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REVIEW

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Inter-organelle ER-endolysosomal contact sites in metabolism and disease across evolution

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ABSTRACT

Since their initial observation, contact sites formed between different organelles have transitioned from ignored curiosities to recognized centers for the exchange of metabolites and lipids. Contact formed between the ER and endomembrane system (eg. the plasma membrane, endosomes, and lysosomes) is of particular biomedical interest, as it governs aspects of lipid metabolism, organelle identity, and cell signaling. Here, we review the field of ER-endolysosomal communication from the perspective of three model systems: budding yeast, the fruit fly *D. melanogaster*, and mammals. From this broad perspective, inter-organelle communication displays a consistent role in metabolic regulation that was differentially tuned during the development of complex metazoan life. We also examine the current state of understanding of lipid exchange between organelles, and discuss molecular mechanisms by which this occurs.

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Introduction: Membrane contact sites in cell biology and disease

Since their initial observation over 60 years ago, contact sites formed between different organelles have transitioned from ignored curiosities to recognized centers for the exchange of metabolites and lipids.[1] Recent studies have identified several conserved protein families that function as "tethers" at inter-organelle membrane contact sites (MCSs), as well as soluble proteins that dock at these sites to mediate the non-vesicular trafficking of lipids. Many of these proteins have important roles in human physiology, and their loss contributes to serious genetic conditions, underscoring the need to understand their roles in cell biology, and ultimately, human physiology and pathophysiology (Table 1).

The purpose of this short review is two-fold: 1) to examine the growing list of proteins that function at sites of inter-organelle contact, and 2) to discuss how perturbing their functions impact metabolism and disease across evolution from yeast and fruit flies to mammals. As this is a broad topic, most attention will be paid to proteins involved in contact between the Endoplasmic Reticulum (ER) and the endomembrane trafficking pathway (the plasma membrane, endosomes, and lysosomes). Since several MCS-localizing proteins play important roles in lipid metabolism, we will also discuss how lipid metabolism integrates into general physiology, and how this relationship governs organismal health and aging. Finally, we will briefly examine current models describing how lipids are exchanged at MCSs, and how perturbing this affects metabolism and organelle identity. For additional information regarding other inter-organelle contact sites, as well as discussions of inter-organelle communication, endosome movement, lipid transport, and signaling, the reader is directed to other excellent reviews.[2-5]

Yeast ER-endolysosomal contact sites in cell metabolism and disease

Although ER-endolysosomal contact sites are currently the focus of intense study in mammalian systems, proteins that localize to sites of contact between the ER and vacuole (the yeast lysosome) have been well known for over a decade.[6-8] Many of these act as inter-organelle "tethers" and were originally described in the budding yeast *Saccharomyces cervisiae*. Here the site of contact between the ER and vacuole is known as the Nuclear ER (nER) Vacuolar Junction (NVJ), and comprises a ~200–500 nm long patch-like strip juxtaposed between the

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Table 1. Location, proposed functions, and associated diseases of ER-organelle MCS proteins.

Protein						
S. cerevisiae	*D. melanogaster*	*H. sapiens*	Observed Membrane Contact Site(s)	Proposed Function(s)	Disease(s) Associated	References
Tricalbin	Esyt2	E-Syts	ER-PM (yeast, human)	ER-PM tethering, potential role in non-vescicular lipid transport		Tavassoli, S. *et al.*, 2013
Scs2/22	VAP	VAP-A/B (ALS8)	ER-PM (yeast)	ER-PM tethering, recruitment of FFAT motif-containing proteins	ALS disease	Nishimura, A. L. *et al.*, 2004
Ist2	Axs?	TMEM16/Ano family	ER-PM (yeast)	ER-PM tethering, possible channel activity	defects in chromosomal segregation in *D. melanogaster*	
Nvj1	n/a	n/a	ER-vacuole (yeast)	ER-vac tethering, essential for Piecemeal Autophagy of the Nucleus (PMN)		Pan, X. *et al.*, 2000
Vac8	n/a	n/a	ER-vacuole (yeast)	ER-vac tethering, vacuole inheritence		Pan, X. *et al.*, 2000
Nvj2	CG43783	HT008/Tex2	ER-vacuole (yeast)	ER-vacuole tethering (?); possible role in lipid trafficking via its SMP domain		Toulmay, A. & Prinz, W. A., 2012
Tsc13	Sc2?	TECR	ER-vacuole (yeast)	enoyl reductase, generates very long chain fatty acids (VLCFAs); mutants in humans affect sphingolipid metabolism	essential in flies; mutants in mouse models	Abe, K. *et al.*, 2013; Kvam, E. *et al.*, 2005.
Mdm1	Snz	Snx13,14,19, 25	ER-vacuole (yeast)	ER-vacuole, comunication and lipid metabolism in yeast, and possible roles in obesity, aging, and neurological disease in metazoans	*snz*-deficienct fruit flies exhibit lifespan extension and obesity; human *SNX14*-deficiency is linked to cerebellar ataxia with intellectual disability	Henne, W. M. *et al.*, 2015; Suh, *et al.* 2008; Thomas, *et al.*, 2014
Lam6/Ltc1	CG34394?	Protrudin STARD3/ STARD3NL	ER-endosome (human) ER-vacuole/ER-mitochondria (yeast)	endosomal migration, StART-like domain containing sterol-binding protein; possible role in inter-organelle sterol shuttling	loss of mammalian homologs linked to diseases of cholesterol homeostasis and breast cancer (MLN64/STARD3)	Alpy, F. *et al.*, 2013; Elbaz-Alon, Y. *et al.*, 2015; Murley, A. *et al.*, 2015
Vps13	VPS13A	VPS13A/CHAC; VPS13B/COH	vacuole-mitochondria; ER-vacuole (yeast)	unknown, gain-of-function mutants can bypass loss of ERMES function in yeast	VPS13A: chorea-acanthocytosis; VPS13B: Cohen Syndrome	Velayos-Baeza, A. *et al.*, 2004
Osh1-7	OSBPs	ORPs	ER-PM (Osh6 yeast; Orp5/8 human); ER-vacuole (Osh1)	sterol and phospholipid-binding proteins, which shuttle lipids including PS and PI4P at multiple membrane contact sites	links to defects in phosholipid and cholesterol homeostasis diseases	Olkkonen, V. M. *et al.*, 2012
	dSTIM	STIM1	ER-PM	coordinates store operated calcium entry (SOCE) at ER-PM contact sites	combined immuno-deficiency (CID) in humans, bristle development in flies	Eid, J.-P., *et al.*, 2008

outer nuclear envelope (continuous with the peripheral ER) and vacuole surface. The NVJ is principally formed by the direct interaction of the integral ER protein Nvj1, which directly binds to the vacuolar-resident protein Vac8.[6] Notably, Vac8 is not an integral membrane protein, but binds tightly to the vacuole surface including numerous post-translational palmitoylations on its N-terminus.[9] Both Nvj1 and Vac8 have no obvious mammalian orthologs. However, a second NVJ-resident ER integral membrane protein, Nvj2, has a clear ortholog in humans: Tex2/HT008,[7] although its function is unclear. Nvj2 contains two lipid-binding domains: a predicted PH domain, and a lipid-binding synaptotagmin-like-mitochondrial-lipid binding protein (SMP) domain that is present in numerous MCS-localizing proteins, including the Extended-Synaptotagmins (E-Syts).[10-12] E-Syts and their yeast homologs, the Tricalbins (Tcbs), act as tethers at ER-plasma membrane (PM) contact sites, and also interact with VAP proteins, which have been implicated in ALS disease.[13,14]

Numerous lipid-modifying and transport proteins also show enrichment at yeast NVJs. The enoyl reductase Tsc13, which catalyzes the final step of very long chain fatty acid (VLCFA) elongation, exhibits clear human homologs in the TECR protein family,[15,16] which are linked to intellectual disability. Loss of Tsc13 function affects sphingolipid synthesis, leading to decreases in mature sphingolipids and C24 ceramide. Notably, loss of Tsc13 can be rescued by reintroduction of TECR, indicating a conservation of function between yeast and man. Osh1, a member of the Oxysterol binding OSBP/ORP protein family, also enriches at NVJs in response to stationary growth stress.[8] Osh/ORP family proteins play key roles in lipid metabolism, and are implicated in the non-vesicular transport of sterols and phospholipids between organelles. Their loss may contribute to numerous familial hypercholesteraemias and lysosomal cholesterol-sphingolipid storage diseases.[17]

More recently, other proteins with clear functions in lipid metabolism have been found to localize at NVJs. Yeast Mdm1/Snx13 is an ER-resident protein with clear orthologs in all metazoans (Fig. 1). It contains a PI3P-binding Phox homology (PX) domain that binds the vacuole surface, and is thus capable of tethering the nER and vacuole at NVJs.[18] Yeast also express a soluble paralog of Mdm1, named Nvj3, which also localizes to NVJs in an Mdm1-dependent manner. Notably, Sorting Nexin 14 (Snx14), one of four human orthologs of Mdm1, was recently implicated in pediatric cerebellar ataxia and intellectual disability.[19,20] The precise function of Mdm1 and its homologs is still unknown, but intriguingly overexpression of disease-analogous Mdm1 alleles that mimic the Snx14 alleles found in *SNX14*-deficient patients perturbs yeast sphingolipid metabolism, suggesting Mdm1 may function in lipid metabolism at NVJs.[18] A role for Mdm1 family proteins in lipid metabolism is also supported by the fact that Snazurus (Snz), the *Drosophila* homolog of Mdm1, is highly expressed in the fly fat body, the lipid metabolic center of the insect.[21]

Recently, other proteins have been shown to dynamically localize to NVJs, and several of these are implicated in lipid metabolism and human disease. The *L*ipid Transfer *A*nchored at *M*embrane contact site (Lam) protein Lam6/Ltc1 localizes to both ER-mitochondrial and ER-vacuole MCSs, and is a member of the STAR-related lipid-transfer (StART) domain-containing protein family.[22-24] Notably, StART domain-containing proteins bind sterols, and several are implicated in diseases of cholesterol metabolism, including diabetes. Members of this protein family such as STARD3 may also function with Niemann-Pick proteins NPC1 and NPC2 to traffic cholesterol out of lysosomes, the failure of which causes the pathological accumulation of sterols and other lipids in lysosomal storage diseases.

Another protein, Vps13, was recently found to dynamically localize to both ER-mitochondrial and ER-vacuole MCSs.[25] When yeast are grown in glycerol-containing media, known to suppress formation of vacuole-mitochondria contact sites, Vps13 concentrates at NVJs. The reason for this localization is currently unclear, but it is tempting to speculate that Vps13 functions in lipid-exchange at these different MCSs. At least two Vps13 homologs exist in humans, VPS13A/CHAC and VPS13B/COH1, which have been implicated in chorea-acanthocytosis and Cohen Syndrome, respectively.[26] Intriguingly, both these disorders exhibit significant neurological and neuromuscular defects, as well as alterations in lipid metabolism in patients. The molecular mechanisms governing this remain obscure, however in yeast Vps13 appears to function on a parallel pathway to the ERMES complex, which controls ER-mitochondrial phospholipid flux. Vps13 alleles have been identified, which suppress the toxicity associated with growing ERMES-deficient strains on non-fermentable media, indicating that establishing alternative inter-organelle contact sites may compensate for the loss of ER-mitochondrial contact.[25] Further studies will be needed to understand the function of Vps13 and its homologs, as well as the roles of ER-endolysosomal contact sites in cellular lipid metabolism.

Thus, many yeast MCSs display clear orthologs in metazoans with roles in metabolism. Understanding their metabolic roles in metazoans is a clear priority in

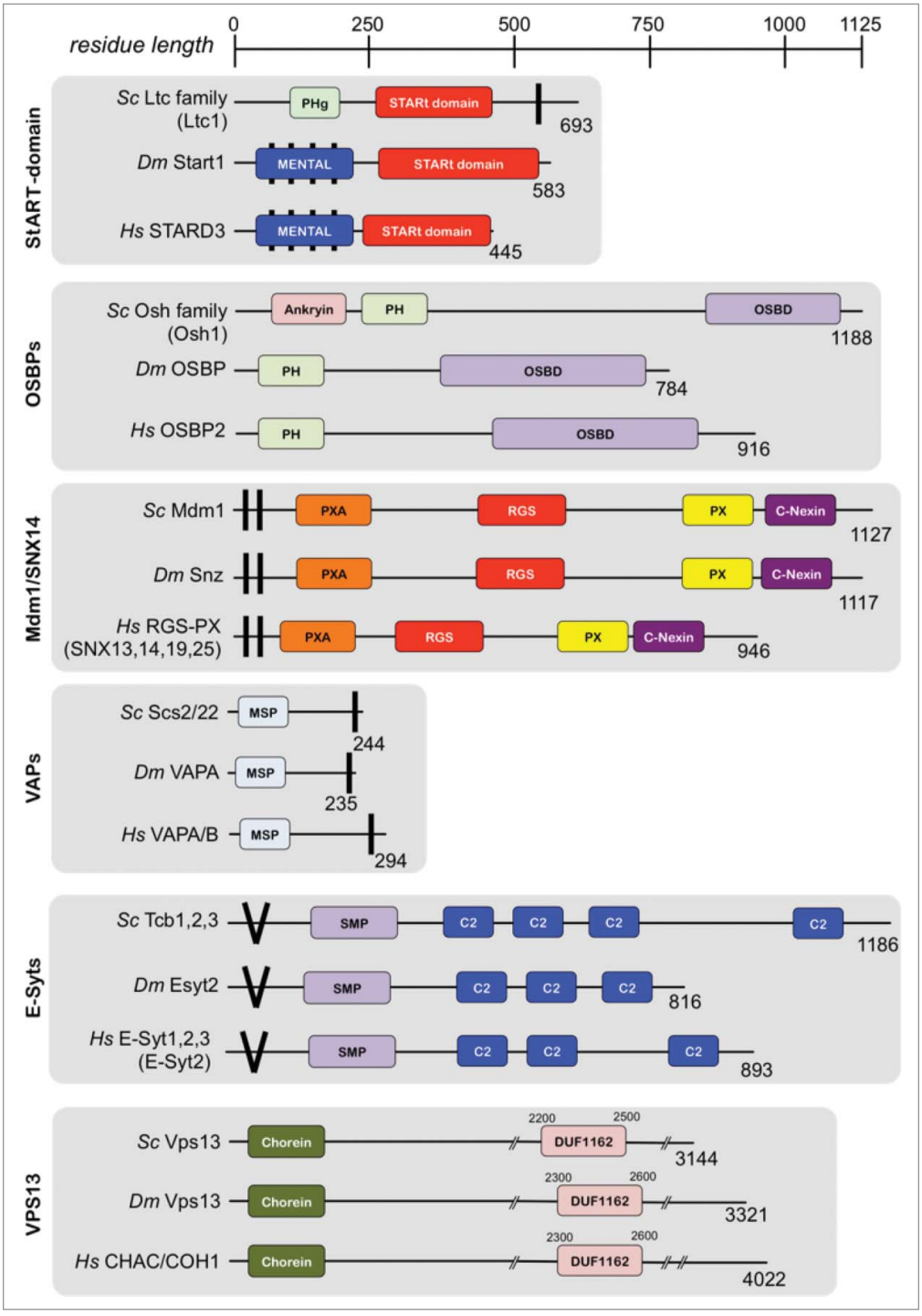

Figure 1. Domain architecture of proteins implicated in ER-endolysosomal membrane contact sites. Protein orthologs among *Saccharomyces cerevisiae* (Sc top), *Drosophila melanogaster* (Dm, middle) and *Homo sapiens* (Hs, bottom) are depicted. Abbreviations: PHg, Pleckstrin homology gram; MENTAL, MLN64 N-terminal alignment; StART, StAR-related Lipid Transfer; PH, Pleckstrin homology; OSBP, Oxysterol binding domain; PXA, PX-Associated; RGS, Regulator of G-protein Signaling; PX, Phox Homology; MSP, Major Sperm Protein; SMP, Synaptotagmin-like mitochondrial lipid-binding protein; DUF, domain of unknown function.

order to understand their connections to human disease. This review now turns to studies in the fruit fly *Drosophila melanogaster* as a potential model system to understand metazoan lipid metabolism and inter-organelle communication.

Organelle contact site proteins in *Drosophila* metabolism and aging

Numerous parallels exist between *Drosophila* and vertebrate metabolism, especially with respect to nutrient

sensing and lipid metabolic pathways. The fly genome is also remarkably well shared between both yeasts and mammals, making it an ideal model system linking unicellular and multicellular organisms. Additionally, neuropeptides, enzymes, organs, and even disease phenotypes are highly conserved from flies to humans.[27-29]

Numerous proteins involved in ER-endolysosomal MCSs in yeast are also conserved in *Drosophila*, and a few show clear disease pathologies. VAP-B/ALS8, the metazoan homolog of yeast Scs2/22 that promotes ER-PM contact sites, is linked to ALS disease in humans. Consistent with this, a fly model for ALS using mutant VAP^{P58S} manifests protein aggregation and defects in synaptic morphology.[30] Ist2, which in yeast also participates in ER-PM contact sites, is homologous to *Drosophila* Axs (Abnormal X segregation), which is ER-localized and linked to defects in chromosomal segregation during meiosis.[31] It is unclear whether this phenotype is linked to inter-organelle communication. Stim1, which couples ER-PM Ca^{2+} flux in mammals, is also highly conserved in flies and is essential for proper larval development and mechano-sensory bristle differentiation.[32,33] The yeast enoyl-reductase Tsc13 that localizes to ER-vacuole NVJs in yeast is conserved in flies as Sc2, whose loss is embryonic lethal. Mutant Sc2 alleles have also been shown to exacerbate fly models of spinocerebellar ataxia.[34]

Previous studies have identified Snazarus (Snz), the fly ortholog of yeast NVJ tether Mdm1, as a novel lifespan-associated gene by executing a fat body enhancer trap screen followed by longevity testing of the enhancer trap fly collection.[21] Snz is highly expressed in the fly fat body, the central organ for insect lipid metabolism, implying a role in fat metabolism. Consistent with this, Snz-deficient flies exhibit remarkable longevity, living approximately twice as long as their *wildtype* brethren. As lifespan is traditionally associated with alterations in metabolism and caloric intake/expenditure, it begs the question: what is the role for Snz in fly metabolism? Furthermore, how does alteration of Snz promote changes in aging and extend fly lifespan?

Metabolism and lifespan in *Drosophila*

Classic life-span studies in *Caenorhabditis elegans* implicate roles for insulin signaling in aging.[35] As in mammals, fruit fly insulin metabolism is regulated through neuroendocrine signaling. *Drosophila* secrete 8 insulin-like peptides (DILPS) that control growth, lipid and carbohydrate homeostasis, stress response, and aging.[36-39] Flies also produce a glucagon homolog, adipokinetic hormone (AKH), which regulates sugar levels in the hemolymph.[40,41] Recent studies have highlighted the significance of the minor glucose fraction in fly metabolism and its relevance to mammalian physiology.[42] Moreover, a high degree of functional conservation is observed among organ systems involved in metabolic processes, for example, the fat body is analogous to the mammalian adipose tissue and liver, storing excess energy as lipids and glycogen.[27,28,40,43]

Like vertebrates, flies mobilize their fat reserves to provide energy during conditions of starvation or stress, and regulate sugar levels in response to environmental changes.[27,40] The processes of lipolysis and lipid storage are highly conserved between mammals and flies; flies express numerous cytoplasmic lipases including adipose triglyceride lipase (ATGL) homolog, brummer lipase, and a perilipin-like protein, LSD2 (lipid storage droplet 2), which localize to the outer membrane of lipid droplets.[44-46] Lysosomal lipases are less defined, but include Lip4 which are implicated in the turnover of lipid droplets during lipophagy.[47] In addition to maintaining lipid and carbohydrate homeostasis, the fat body also secretes lipoproteins and hormones to maintain metabolic balance; for example, the fat body produces DIPL6, the closest homolog to IGF, on induction by dFOXO and starvation. Interestingly, increased DILP6 levels have been linked to increased lifespan in flies, possibly by inhibiting secretion of DILP2 from brain insulin producing cells (IPCs).[37,39,48]

Numerous studies have also established a relationship between energy homeostasis and aging in yeast, worms, flies, and mice. For example, attenuated insulin and TOR signaling, AMPK activation, and caloric restriction prolong lifespan.[28,37,49,50] Nutritional deprivation, short of malnutrition, is a potent stimulator of cellular autophagy: a process of self-degradation of cytoplasmic components by lysosomes and recycling of the catabolized products back to the cytosol to meet the nutritional demands of the cell, and to use as building blocks for new cellular components.[51,52] Defects in autophagy have been associated with accelerated aging, as aging is often characterized by accumulation of proteotoxic aggregates and dysfunctional organelles that contribute to cellular damage.[53-55] Autophagy is also involved in the breakdown of lipid droplets and triglycerides by a process called lipophagy, thereby playing a vital role in maintaining lipid homeostasis.[56] Studies in *C. elegans* have shown that up-regulation of lipid hydrolysis increases lifespan; intestinal induction of lysosomal acid lipase LIPL-4 triggers a lipid chaperone-mediated lysosome-to-nucleus longevity

pathway.[57] However, the observed longevity may not be a consequence of reduced "adiposity," as paradoxically, many long-lived mutants have increased fat storage.[58,59]

Consistent with the relationship between autophagy and aging is the observation that several lifespan-extending mutations affect fat storing tissues. In mice, targeted loss of the murine insulin receptor in adipose tissue extends lifespan.[60] Murine Sir2 may confer anti-aging effects by stimulating fat hydrolysis via repression of the master regulator of adipogenesis (PPAR-g)[61]; in the fruit fly, fat body-specific transgenic expression of Sir2 produces a similar result.[62] *Drosophila* is especially suitable to aging and longevity studies as it is possible to perform loss-of-function and gain-of-function screening, in a tissue-specific manner.[63]

Mammalian ER-endosome contact sites in health and disease

As in *Drosophila*, mammals must also organize and regulate sophisticated metabolic pathways, and numerous studies are beginning to highlight inter-organelle MCSs as hubs for this metabolic regulation. Collectively, these studies indicate that zones of ER-endolysosomal contact regulate metabolism.

Unlike the singular and stable nuclear-vacuole junction of yeast, live-cell imaging and electron microscopy indicate that mammalian ER-endosome/lysosome MCSs are highly dynamic and prevalent within cells. ER-endosome MCSs are currently thought to be relatively tight connections between the 2 membranes (10–30 nm).[64] Intriguingly, this contact begins early in endosome biogenesis, and changes throughout its maturation. As endosomes mature, the number of ER-endosome contact sites increase and it has been reported that 99% of late endosomes (LEs) form contacts with the ER.[64-66] However, the dynamics and regulation of ER–endosome contact sites still remain poorly understood.

How do mammalian cells utilize ER-endolysosomal MCSs? Numerous studies indicate that these sites play roles in endosomal fission, receptor dephosphorylation, cholesterol transfer and Ca^{2+} exchange.[67] Specific proteins have also been identified which localize to ER-endosomal MCSs. Protrudin, an ER-localized protein that regulates protrusion and neurite outgrowth in mammals (although there is no obvious fly or yeast homolog), was recently found to localize to ER-LE contact sites, where it governs endosome mobility.[68] Protrudin facilitates LE translocation to the cell periphery through recruiting kinesin-1. Notably, mutations in the Protrudin gene ZFYVE27 are associated with an inherited neurological disorder and hereditary spastic paraplegia (HSP).[69] Thus, Protrudin is also named spastic paraplegia (SPG33). Consistent with this, previous studies indicate that Protrudin interacts with numerous HSP-associated proteins. A subset of HSP patients exhibit the Protrudin^{G191V} mutant, which promotes protein aggregation and exacerbates ER stress response and abnormal ER morphology. Another ER-endosome MCS protein is PTP1B, an ER-localized protein tyrosine phosphatase that interacts with the epidermal growth factor receptor (EGFR) at ER-endosome MCSs,[70] has been reported as both an oncogene and tumor suppressor in different cancer types,[71] and has also been implicated in metabolic disease.[72]

Numerous sterol-binding proteins have also been implicated in ER-endolysosomal lipid metabolism and trafficking. The STARD3 StAR (steroidogenic acute regulatory protein) related lipid transfer (START) domain-3, previously known as MLN64 (metastatic lymph node 64),[73] and STARD3NL (STARD3 N-terminal like), previously known as MENTHO[74] proteins, are components of ER-LE MCS bridging complexes.[75] Tichauer et al. reported that overexpression of STARD3 in mice promotes liver damage,[76] and studies also found that high-grade prostate cancer and HER2-positive breast cancer are associated with STARD3.[77,78] The *Drosophila* STARD3 homolog, Start1, is linked to insect egg development. Research also revealed that bone mineral density patients have single-nucleotide polymorphisms of STARD3NL.[79]

Lipid flux at inter-organelle contact sites in human health and disease

Several studies collectively implicate inter-organelle MCSs in non-vesicular lipid transport.[80,81] An increasing number of proteins are being identified as "tethers" that bring together membranes of opposing organelles into close proximity (<30 nm).[82,83] In addition to sustaining the integrity of the MCS, these "tethers" may function in shuffling lipids between nearby membranes. Major efforts in the field have been aimed at distinguishing between these two functions.[81] Alternatively, MCS "tethers" may recruit soluble effector molecules known as lipid transfer proteins (LTPs) that mediate lipid transfer between organelles.[84,85] Therefore, the closely juxtaposed MCSs provide a convenient center for both spontaneous and LTP-mediated non-vesicular lipid transport.[86,87]

Based on their specificity to different lipids, well-defined LTPs can be grouped into three main categories: (1) sphingolipid-, (2) sterol- and (3) phospholipid-transfer proteins.[86] The crystal structures of several LTPs have been resolved both in the presence or absence of their

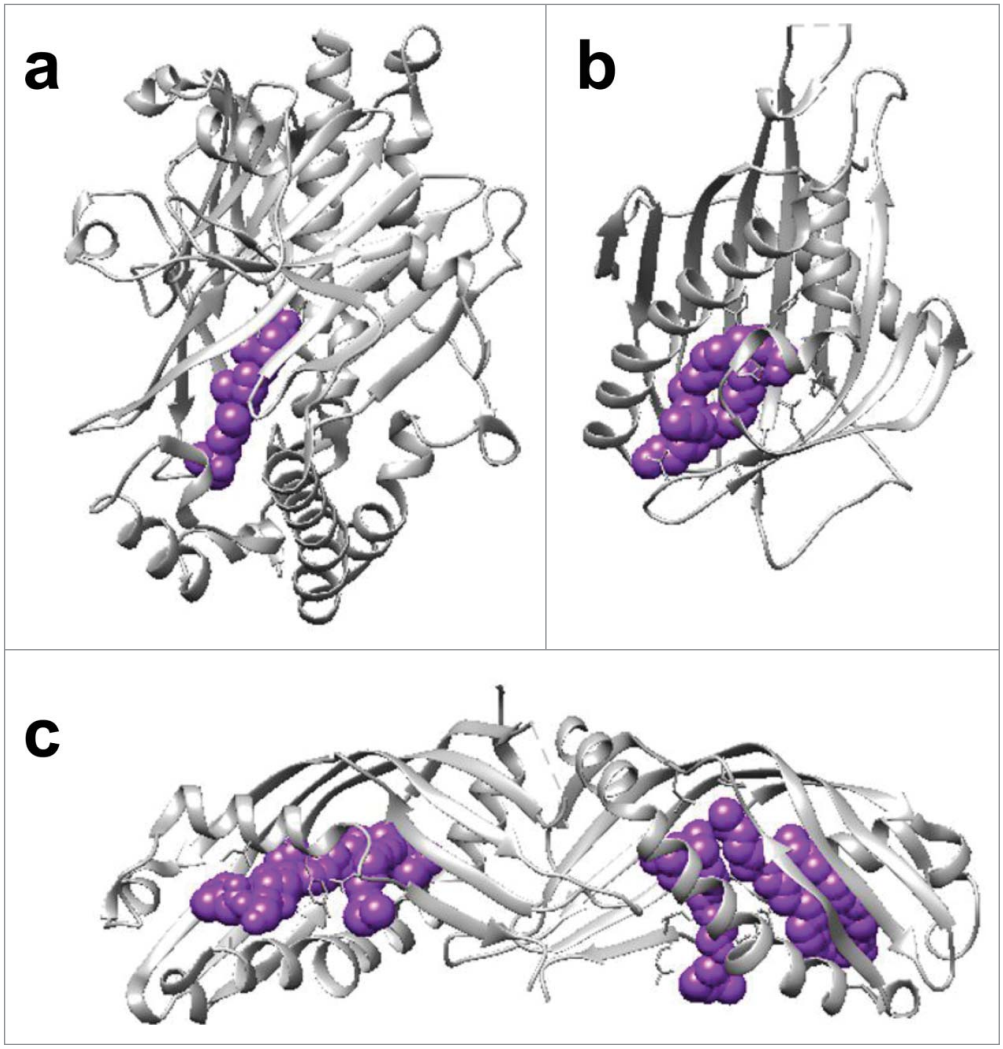

Figure 2. Three-dimensional structure of LTPs showing the protein backbone (gray ribbon representation) and the bound lipid (purple sphere representation) (a) The crystal structure of the yeast oxysterol-related domain ORD of oxysterol binding protein (OSBP) homolog 4 (Osh4) in complex with 25-hydroxycholesterol (PDB: 1ZHX). (b) The crystal structure of CERT (STAR)-related transfer (STARt) domain in complex with C16-ceramide (PDB: 2E3P). (c) The crystal structure of extended-synaptotagmin 2 (E-syt2) SMP domain in complex with TritonX-100 and DOPE (PDB: 4P42).

lipid-binding ligands, which aid in understanding their mechanism of action (Fig. 2).[85,86] In general, LTPs bind individual lipids in a hydrophobic pocket or tunnel formed within the tertiary fold of the protein. In addition to the core hydrophobic pocket, most LTPs also contain protein-binding and/or lipid-binding domains that allow them to directly interact with membranes that donate and/or accept lipids. Additionally, the interaction of some LTPs with membranes is thought to induce conformational changes which expose the lipid-binding pocket within the LTP. The physical and chemical properties of this hydrophobic pocket dictate the lipid-binding specificity and affinity of different LTPs.[86] Typically, a lid-like domain shields the transferred lipids and acts as a gate during lipid exchange.[88,89]

Several LTPs have been directly implicated in lipid metabolism at the MCSs (Table 1). LTPs are classically defined by their ability to facilitate lipid transfer between membranes *in vitro*.[90,91] However, in many cases, it remains unclear whether LTPs can also shuttle lipids between membranes *in vivo*.[85] One alternative hypothesis is that LTPs may function as lipid sensors or even lipid chaperones that localize to MCSs to escort or "present" specific lipids to metabolic enzymes to be processed.[85] So far, only a few studies have demonstrated *in vivo* lipid transfer activities by LTPs.[85,92] One example is the ceramide-transfer protein CERT which mediates the transport of various molecular species of ceramides between the ER and Golgi.[93-95] CERTs are cytosolic proteins that consist of an N-terminal

pleckstrin homology (PH) domain, an FFAT motif (two Phe residues in an acidic tract),[96] and a C-terminal START domain (Fig. 1). The START domain is sufficient to mediate ceramide transfer *in vivo*.[97] The crystal structure of CERT START domain has been solved both in the apo-form and in complex with different ceramides providing structural insights into the mechanism by which it recognizes different ceramide species.[98]

CERT. CERTs have been reported to function at the ER-Golgi MCSs,[93] and mediate the transfer of ceramides from their site of synthesis in the ER to the Golgi complex where they are converted to sphingomyelin by sphingomyelin synthase (SMS).[97,99] The interaction with the ER is mediated by the CERTs FFAT motif, which binds to the VAMP-associated proteins (VAPA and VAPB), while the PH domain of CERT mediates the interaction with the Golgi complex by binding PtdIns4P.[100,101] Mutations in either the FFAT motif or the PH domain not only abrogate the ER-Golgi MCS localization of CERTs, but also inhibit ceramide transport and consequently sphingolipid synthesis.[97,100] This suggests that the spatial restriction of CERT-mediated ceramide transfer at the ER-Golgi MCSs is required for sphingolipid metabolism.[86]

FAPP2. Another example of LTPs is FAPP2, which was shown to transfer glycosylated ceramides (GlcCer) *in vivo*.[102] FAPP2 have similar domain organization as CERT and contains an N-terminal PH domain and a C-terminal glycolipid transfer protein or GLTP-homology domain.[102] The crystal structure of GLTP has been solved both in the presence and absence of the bound ligand, which provides molecular insights into the specificity of glycolipid binding and the mechanism of transfer.[103] The amino acid residues that are required for GLTP-mediated glycolipid transfer are highly conserved in FAPP2.[102,103] FAPP2 has been demonstrated to promote non-vesicular transfer of GlcCer between the Golgi compartments[102] as well as from the Golgi to the ER.[104] GlcCer transport to the ER lumen was reported to be significantly reduced due to the knockdown of FAPP2, which also severely impaired glycosphingolipids (GSL) metabolism.[104]

Nir2. Nir2 is an example of a phosphoinositol transfer protein (PITP). PITPs were shown to transfer phosphatidylcholine (PtdCho) and phosphatidylioinositol (PtdIns) *in vitro*.[105] In cells, lipid transfer activity of PITPs was demonstrated using the *Drosophila* PITP DrdgBα. The levels of PtdIns(4,5)P_2 was found to be significantly reduced in the DrdgBα mutants cells indicating that DrdgBα may play a role in moving PtnIns from their site of synthesis in the ER to the PM for local conversion to PtdIns(4,5)P_2.[106,107] Similar to CERT, Nir2 contains an FFAT motif, which mediates its interaction with the ER. Nir2 also contains an N-terminal PITP domain, which binds lipids. Structural analysis of two human PITPs (PITPα and β)[108,109] and the yeast PITP Sec14p[110,111] and its closest homolog Sfh1[112] provided insights into the mechanism of lipid exchange by PITPs.

Oxysterol binding proteins (OSBP/ORP). Oxysterol binding proteins (OSBPs) bind to phospholipids and sterols, and often contain a lipid binding PH domain and a FFAT motif, which mediate dual interactions with lipid and protein, respectively. Additionally, mammalian OSBP has a C-terminal oxysterol-related domain (ORD), which binds 25-hydroxycholesterol (25-OH). OSBP is a member of a bigger family of sterol-binding proteins which includes[11] OSBP-related proteins (ORPs). The yeast OSBP ortholog Osh1 localizes to the nuclear vacuole junction (NVJ) by binding to Nvj1.[8] The crystal structure of another yeast ortholog Osh4 has been solved,[89] and the mechanism of sterol transfer has been demonstrated *in vivo*.[113] In addition, more recent work has demonstrated that Osh/ORP proteins can exchange phospholipids including phosphatidylserine (PS) and PtdIns4P between the ER and plasma membrane, and do so at ER-PM contact sites.[89]

SMP domain-containing proteins. The synaptotagmin-like mitochondrial-lipid-binding protein (SMP) domain is found in several proteins that localize to inter-organelle contact sites such as the ERMES complex (ER-mitochondria) and the extended synaptotagmins (E-Syts), known in yeast as tricalbins (ER-PM).[10-12] SMP domains are found to bind several different lipids,[12] and they are proposed to transfer lipids between organelles at membrane contact sites, although the molecular mechanisms governing this require more study.

Lipid exchange at MCSs may plays an important role in lipid metabolism. Abnormal lipid metabolism can lead to several life-threatening conditions including obesity, diabetes, and heart disease. Moreover, genetic defects in several LTPs were found to have severe consequences on lipid metabolic pathways, which underlines the significance of these proteins in human health and development (Table 1). One of the well-studied examples of diseases associated with lipid metabolism is the Neimann-Pick Disease.

As mentioned in the previous section, two recent studied have implicated Snx14, the human homolog of the yeast MCS protein Mdm1, in a neurological disease with hallmarks of lipid storage diseases.[19,20] Cells derived from patients showed enlarged lysosomes and accumulation of cellular debris[20] which may implicate Snx14 in the turnover of lysosomal lipids. Finally, few diseases have been directly linked to defects in MCS "tethering" proteins suggesting that these contact sites are important locations for inter-organelle communication. A mutation

in VAPB, which may reduce the level of ER anchoring of lipid-binding proteins, has been linked to motor-neuron degeneration in amyotrophic lateral sclerosis (ALS) (Table 1).[114]

Conclusions and perspectives

In conclusion, the study of inter-organelle contact sites has transformed from a curiosity of electron microscopy to a source of new understanding in the fields of lipid metabolism, nutrient exchange, signaling, and organelle identity. The discovery of key proteins and protein complexes which mediate inter-organelle tethering, or localize to MCSs for their functions, has been instrumental in the understanding of membrane contact sites in general cell biology. Most notably, it is now appreciated that key proteins with essential roles in human physiology appear to utilize MCSs for their functions. Loss of these proteins contributes to major genetic or acquired diseases, thus prompting the need to further understand these proteins, as well as how sites of inter-organelle contact are maintained, regulated, and integrate into general cellular physiology and pathophysiology. Future studies will no doubt continue to identify new and exciting proteins and pathways that utilize inter-organelle membrane contact sites.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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