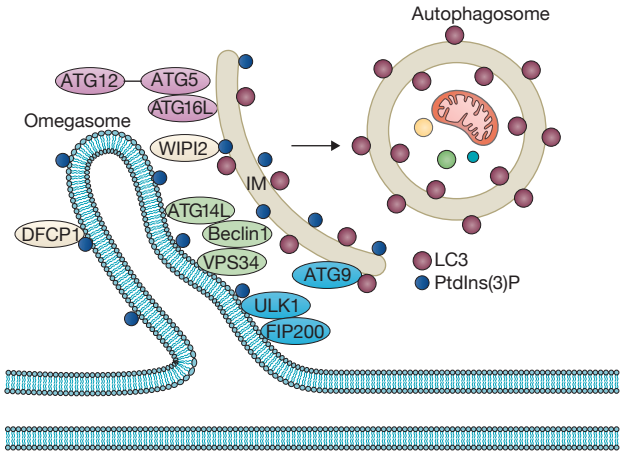

Figure 3 Schematic illustration of the localization of autophagy proteins during autophagosome formation. Upon autophagy induction, ATG proteins are recruited to the autophagosome formation site in a hierarchical order. Some ATG proteins associate with the ER (the ATG14L–Beclin1–VPS34 and ULK1–FIP200 complexes), whereas others locate on the IM (ATG9 and the ATG12–ATG5–ATG16L complex). WIPI2 protein is a PtdIns(3)P effector and DFCP1 is a PtdIns(3)P-binding protein that relocalizes during autophagosome biogenesis. ATG12 is covalently conjugated to ATG5, which further interacts with ATG16L. WIPI2 recruits the ATG12–ATG5–ATG16L complex to forming IMs by binding to ATG16L for LC3 lipidation[135]. Most ATG proteins disassociate from IMs/ER as autophagosomes close and detach from the ER. Lipidated LC3 associates with autophagic structures at all stages, including early unclosed IMs, closed autophagosomes and autolysosomes. Closed autophagosomes contain cytoplasm, mitochondria, and other organelles (yellow, green, and blue circles).

Specification of the ER omegasome formation site. How is the omegasome formation site specified on the ER? Genetic analysis shows that DFCP1-positive omegasome formation depends on the ULK1–ATG13–FIP200 and VPS34–ATG14 complexes, but is upstream of the ATG8 lipidation system[113]. Accordingly, thin tubular clusters are observed in cells deficient for ATG7 (E1 enzyme for ATG5 and ATG8 conjugation), ATG5 or ATG16L1, but not in FIP200-deficient cells[111]. The ULK1–ATG13–FIP200 complex tightly associates with the ER and forms distinct punctate structures upon starvation[113]. How autophagy induction signals induce the formation of ULK1 puncta remains unknown. ATG14, containing an ER-targeting motif, is recruited to the ULK1–FIP200 puncta, which subsequently target the PtdIns(3)P kinase VPS34 for PtdIns(3)P generation[113,114]. PtdIns(3)P in turn modulates stable accumulation of ULK1 puncta, creating a positive feedback loop[115,116]. ATG13 possesses a putative lipid-binding site with preference for acidic phospholipids[116]. The ER omegasome formation site may be determined stochastically, by membrane curvature, or more likely at interaction sites with other organelles. In mammalian cells, IM biogenesis also occurs at the ER–mitochondria contact site, a region called the mitochondria-associated ER membrane (MAM)[117]. Upon autophagy induction, the ER-resident SNARE protein syntaxin 17 (STX17) redistributes to the MAM, where it recruits the PtdIns(3)K complex through its interaction with ATG14L and causes re-localization of DFCP1 to the MAM[117]. Knockdown of the ER–mitochondrion tethering factors mitofusin 2 (MFN2) and PACS-2 prevents ATG14L puncta and attenuates autophagosome formation[117,118].

MAM is also the major site for PE production. Phosphatidylserine (PS) is transported from the ER to mitochondria, where it is converted to PE. The fluorescent lipid NBD-PS (converted to NBD-PE in mitochondria) transfers from mitochondria to autophagosomes[118]. Thus, the MAM serves as a scaffold for omegasome formation and also supplies phospholipids for IM expansion. The ER–Golgi intermediate compartment has also been shown to recruit ATG14 and DFCP1 and provide a membrane source to trigger LC3 lipidation[119].

In yeast, omegasomes have not been detected at the single pre-autophagosomal assembly site (PAS), from which all autophagosomes are generated. ERESs are intimately linked with autophagosomes. Coatomer protein mutants, *sec16*, *sec23* and *sec24*, are defective in autophagy[120]. ERESs act downstream of the Atg1 kinase complex for the recruitment of the autophagy machinery at the PAS[121]. Atg proteins interact with COPII-vesicle transport components[121]. For example, Atg9 directly interacts with Sec23/Sec24. COPII vesicles may provide the membrane source for the initiation and expansion of IMs[121,122].

ER–IM contacts for autophagosome formation. The ER also plays an essential role in IM expansion. 3D electron tomography shows that the ER is closely apposed to both the IM outer and inner membranes[110,123]. The ER is connected by thin tubular structures to the edges and body of the IM, and the two ER cisternae sandwiching the IM are linked by narrow tubule-like extensions through the open end of the IM[110,111,123]. When IMs mature into autophagosomes, the ER cisterna adhering to the outer IM membrane detaches, whereas the internal ER cisterna is engulfed[110,123]. The molecular machinery that initiates and terminates these contacts is unknown. Interactions of the ER-localized ATG proteins (for example, the ULK1–FIP200 and ATG14L–VPS34 complexes) and IM-localized ATG proteins (for example, those involved in ubiquitin-like conjugation systems) may tether the ER to IMs. For example, the ULK1–FIP200 complex interacts with ATG8, ATG16L and ATG9[124–127]. PtdIns(3)P is highly enriched on the omegasome and IM, and may modulate ER–IM contact formation. ER–IM contacts may allow lipid transport via lateral diffusion through membrane continuities or by lipid transport proteins located at the membrane contact sites.

Completed autophagosomes detach from the ER and mature by fusing with endosomes. They are transported to the perinuclear region where late endosomes/lysosomes are localized. ER contacts control the endosomal transport and positioning[128], but it is unknown whether the ER regulates autophagosome transport.

Concluding remarks

As discussed in the previous sections, although we know that *de novo* organelle biogenesis in the ER occurs at specialized subdomains, determining the protein and lipid composition of these subdomains is a difficult challenge that will nevertheless provide insight into how these regions form and function.

It seems likely that the lipid composition of the domains differs from that of the rest of the ER, and these differences probably play critical roles in organelle biogenesis as they do in transport vesicle formation[18,129,130]. This may be particularly true of LD biogenesis, which may be largely driven by the physical properties of membranes, though this remains to be determined[131,132]. Protein recruitment to PPV biogenesis sites may also be driven by the lipid composition and physical properties of the domains where they are generated. How PPVs form and whether there are multiple PPV types remain important questions. PPV-generating ER subdomains may also be important for protein sorting into PPVs but this is yet to be established. Finally, much remains to be learned about how localized PtdIns(3)P production contributes to omegasome biogenesis and to the enrichment of proteins and lipids to these specialized subdomains.

The ER also makes important contributions to peroxisome, LD, and autophagosome maturation after biogenesis by making close contacts with these organelles, which are probably important for lipid exchange and signalling[110,111,123,133,134]. Identification of the molecular machinery mediating ER contacts with these organelles will be crucial for understanding how the ER contributes to organelle growth, maturation and positioning. These open questions notwithstanding, advances in different areas of organelle biology underscore the multifunctionality and versatility of the ER, and how its distinct properties are employed to mediate many different cellular responses.

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The authors declare no competing financial interests.

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