BBA - Molecular Cell Research 1864 (2017) 1450–1458

Contents lists available at ScienceDirect

BBA - Molecular Cell Research

journal homepage: www.elsevier.com/locate/bbamcr

Membrane contact sites, ancient and central hubs of cellular lipid logistics☆

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ARTICLE INFO

Keywords:
Evolution
Golgi complex
Lipid transfer protein
Mitochondria
Secretory pathway
Sphingolipid biosynthesis

ABSTRACT

Membrane contact sites (MCSs) are regions where two organelles are closely apposed to facilitate molecular communication and promote a functional integration of compartmentalized cellular processes. There is growing evidence that MCSs play key roles in controlling intracellular lipid flows and distributions. Strikingly, even organelles connected by vesicular trafficking exchange lipids en bulk via lipid transfer proteins that operate at MCSs. Herein, we describe how MCSs developed into central hubs of lipid logistics during the evolution of eukaryotic cells. We then focus on how modern eukaryotes exploit MCSs to help solve a major logistical problem, namely to preserve the unique lipid mixtures of their early and late secretory organelles in the face of extensive vesicular trafficking. This article is part of a Special Issue entitled: Membrane Contact Sites edited by Christian Ungermann and Benoit Kornmann.

1. Introduction

The identity and function of organelles in eukaryotic cells critically rely on the flux of proteins and lipids that they acquire and lose through cytosolic exchange and vesicular trafficking. The repertoire of membrane lipids in eukaryotes typically comprises hundreds of different species [1]. Besides defining the boundary of each organelle, membrane lipids provide specific cues for proteins to support organelles in executing their specialized tasks [2,3]. Consequently, the lipid composition of organelles varies dramatically and many lipids are unevenly distributed between the two leaflets of the organellar bilayer [4]. How cells monitor and fine-tune the unique lipid mixtures in each organelle to sustain their compartmentalized organization is a major outstanding question in current cell biology.

The non-random lipid distributions in cells cannot be explained by local metabolism alone. For instance, some lipids (e.g. sterols) accumulate and exert their biological activity at locations distant from their site of synthesis. Additionally, enzymes catalyzing sequential steps in pathways of lipid metabolism often reside in membranes of distinct organelles. Spontaneous desorption of a lipid monomer from a bilayer and its free diffusion through the cytosol is too slow to support any meaningful transport of most lipids [5–7]. As lipids are the principal constituents of transport vesicles, bulk amounts of lipids can be moved from one organelle to another by vesicular trafficking. However, various organelles, including mitochondria and plastids, are not connected to the vesicular transport network yet rely on lipid import for proper function. In addition, bulk transport of various lipids between the ER and plasma membrane continues undisrupted when vesicular trafficking is shut off [8–10], suggesting that non-vesicular mechanisms play a major role in lipid trafficking along the secretory pathway.

Accumulating evidence indicates that inter-organellar lipid transport is facilitated at membrane contact sites (MCSs), regions where two organelles come within a distance of 30 nm from each other. MCSs are found between almost every pair of organelles [11]. Despite their heterogeneity, MCSs share some common features. They are typically enriched in proteins involved in lipid metabolism and transport, their formation relies on a tethering of apposing membranes through protein-protein and protein-lipid interactions, and they tend to be dynamic structures that undergo assembly and disassembly in response to changing physiological conditions. While MCSs participate in a wide array of cellular processes including ion homeostasis, organelle inheritance, and apoptosis [12–14], we here focus on their fundamental role as centers of lipid logistics. There is reason to believe that MCSs are ancient structures that evolved before the establishment of vesicular trafficking. We describe how MCSs may have played a crucial role in the acquisition of bacteria-type lipids from proto-mitochondria by their archaeal host, and how mitochondria in modern eukaryotes boost their capacity to acquire essential lipids by creating a variety of MCSs with different organelles of the endomembrane system. We then discuss how lipid transfer proteins operating at ER-Golgi contact sites allow lipids to bypass vesicular connections, and how this arrangement enables cells to build sphingolipid and sterol gradients along the secretory pathway, the

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http://dx.doi.org/10.1016/j.bbamcr.2017.05.017
Received 7 March 2017; Received in revised form 10 May 2017; Accepted 17 May 2017
Available online 26 May 2017

maintenance of which is key to a proper functioning of their early and late secretory organelles.

2. Membrane contact sites and the evolutionary switch in membrane lipid composition

Major events in the evolution of eukaryotic cells included the acquisition of a nucleus, an endomembrane system and mitochondria. The general consensus is that mitochondria arose from endosymbiotic α-proteobacteria in an archaeal host [15]. However, conflicting views exist on how the merger of two prokaryotes gave rise to cells possessing an elaborate endomembrane system and how the archaeal lipids of the host's cell membrane were replaced by bacterium-type lipids that are characteristic of eukaryotes. Archaeal lipids are composed of glycerol-1-phosphate with ether-linked, methyl-branched isoprenoid chains [16]. The structural features of these lipids allow archaea to retain the physical properties of their membranes over a wide range of temperatures [17]. The bulk of bacterial and eukaryotic membrane lipids, on the other hand, are based on glycerol-3-phosphate with unbranched, ester-linked fatty acids. Both eukaryotes and some bacteria, but not archaea, also produce sphingolipids and sterols, which help control membrane fluidity [18,19]. Contrary to archaeal membranes, bacterial and eukaryotic membranes are adjusted to the phase transition boundary at physiological temperatures. This property is thought to facilitate the dynamic and reversible membrane deformations that are characteristic of eukaryotic cells [20]. Many eukaryotic genes involved in lipid metabolism and transport have their closest prokaryotic relatives in α-proteobacteria [21]. While this implies that eukaryotes acquired their bacterium-type lipids from mitochondria, models of eukaryotic evolution struggle with the question how the dramatic transition from archaeal membranes to bacterium-like membranes may have occurred [16].

A widely favored model is that the nucleus and endoplasmic reticulum (ER) were formed within a prokaryotic cell by invaginations of the limiting membrane, and that proto-mitochondria entered the cell via phagocytosis [22–24]. One problem with this "outside-in" model on the origin of the nucleus, ER and mitochondria is that archaea can form outward protrusions but are not known to undergo processes like endocytosis or phagocytosis. Moreover, scission of endosomes and phagosomes from the plasma membrane requires dynamins, a family of large GTPases that originate from bacteria, not from archaea [25]. Phagocytosis results in the formation of a food vacuole, which needs to be acidified to allow the breakdown of its contents by acid-activated proteases. Eukaryotic vacuole acidification requires a vacuolar or V-type ATPase. This enzyme stems from an archaeal A-type ATPase whose function in creating ATP from redox-generated ion gradients was reverted to acidify food vacuoles at the expense of cytosolic ATP [26]. Theories that place phagocytosis before the acquisition of mitochondria fail to account for the source of cytosolic ATP required to acidify food vacuoles. Such theories are also hard to reconcile with the notion that the physicochemical properties of membranes based on bacterium-type lipids were a likely prerequisite for the evolution of the dynamic and energy-intensive process of vesicular trafficking.

The above considerations have led to the formulation of alternative models in which the acquisition of mitochondria predates phagocytosis. In their recent "inside-out" model of eukaryotic cell evolution, Baum and Baum [27] propose that an archaeal host extruded membrane-bound blebs beyond its cell wall that formed intimate contacts with ectosymbiotic proto-mitochondria to facilitate an exchange of biomolecules, including lipids (Fig. 1). Lateral expansion of these blebs eventually trapped populations of proto-mitochondria, with continuous spaces between the blebs giving rise to the nuclear envelope and ER. At some point, proto-mitochondria moved into the cytoplasmic compartment by penetrating the primordial ER membrane, akin to the mechanism by which pathogenic bacteria found within the ER and Golgi of modern eukaryotes gain entry to the cytoplasm [28]. Fusion steps among blebs ultimately yielded a continuous plasma membrane, hence separating the ER from the outside world and promoting the development of the secretory pathway. As outlined below, these events were accompanied by a diversification of lipid biosynthetic machinery and allowed the ER and plasma membrane to specialize in carrying out biogenic and barrier functions, respectively.

A key aspect of the "inside-out" model of Baum and Baum is that proto-mitochondria established close metabolic and physical ties with their archaeal host prior to the formation of the nucleus and the evolution of phagocytosis. Intimate membrane contacts with the ectosymbiotic proto-mitochondria allowed the archaeal host to acquire bacterium-type lipids and initiate a chemical transition of its membranes before bacterial genes for lipid biosynthesis were transferred to its genome (Fig. 1). The transient occurrence of membranes containing a mixture of archaeal and bacterial lipids may have helped core protein machinery of the host (e.g. V-type ATPase, Sec61/SecY translocon, *N*-linked glycosyltransferases) to survive the transformation from archaeal to eukaryotic-type membranes while facilitating the development of a secretory pathway. Under the "inside-out" model, lipid traffic across membrane contacts between proto-mitochondria and their host occurred early on in eukaryotic evolution, before the establishment of the ER. In line with this idea, membrane contacts between mitochondria and ER are ancient [29] and still function as central hubs of lipid traffic in modern eukaryotes (see below).

3. Diversification of membrane contact sites as platforms of lipid exchange

While mitochondria in modern eukaryotes retain a critical role in lipid biosynthesis and produce some of their own membrane lipids such as cardiolipin, they rely on bulk lipid import for proper function (Fig. 2) [30,31]. The ER is the principal supplier of membrane lipids to mitochondria and all other organelles. Newly synthesized lipids are exported from the ER as components of transport vesicles or by lipid transfer proteins (LTPs) that operate at MCSs. Transport by LTPs is crucial for supplying ER lipids to mitochondria as these organelles are not served by vesicular trafficking. LTPs have hydrophobic pockets or clefts that can take up a single lipid molecule upon its partial desorption from a donor membrane. A conformational change might then occur to seal the binding pocket off, protecting the lipid from the aqueous cytoplasm. The reverse process then results in unloading of the lipid at the acceptor membrane. Thus, LTPs act as catalysts of monomeric lipid exchange between membranes, presumably by reducing the energy barrier for dissociating lipid monomers from the membrane [32–34]. Recent estimates of the rate constants for nonvesicular sterol transport in yeast indicate that sterol desorption from the membrane rather than LTP-mediated sterol diffusion through the cytosol is rate limiting [35]. This implies that there would be no apparent kinetic benefit to having LTP-mediated sterol transfer occur at MCSs. However, combining the action of LTPs with that of tether proteins responsible for creating contacts between a donor and acceptor organelle may offer additional advantages. For instance, restricting transport to subdomains of two organelles may enhance the directness and efficiency of transport for lipid species that are readily consumed in metabolic pathways, prevent deleterious redistribution of toxic lipid species, facilitate the establishment and maintenance of lipid gradients, and provide opportunities for regulatory crosstalk among distinct lipid classes.

Interestingly, work in budding yeast uncovered a protein complex that likely serves a dual role in membrane tethering and lipid transport at ER-mitochondria contacts. This so-called ER-mitochondrial encounter structure or ERMES complex is composed of four core-subunits: the mitochondrial outer membrane proteins Mdm10 and Mdm34, the integral ER protein Mmm1 and the soluble cytosolic subunit Mdm12 [36]. Three of the four ERMES subunits (i.e. Mdm34, Mdm12, and Mmm1) contain a synaptotagmin-like, mitochondrial and lipid-binding protein (SMP) domain. Proteins with SMP domains typically share two

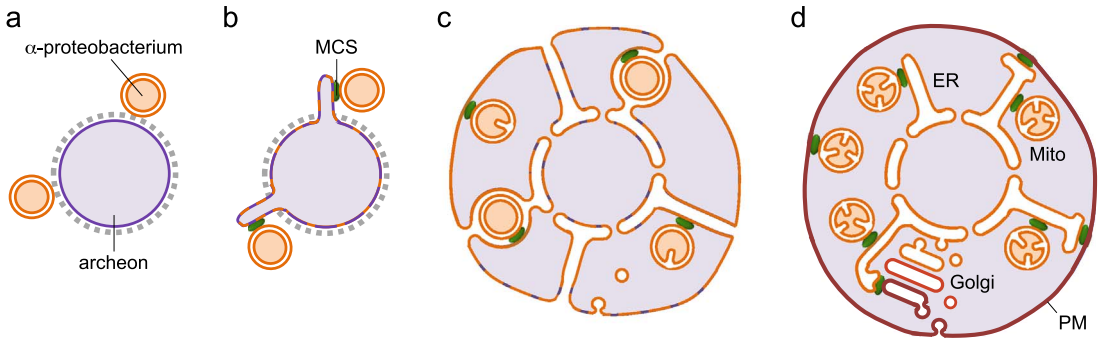

Fig. 1. Inside-out model showing the step-wise evolution of eukaryotic cells.
(a) Free-living α-proteobacteria (proto-mitochondria) form initial contacts with an archaeal host cell (archeon). While α-proteobacteria are surrounded by a double membrane composed of lipids with ester-linked fatty acids (orange), the archeon has a single membrane comprising lipids based on isoprene ethers (purple) and a glycoprotein-rich outer cell wall (gray, dashed).
(b) Protrusions extending from the archaeal host form primordial membrane contact sites with proto-mitochondria to facilitate an exchange of biomolecules, including lipids. Acquisition of bacterium-type lipids by the host initiates a gradual chemical transition of its membrane.
(c) Protrusions laterally extend to blebs that increasingly enclose proto-mitochondria. Spaces between the blebs correspond to the lumen of the future ER and nuclear envelope. An increased contact area between the symbionts and translocation of proto-mitochondria into the cytoplasmic compartment of the host allow closer metabolic ties, involving a diversification of MCSs. Acquisition of bacterial lipid biosynthesis machinery by the host results in a further loss of archaeal lipids. The transition from archaeal to bacterial membranes facilitates the development of vesicular trafficking machinery.
(d) Fusion steps among blebs seal the ER off from the outside world, giving rise to a continuous plasma membrane and promoting the development of a secretory pathway. This is accompanied by the evolution of sphingolipid and sterol biosynthetic/transport machinery and the emergence of the Golgi complex, which serves as a lipid distillation apparatus to keep sphingolipid/sterol levels low in the ER (orange) and high at the plasma membrane (red). Sphingolipid/sterol traffic mediated by lipid transfer proteins at ER-Golgi contact sites bypasses vesicular traffic to exert optimal control over the unique lipid mixtures of the ER and plasma membrane. Figure adapted from [27].

additional characteristics, namely localization to MCSs and the ability to bind and, in some cases, transfer lipids [37]. Structural analysis of a complex formed between the SMP domains of Mdm12 and Mmm1 revealed an extended tubular structure traversed by a hydrophobic channel [38]. Biochemical studies showed that the Mdm12-Mmm1 complex preferentially binds phosphatidylcholine (PC), a bulk lipid whose import by mitochondria is essential to sustain their function [39]. While these findings support a primary role of ERMES in lipid transport at ER-mitochondria contact sites, direct proof for this is lacking. Phylogenetic analyses revealed that ERMES, though present in the common ancestor of yeast and animals, was lost in the animal lineage [29]. Thus, at some point in evolution, ERMES became functionally redundant.

Indeed, the modest changes in mitochondrial lipid composition observed in ERMES mutants suggest that eukaryotes developed alternative pathways for lipid delivery to mitochondria. For instance, loss of ERMES in yeast enhances formation of mitochondria-vacuole/lysosome contacts, termed vCLAMPs [40,41]. Mutations disrupting both ERMES and vCLAMPs render cells nonviable while acute depletion of both complexes causes additive effects in mitochondrial lipid import. As vacuoles are linked to the ER, both directly, through nuclear-vacuolar junctions (NVJs), and indirectly, through vesicular traffic, vCLAMPs may serve as a bypass for lipid exchange between ER and mitochondria. However, the mitochondrial subunit(s) of vCLAMPs remains to be identified, and its other core components do not contain known lipid-binding pockets. Interestingly, Lam6/Ltc1, member of a conserved family of ER-anchored proteins with StART-like lipid transfer domains [42], was found to co-localize with three inter-organellar contacts: ERMES, vCLAMP, and NVJ [43,44]. Overexpression of Lam6/Ltc1 caused an expansion of all three MCSs. Moreover, Lam6/Ltc1 binds sterols *in vitro* and is required for the formation of sterol-enriched vacuolar domains under stress conditions [44]. Thus, Lam6/Lct1 may

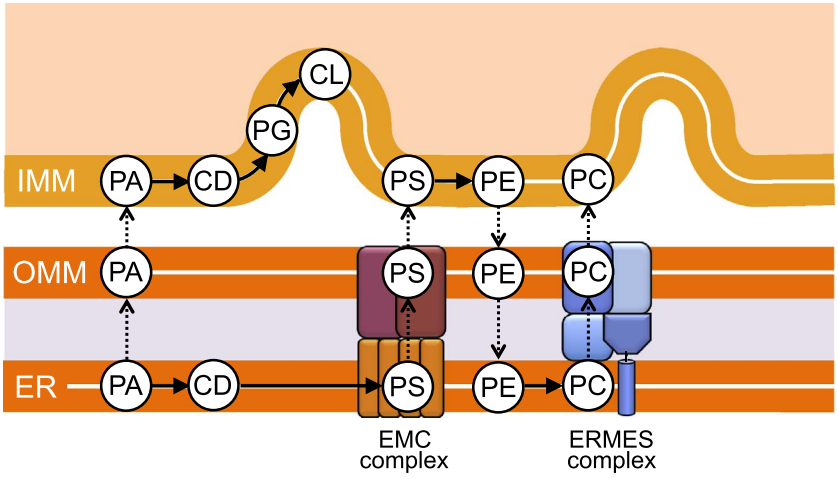

Fig. 2. Lipid traffic at ER-mitochondria contact sites.
Lipid transport between ER and mitochondria is indicated by dashed arrows. Metabolic conversion of one lipid to another is indicated by a solid arrow. The ERMES and EMC protein complexes have been implicated in PC and PS transport at ER-mitochondria contact sites in yeast, respectively. Note that only the EMC complex is conserved in mammalian cells. While phosphatidylserine (PS) synthesis in yeast involves an enzymatic reaction in which CDP-diacylglycerol (CD) reacts with serine, PS synthesis in mammalian cells relies on a base-exchange reaction in which serine replaces the choline or ethanolamine head group of phosphatidylcholine (PC) and phosphatidylethanolamine (PE), respectively (not shown). OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane; PA, phosphatidic acid; PG, phosphatidylglycerol; CL, cardiolipin.

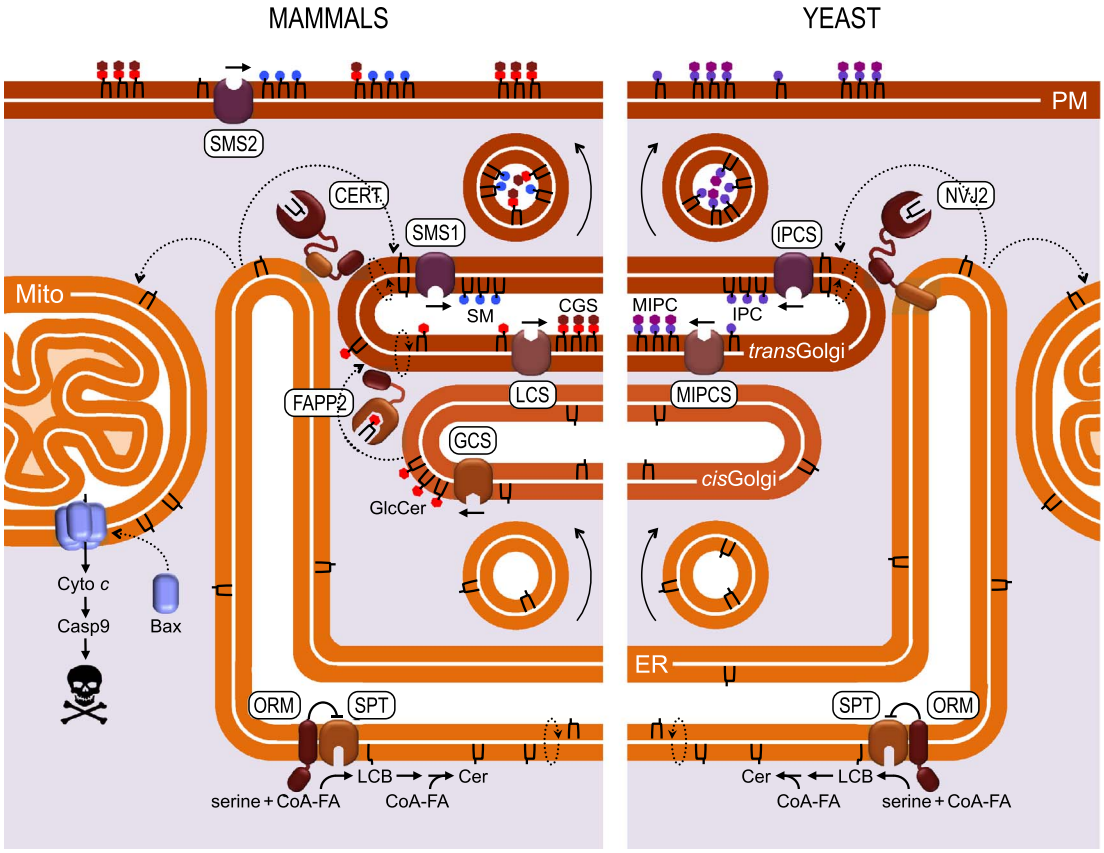

Fig. 3. Sphingolipid biosynthesis in mammals and yeast.
Sphingolipid synthesis begins on the cytosolic surface of the ER with the condensation of serine and coenzyme A-linked fatty acids (CoA-FA), a reaction catalyzed by serine palmitoyltransferase (SPT) to form long chain bases (LCB). LCBs are subsequently *N*-acylated by ER-resident ceramide synthases to form ceramides (Cer), the precursor of all sphingolipids. In mammals, a portion of newly synthesized ceramides is delivered to the *cis*-Golgi by vesicular trafficking for conversion into glucosylceramide (GlcCer) by glucosylceramide synthase (GCS) on the cytosolic surface. FAPP2 mediates non-vesicular GlcCer transport from the *cis* to the *trans*-Golgi for the production of complex glycosphingolipids (CGS) in the *trans*-Golgi lumen. However, the bulk of newly synthesized ceramides is delivered to the *trans*-Golgi by ceramide transfer protein CERT to form sphingomyelin (SM) by SM synthase SMS1 in the *trans*-Golgi lumen. SM and CGS reach the cell surface by vesicular transport. A second SMS-isoform, SMS2, is primarily active on the cell surface. Yeast lacks structural homologues of SMS but instead contains a functionally analogous enzyme to produce inositol phosphorylceramide (IPC) in the *trans*-Golgi lumen. IPC can be further modified by mannose and phosphoinositol moieties before vesicular transport to the cell surface. In yeast, newly synthesized ceramides can reach the Golgi by vesicular means but also by cytosolic transfer, involving a putative ceramide transfer protein, NVJ2. CERT and NVJ2 also serve a role in preventing the toxic buildup of ceramides in the ER, which may cause a leak of ceramides into mitochondria and trigger apoptosis.

coordinate vacuolar and mitochondrial sterol homeostasis. Whether Lam6/Ltc1 and other members of the StART-like LTP family catalyze lipid transfer across membrane contacts remains to be established.

A screen for components influencing mitochondrial import of phosphatidylserine (PS) in yeast yielded a novel ER-mitochondria tether, termed the ER-membrane protein complex (EMC; Fig. 2) [45]. This complex contains six subunits, Emc1-6, and interacts with the outer mitochondrial membrane protein Tom5 at foci that overlap with ERMES. EMC mutants have a reduction in the amount of ER tethered to mitochondria and are defective in PS transport to mitochondria [45]. A notable difference between the EMC and ERMES is that only the EMC is conserved in higher eukaryotes. As EMC subunits have also been implicated in protein folding and quality control in the ER, their role in ER-mitochondria lipid trafficking and tethering may be indirect.

While the concept of ER-mitochondria MCSs as major sites of membrane lipid trafficking first emerged from biochemical studies in mammalian cells more than two decades ago [46,47], the identity of functional homologues of ERMES in mammals has yet to be established [31,48]. Work in yeast showed that ERMES and other mitochondria-organelle contacts function as part of a dynamic network, whereby loss of one contact is compensated by reinforcement of another [43,49]. This redundancy in interconnectivity obviously complicates a molecular dissection of mitochondrial lipid delivery pathways but also underscores the significance of membrane contacts as major hubs of lipid traffic in eukaryotic cells. Indeed, a prominent non-vesicular exchange of lipids even occurs at contacts between organelles that, contrary to mitochondria, are integrated into the vesicular transport network. For instance, the ER and Golgi complex exploit similar logistics for lipid transfer as mitochondria by recruiting a variety of LTPs to ER-Golgi contact sites, despite the fact that these organelles already exchange lipids through intensive bi-directional vesicular trafficking. As discussed below, this arrangement sub-serves a fundamental transition in bulk lipid composition along the secretory pathway.

4. ER-Golgi contact sites contribute to a fundamental switch in lipid composition along the secretory pathway

Eukaryotes arose from their prokaryotic ancestors through the establishment of two distinct membrane systems, one centered on the ER and the other one on the plasma membrane, allowing specialization in function and lipid composition [2,3,50]. This may explain why the advent of eukaryotic cells was accompanied by the evolution of biosynthetic machinery for two entirely new classes of membrane lipids: sphingolipids and sterols [19]. Contrary to the bacterium-type glycerophospholipids, sphingolipids primarily contain saturated or *trans*-unsaturated acyl chains linked to a serine backbone. This hydrophobic structure, called ceramide, is the precursor of all major sphingolipids, including sphingomyelin (SM) and glycosphingolipids.

The absence of the rigid kinks of *cis*-double bonds, which are common in acyl chains of glycerophospholipids, increases the packing density of sphingolipids in a bilayer. Consequently, at physiological temperatures, an SM bilayer exists in a solid gel phase with tightly packed, immobile chains [51]. However, these membranes become fluid upon addition of sterols. The latter molecules have an apolar inflexible core of four fused rings that interferes with the tight packing of saturated acyl-chains, thus preventing the transition of the membrane to the solid gel phase. At the same time, sterols exert a condensing effect on fluid membranes by reducing the flexibility of the acyl chains [52]. This, in turn, increases membrane thickness and impermeability to solutes. Thus, sterols allow eukaryotes to drastically reduce unregulated solute movement across their membranes while keeping them fluid over a wide range of temperatures.

Because of their vital properties, eukaryotic cells developed complex mechanisms to control the abundance and subcellular distribution of sterols. The ER is the site of *de novo* sterol production and harbors important sensors of sterol levels [53,54]. However, sterols are rare in the ER (5 mol% of total lipid) but abundant at the plasma membrane (30–40 mol%) [4]. This major imbalance in sterol distribution serves a clear purpose. A high concentration of sterols provides the plasma membrane with physicochemical properties that support its barrier function. Conversely, low sterol levels in the ER result in a loosely packed lipid bilayer that facilitates insertion of nascent membrane proteins, thus upholding the biogenic function of this organelle. Indeed, sterol excess inhibits protein import in the ER [55]. This implies that eukaryotes must continuously remove sterols from the ER as they are synthesized, and concentrate them in the plasma membrane. This appears a daunting task, as bi-directional vesicular trafficking along the secretory pathway would constantly undermine an asymmetric sterol distribution. However, it appears that eukaryotic cells exploit two complementary strategies to tackle this logistic problem.

Sterols preferentially interact with lipids carrying saturated fatty acyl chains and bulky head groups, such as sphingolipids [56]. Contrary to sterols and glycerophospholipids, the bulk of sphingolipids is produced in the lumen of the *trans*-Golgi from ceramide supplied by the ER (Fig. 3; discussed below). Analogous to sterols, sphingolipids are enriched in the plasma membrane while their levels are low in the ER. Thus, sphingolipid production in the Golgi followed by anterograde sphingolipid transport to the cell surface provides a potential means to trap ER-derived sterols and move them up a concentration gradient into the plasma membrane. In line with this idea, retrograde-moving COPI vesicles are relatively depleted of cholesterol and SM in comparison to the Golgi, the organelle from which they bud off [57]. Additionally, cell surface SM degradation causes cholesterol to redistribute to the ER [58], leading to downregulation of HMG-CoA reductase, the rate-limiting enzyme in sterol biosynthesis [59]. It also deserves mention that glycerophospholipids are more saturated at the plasma membrane than in the ER due to a substantial remodeling of their acyl chains [60,61], which would further promote an asymmetric sterol distribution along the secretory pathway.

Strikingly, sterol transport along the secretory pathway has been shown to be independent of vesicular trafficking [10,62]. Instead, recent work suggests that sterol transport is mediated by LTPs that operate at MCSs between the ER and *trans*-Golgi. Thus, two LTPs from the ORP/Osh protein family, Osh4p in yeast and OSBP in mammals, have been implicated in the creation and maintenance of a sterol gradient between early and late secretory organelles [63,64]. OSBP is the founding member of a large family of proteins named ORPs (OSBP-related proteins) in mammals and Oshs (OSBP homologs) in yeast. As shown in Fig. 4a, OSBP contains an *N*-terminal pleckstrin homology (PH) domain that binds phosphatidylinositol-4-phosphate (PI4P), a phosphoinositide enriched on the cytosolic surface of the *trans*-Golgi in both mammals and yeast. In addition, OSBP possesses a central diphenylalanine-in-an-acidic-tract (FFAT) motif that binds the conserved ER-resident membrane proteins VAP-A and VAP-B, and an oxysterol-related domain (ORD) that, analogous to the ORD domain of Osh4, can bind and transfer cholesterol and PI4P in a mutually exclusive manner [64,65]. It was shown that OSBP can exchange PI4P for cholesterol while its PH-FATT region mediates recruitment of the protein to ER-Golgi contact sites [64]. This led to a model in which OSBP transfers cholesterol from the ER to the *trans*-Golgi by exchanging it for PI4P (Fig. 4b) [66]. Net transfer of sterols would be energized through dissipation of a PI4P gradient created by the combined actions of PI-4-kinases, which produce PI4P in the *trans*-Golgi, and the PI4P phosphatase Sac1, which converts PI4P to PI in the ER. ER-Golgi contact sites are also the sites where a PI/PC transfer protein, Nir2, is believed to deliver PI back to the *trans*-Golgi for its conversion to PI4P [67].

However, a conditional yeast mutant lacking all functional Osh proteins, including Osh4p, did not display any major defect in sterol transport between the ER and plasma membrane [68]. Consequently, whether heterotypic lipid exchange fueled by the metabolic energy of PI4P is sufficient to establish a sterol gradient in eukaryotic cells remains to be determined. It appears likely that thermodynamic trapping of sterols by sphingolipids also plays a role. In fact, this may explain why bulk assembly of sphingolipids from ceramides occurs in the *trans*-Golgi, hence spatially separated from bulk glycerophospholipid and sterol production in the ER. The first and rate-limiting step in *de novo* sphingolipid synthesis occurs on the cytosolic surface of the ER, involving the condensation of serine with palmitoyl-CoA by serine palmitoyl transferase (SPT) (Fig. 3). Subsequent reduction of the product yields a long chain base (LCB), which is then *N*-acylated and further reduced by ER-resident ceramide synthases and a desaturase, respectively, to generate ceramide [69]. In mammals, a portion of newly synthesized ceramides is delivered by a vesicular pathway to the *cis*-Golgi to initiate the production of complex glycosphingolipids, involving glycosyltransferases that are distributed along the Golgi cisternae and a glucosylceramide transfer protein, named FAPP2 (Fig. 3; reviewed in [70]). However, the bulk of newly synthesized ceramides is converted to SM by a SM synthase in the lumen of the *trans*-Golgi [71]. Delivery of ER ceramides to the site of SM production requires the ceramide transfer protein CERT [72]. Analogous to OSBP, CERT contains a PI4P-binding PH domain, an FFAT motif that interacts with VAPs, and a START domain that binds and transfers ceramide (Fig. 4a). Thus, CERT likely operates alongside OSBP at MCSs between the ER and *trans*-Golgi. This arrangement offers several advantages. To begin with, while CERT exchanges ceramides bi-directionally between the ER and the *trans*-Golgi, metabolic trapping of ceramides by the SM synthase in the latter organelle ensures an efficient one-way transfer. The pool of newly synthesized SM building up in the *trans*-Golgi then provides a thermodynamic trap for sterols (Fig. 4b). This, in turn, would facilitate an exchange of sterols for PI4P by OSBP at the *trans*-Golgi, thus promoting net sterol transfer in the face of sterol excess. The proximity of CERT and OSBP at ER-Golgi MCSs also facilitates regulatory crosstalk to ensure that sphingolipid precursors reach the *trans*-Golgi in harmony with sterols, allowing an efficient implementation of the major switch in lipid composition that marks the adaptation from biogenic to barrier functions [50].

5. Coordination of sterol and ceramide trafficking at ER-Golgi contact sites

A first 3D reconstruction of the contact sites between the ER and the *trans*-most Golgi cisterna in mammalian cells was reported nearly two decades ago [73]. While the tethering complexes responsible for creating ER-Golgi contact sites have yet to be identified, CERT and OSBP with their ER and *trans*-Golgi targeting motifs qualify as prime candidate components. Indeed, overexpression of VAP-A with CERT or OSBP induces a redistribution of VAP-A to the perinuclear region and, at the EM level, results in the appearance of large contact zones between the ER and Golgi [64,74]. As both LTPs rely on PI4P to bridge

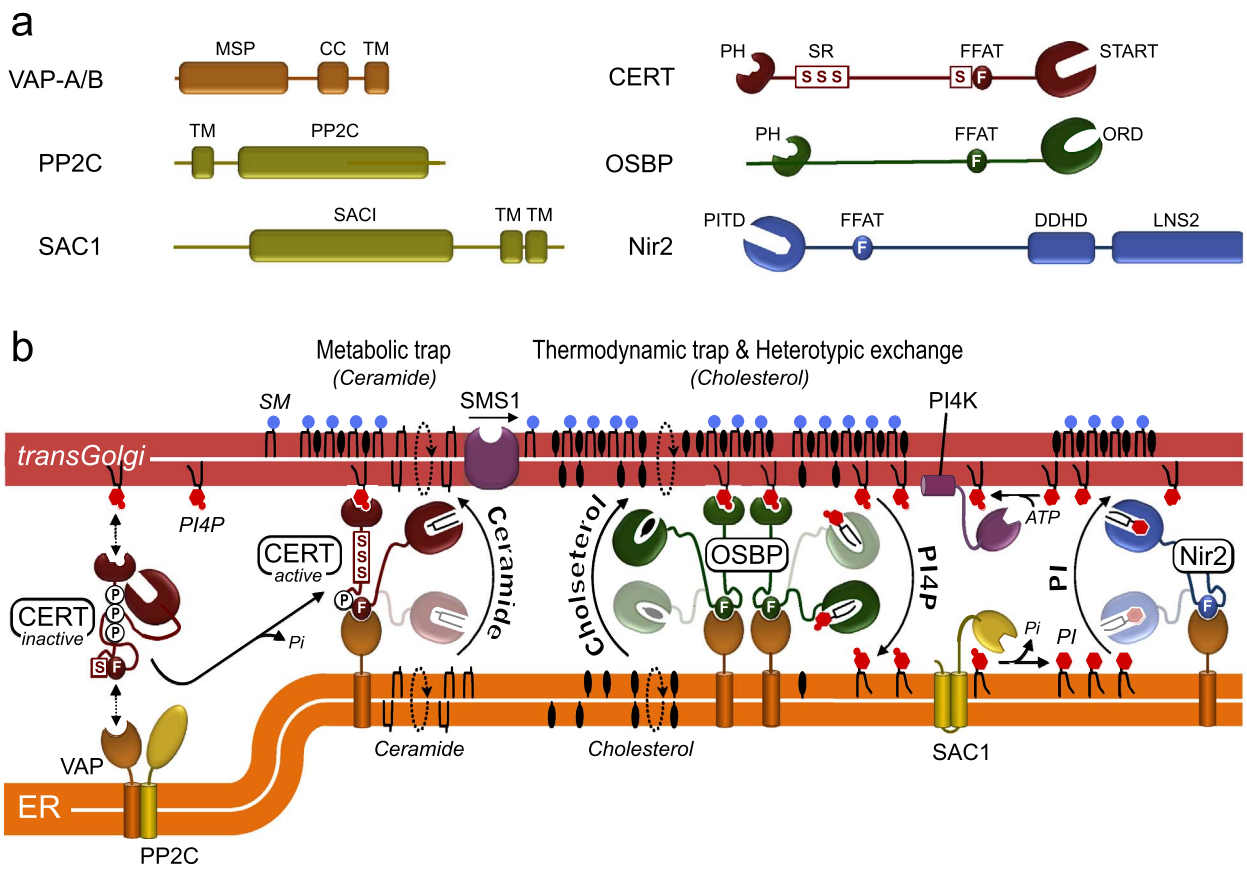

Fig. 4. Coordination of LTP-mediated ceramide and sterol traffic at ER-Golgi contact sites.

(a) Putative components of ER-Golgi contact sites. VAP-A/B, vesicle-associated membrane protein (VAMP)-associated protein A/B; PP2C, protein phosphatase 2C; SAC1, phosphatidylinositol-4-phosphate (PI4P) phosphatase; CERT, ceramide transfer protein; OSBP, oxysterol binding protein; Nir2, phosphatidylinositol (PI)/phosphatidylcholine (PC) exchange protein; MSP, major sperm protein domain; CC, coiled-coil domain; TM, transmembrane domain; PH, pleckstrin homology domain; FFAT, two phenylalanines in an acidic track motif; SR, serine-rich motif; START, steroidogenic acute regulatory protein-related lipid transfer domain; OSB, oxysterol transfer domain; PITP, phosphotidylinositol transfer protein domain; DDHD, death domain homologous domain; LNS2, lamin/neurexin/sex-hormone-binding domain.

(b) Coordination of lipid transfer reactions catalyzed by CERT, OSBP, and Nir2. CERT is inactivated through hyperphosphorylation of its SR motif, which results in reciprocal masking of its PH domain and ceramide-binding START domain. On dephosphorylation by PP2C, CERT engages PI4P on the surface of the *trans*-Golgi via its PH domain and ER-resident VAP proteins via its central FFAT motif to catalyze the transfer of ceramides between the cytoplasmic faces of the two organelles. At the *trans*-Golgi, ceramide flips to the luminal side, promoting SM synthesis by SMS1. This metabolic trapping enables continued delivery of ceramide from the ER. Like CERT, OSBP is auto-inhibited and requires VAP proteins to be active and bridge ER-Golgi contact sites via its PH-FFAT motifs. OSBP uses heterotypic lipid exchange to move cholesterol from the ER to the *trans*-Golgi, and PI4P in the opposite direction. Cholesterol build up in the *trans*-Golgi is sustained by ongoing sphingolipid production, which creates a thermodynamic trap for newly arrived cholesterol, and by the maintenance of a steep PI4P gradient, which runs in the opposite direction due to PI4P synthesis in the *trans*-Golgi and PI4P consumption in the ER. A PI transfer protein, Nir2, is required to feed the metabolic cycle of PI4P. Figure adapted from [66].

the ER and *trans*-Golgi, PI4P plays a central role in controlling the flow of ceramides and sterols across ER-Golgi contact sites. The *trans*-Golgi is enriched in PI4P, achieved by phosphorylation of PI by the PI4 kinase PI4KIIα and PI4KIIIβ [75]. Interestingly, OSBP-mediated delivery of sterols to the *trans*-Golgi has been proposed to activate PI4KIIα, causing an increase in PI4P levels. This, in turn, enhances recruitment of CERT to promote delivery of ceramide for SM synthesis [76,77]. However, the parallel rise in SM and sterol levels in the *trans*-Golgi is curbed by an OSBP-mediated back delivery and consumption of PI4P in the ER. The decline in *trans*-Golgi PI4P levels acts as an intrinsic “OFF”-switch for both OSBP and CERT-mediated lipid transfer. In this way, OSPB may implement a synchronization of sterol and ceramide transport across ER-Golgi contact sites to enable an efficient transition in the functional identify from early to late secretory organelles.

Several lines of evidence indicate that the activity of OSBP and CERT is regulated in coordination with membrane trafficking to the plasma membrane. Protein kinase D (PKD) is a serine/threonine kinase with a critical role in the fission of transport carriers from the *trans*-Golgi [78]. PKD localizes to the *trans*-Golgi through diacylglycerol (DAG) binding by its C1a domain [79]. Phosphorylation of PI4KIIIβ by PKD results in enhanced production of PI4P at the *trans*-Golgi [80], thus providing a platform for the recruitment of OSBP and CERT. Recruitment of CERT stimulates PKD activity, presumably through formation of DAG during SM synthesis [81]. CERT, in turn, is phosphorylated by PKD at a serine, which serves as a priming site for multiple phosphorylations by casein kinase CKIγ2 in a serine-rich (SR) motif [82]. Hyperphosphorylation of CERT blocks both the ceramide transfer activity of its START domain and PI4P binding by its PH domain, thus releasing CERT from the Golgi and reducing SM biosynthesis [83]. At the ER, CERT is dephosphorylated by PP2C, a membrane-anchored phosphatase that interacts with VAP-A [84]. This, in turn, stimulates recruitment of CERT to the *trans*-Golgi and its binding to VAP-A (Fig. 4b). Depletion of SM from the plasma membrane triggers both dephosphorylation of the SR motif and phosphorylation of a serine residue near the FFAT motif to activate CERT, but how this is accomplished remains to be addressed [83,85]. Besides CERT, PKD also phosphorylates OSBP, causing its release from the Golgi [86]. Thus, through activation of PI4KIIIβ and subsequent inactivation of CERT and OSBP, PKD may implement continuous rounds of ceramide and cholesterol transfer at ER-Golgi contacts. Moreover, recent work revealed that VAP-dependent association-dissociation dynamics of ER-Golgi contacts are important for creating *trans*-Golgi-derived membrane

carriers destined for the plasma membrane [87]. This implies that ER-Golgi contact sites not only serve a fundamental role in membrane maturation but also define the position and timing of membrane fission, analogous to the role of ER contact sites in mitochondria and endosome fission [88,89].

While ER-Golgi contact sites have been well documented in mammalian cells, their occurrence in lower eukaryotes like yeast has been demonstrated only recently. Analogous to mammalian cells, sterol trafficking in yeast occurs independently of vesicular trafficking [10] whereas transport of ceramides to the Golgi is mediated by both vesicular and non-vesicular pathways [90]. As yeast lacks a CERT homologue, the mechanism by which this organism mediates non-vesicular ceramide transport is unclear. Excess of ER ceramides in mammalian cells is toxic, causing cell cycle arrest and apoptosis [91,92]. Consequently, CERT removal triggers mitochondria-mediated cell death and sensitizes cancer cells to drug-induced apoptosis [92,93]. Ceramide toxicity has also been shown in yeast and may cause an apoptosis-like cell death [94]. A recent study showed that contacts between the ER and Golgi in yeast increase dramatically during ER stress and when ceramide levels accumulate [95]. ER-Golgi contact formation requires Nvj2, an ER-resident membrane protein that normally resides at contacts between the nuclear envelope and vacuole [96]. Nvj2 contains a lipid-binding SMP domain and a PH domain required for Golgi binding. Importantly, Nvj2 was found to facilitate ceramide export from the ER to the Golgi, where Aur1 converts ceramides into inositol phoshorylceramide (IPC), the yeast analogue of SM. Combinatorial loss of Nvj2 and negative regulators of ceramide biosynthesis caused a dramatic accumulation of ceramides and poor growth, even in the absence of ER stress [95]. Collectively, these results suggest that Nvj2 functions as an inducible ER-Golgi tether that facilitates ceramide export from the ER to prevent the buildup of toxic amounts of ceramides. Whether Nvj2 binds and transfers ceramides remains to be established. Mammals contain a structural homologue of Nvj2, HT008, which can partially compensate for the loss of the protein in yeast [97]. Thus, HT008 may act in concert with CERT to promote ceramide export from the ER during stress.

6. Conclusions and outlook

In here, we have described MCSs as ancient and central hubs of cellular lipid logistics. It is conceivable that MCSs emerged as platforms of non-vesicular lipid exchange already early on during eukaryotic evolution, i.e. when ectosymbiotic proto-mitochondria initiated close metabolic and physical ties with their archaeal host, before the establishment of an endomembrane system and vesicular trafficking. In modern eukaryotes, membrane contacts interconnect mitochondria with the endomembrane system at multiple nodes [11]. Besides allowing extensive inter-organellar crosstalk and collective regulation, this redundancy in interconnectivity ensures that mitochondria can acquire essential lipids from multiple resources, making them less vulnerable when one particular supply route is compromised.

This redundancy also means that a molecular dissection of the lipid transport machinery operating at mitochondrial contact sites is not straightforward. Several components of MCSs between mitochondria and neighboring organelles possess lipid-binding domains (e.g. Mdm12, Mmm1, Ltc1/Lam6). However, ultimate proof that these candidate LTPs transfer lipids across contact sites remains to be established. For MCS components harboring tethering and lipid transfer activities within one protein, an unambiguous functional analysis may become challenging. Moreover, some LTPs (e.g. Ltc1/Lam6, Nvj2) are found at more than one MCS. There is evidence that these LTPs contribute to MCS dynamics and facilitate cross-talk between different contact sites in response to different physiological conditions (e.g. ER stress, nutrient deprivation; [43,95]. The human genome encodes over one hundred predicted LTPs [98], and novel LTPs evolutionary unrelated to previously known LTPs continue to be discovered [42]. Consequently, a systematic and unbiased monitoring of interactions between LTPs and their lipid cargo [99] combined with sophisticated genetic and biochemical approaches will be necessary to unravel, in molecular detail, how mitochondria exchange lipids across contact sites with organelles of the endomembrane system.

Strikingly, two of the best-characterized LTPs in mammalian cells, CERT and OSBP, operate at ER-Golgi contact sites. Converging lines of evidence indicate that these LTPs bypass vesicular connections to help create and maintain sphingolipid/sterol gradients between early and late secretory organelles. Vectorial transport of ceramides by CERT is accomplished by metabolic trapping, involving SM synthase in the *trans*-Golgi lumen. OSBP, on the other hand, can move sterols up against their concentration gradient using two additional strategies, namely: i) thermodynamic trapping by sphingolipids produced in the *trans*-Golgi lumen; ii) heterotypic lipid exchange against a steep gradient of PI4P that runs in the opposite direction. By exerting tight control over lipid exchange at ER-Golgi contact sites, these LTPs and their functional homologues allow eukaryotic cells to tackle a fundamental logistical problem, namely to preserve the unique lipid mixtures of the ER and plasma membrane in the face of extensive vesicular trafficking.

An efficient lipid exchange by OSBP and CERT appears to be critically dependent on the ability of their PH-FFAT motifs to tether ER and *trans*-Golgi membranes. Whether these LTPs are also essential for creating ER-Golgi contact sites remains to be established. If this were the case, contact site formation would be critically dependent on PI4P levels in the *trans*-Golgi. Interestingly, both OSBP and CERT are substrates of PKD, which also serves as a master regulator of PI4P levels and membrane fission at the *trans*-Golgi. These and other findings indicate that PKD may tune sterol and sphingolipid levels in the *trans*-Golgi to ensure that only transport carriers with a suitable lipid composition are dispatched for delivery to the plasma membrane. Recent work suggests that ER-Golgi contact sites are dynamic structures [87], in line with the notion that the *trans*-Golgi is not a static compartment but an inducible one that is formed and molded by incoming and outgoing cargo [100]. Thus, future application of advanced approaches in super-resolution live cell imaging will likely reveal major insights into the dynamic organization of LTPs at membrane contacts in relation to vesicular trafficking, organelle homeostasis, and other vital cellular processes.

Transparency document

The http://dx.doi.org/10.1016/j.bbamcr.2017.05.017 associated with this article can be found, in online version.

Acknowledgments

We thank Svenja Bockelmann for comments on the manuscript. Work in our laboratory is funded by the European Union (MC-ITN Sphingonet Project 289278) and the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 944, Project 14).

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