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REVIEW ESSAY

Controlling contacts—Molecular mechanisms to regulate organelle membrane tethering

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Abstract

In recent years, membrane contact sites (MCS), which mediate interactions between virtually all subcellular organelles, have been extensively characterized and shown to be essential for intracellular communication. In this review essay, we focus on an emerging topic: the regulation of MCS. Focusing on the tether proteins themselves, we discuss some of the known mechanisms which can control organelle tethering events and identify apparent common regulatory hubs, such as the VAP interface at the endoplasmic reticulum (ER). We also highlight several currently hypothetical concepts, including the idea of tether oligomerization and redox regulation playing a role in MCS formation. We identify gaps in our current understanding, such as the identity of the majority of kinases/phosphatases involved in tether modification and conclude that a holistic approach—incorporating the formation of multiple MCS, regulated by interconnected regulatory modulators—may be required to fully appreciate the true complexity of these fascinating intracellular communication systems.

KEYWORDS
FFAT motif, membrane contact sites, oligomerization, organelle interactions, phosphorylation, redox-related modifications, ubiquitination

Abbreviations: ACBD, acyl-coenzyme A-binding domain-containing protein; AMPK, AMP-activated protein kinase; AQP11, aquaporin 11; CERT, Ceramide transfer protein; CKIγ2, casein kinase I γ2; ER, endoplasmic reticulum; ERAD, ER-associated protein degradation; ERMES, ER-mitochondria encounter structure; ERO1α, endoplasmic oxidoreductin-1-like protein α; FFAT, two phenylalanines (FF) in an acidic tract; FFNT, two phenylalanines (FF) in a neutral tract; GRP75, glucose-regulated protein 75; GSK3β, glycogen synthase kinase 3β; IncV, inclusion membrane protein; IP_3R, inositol 1,4,5-trisphosphate receptor; Kv2, voltage-gated potassium channel; Laf1, Lam2/Ltc4-Associated Factor; Lam2, Lipid transfer protein Anchored at a Membrane contact site 2; MAM, mitochondria-associated membranes; MCS, membrane contact site; MFN2, mitofusin-2; MIGA2, mitoguardin 2; MIRO, mitochondrial Rho GTPase; MITOL, mitochondrial ubiquitin ligase; MOSPD, motile sperm domain-containing protein; MSP, major sperm protein; ORD, oxysterol-binding protein-related domain; ORP3, oxysterol-binding protein-related protein 3; OSBP, oxysterol-binding protein; PDK4, pyruvate dehydrogenase kinases 4; PEX, peroxisomal biogenesis factor; PH, pleckstrin homology domain; PINK1, PTEN-induced putative kinase protein 1; PKC/D, protein kinase C/D; PLK, polo kinase; PP2Cε, protein phosphatase 2Cε; PTPIP51, protein tyrosine phosphatase-interacting protein 51; RNF26, RING finger protein 26; ROS, reactive oxygen species; SMP, synaptotagmin-like mitochondrial lipid-binding protein; SQSTM1, sequestosome-1; STARD3, StAR-related lipid transfer protein 3; TMEM24, transmembrane protein 24; TPR, tetratricopeptide repeat; UBE2J1, ubiquitin-conjugating enzyme E2 J1; VAP, vesicle-associated membrane protein (VAMP)-associated protein; VDAC1, voltage-dependent anion channel protein 1; VPS13D, Vacuolar protein sorting-associated protein 13D; Vps39, vacuolar protein sorting-associated protein; Ypk1, yeast protein kinase 1; Ypt7, yeast protein transport 7.

Joseph L. Costello and Michael Schrader contributed equally to this paper.

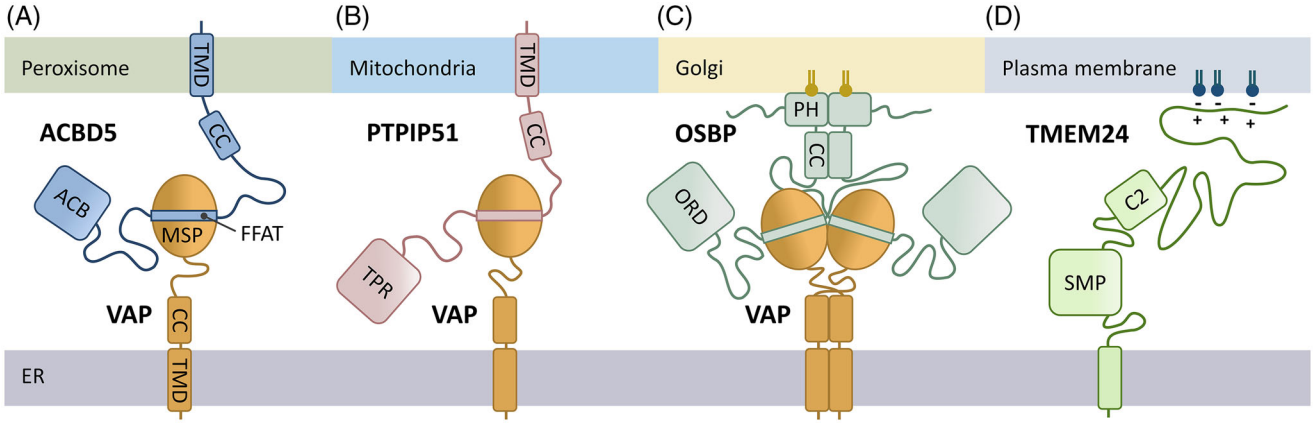

FIGURE 1 **The diversity of tether proteins at MCS. (A)** Peroxisomal membrane protein ACBD5 binds via its FFAT motif to the MSP domain of ER membrane protein VAP, mediating peroxisome-ER MCS. The ACB domain of ACBD5 has high affinity for very long chain fatty acids. **(B)** Mitochondrial membrane protein PTPIP51 also binds to VAP via its FFAT motif, mediating mitochondria-ER MCS. The TPR domain of PTPIP51 binds and transfers phosphatidic acid. **(C)** OSBP binds via a PH domain to lipids of the Golgi membrane and contains a FFAT motif that binds to VAP, mediating Golgi-ER MCS. OSBP has an N-terminal disordered tail that prevents protein crowding around the PH domain. The ORD domain of OSBP transfers cholesterol. Many tether proteins contain a coiled coil (CC) domain, which can mediate di-/oligomerization of the proteins. **(D)** TMEM24 is an ER membrane protein and binds directly to the negatively charged cytosolic leaflet of the plasma membrane via its positively charged C-terminal region, mediating plasma membrane-ER MCS. The SMP domain transports phosphatidylinositol.

INTRODUCTION

A hallmark of eukaryotic cells is the presence of membrane-bound compartments called organelles, which create distinct optimized micro-environments to promote a variety of metabolic reactions required to sustain life. Organelles do not work as isolated entities; for the entire cell to function as a unit, coordination and cooperation between specialized organelles must take place. This cooperation often requires inter-organellar membrane contacts, whereby two (or more) organelles come into close apposition (10–30 nm).[1–3] Membrane contact sites (MCS) between organelles are mediated by tether proteins and have now been described for most, if not all, organelles including mitochondria, the endoplasmic reticulum (ER), Golgi complex, endosomes, lysosomes, peroxisomes, lipid droplets and the plasma membrane.[4–10] Tether proteins are usually membrane proteins, which can bridge two organelles through binding another tether protein or directly to membrane lipids on the opposing membrane (Figure 1). Besides the molecular tethers, other proteins associated with MCS are involved in the transfer of small molecules (e.g., ions and lipids), as well as regulatory components. Tether proteins themselves can also have additional functions (e.g., in lipid transfer) and localize to multiple MCS. General physiological functions of MCS include metabolic roles in the channelling of metabolites, ion homeostasis, and signalling functions, but also membrane lipid exchange, and roles in organelle biogenesis and dynamics such as organelle positioning, transport and inheritance.[11] There is increasing evidence now that MCS are central to cell physiology and impact on human health and disease.[12–16] The research field of MCS has rapidly expanded, with new MCS, tethers and MCS-resident proteins being discovered, and focus is now on the diverse functions of organelle contacts and their physiological importance. Individual organelle contacts appear to be dynamic,[17] suggesting that protein tethers between organelles are highly regulated. There is a need to regulate MCS, for example, during cell division,[18] to allow mobility and relocation of organelles, to adapt to changing metabolic needs of the cell or to respond to cellular stress. However, there is a gap in our knowledge about how the majority of MCS are regulated. Here, we will discuss molecular mechanisms regulating MCS. We will focus on mechanistic principles, and support those with selected examples, mainly addressing the role of direct alterations of the tether proteins, including phosphorylation, ubiquitination/degradation, redox-related modifications, and protein oligomerization in the modulation of MCS.

Regulation of MCS by tether abundance

The function of a tether can be considered to be to physically position organelle membranes in close proximity to generate an MCS. Accordingly, overexpression or loss of tether proteins can often increase or reduce MCS formation. This is exemplified by the manipulation of peroxisomal ACBD5 or mitochondrial PTPIP51, which are involved in the formation of peroxisome-ER and mitochondria-ER contacts, respectively (Figure 1A,B). When ACBD5 or PTPIP51 are co-expressed with the ER tether protein VAPB in mammalian cells, the number of peroxisome-ER or mitochondria-ER contacts increase.[19–21] Conversely, loss of ACBD5 or PTPIP51 reduces both the size and number of peroxisome-ER or mitochondria-ER contacts. Therefore, differential expression of tether proteins is clearly a potential way to regulate MCS formation (Figure 2A). Changes in tether abundance could be achieved at the level of gene expression, including tissue- or developmental stage-specific expression, or by changes in protein turnover (see

Box 1: Post-translational modifications of MCS components

1. **Phosphorylation** of proteins allows for a rapid response to changes in the (intra)cellular environment. The reversibility and speed of the addition or removal of a negatively-charged phosphate group (PO_4^{3-}) to an amino acid residue—serine, threonine or tyrosine—makes the event suitable for dynamic regulation of MCS. Phosphorylation and its counterpart dephosphorylation are catalysed by kinases and phosphatases, respectively, regulating protein localization, function and binding partners by changing the shape and charge of the modified protein. Kinases and phosphatases themselves are also closely regulated, allowing a tight coordination of specific MCS components.
2. **Ubiquitination** is the addition of the 76-amino acid protein ubiquitin to target proteins. This can lead to altered subcellular localization, enzymatic activity and protein interactions. Addition of ubiquitin is often on lysine residues and the initial ubiquitin molecule can also be further ubiquitinated, allowing formation of different ubiquitin chains. The most well characterized outcome for polyubiquitinated proteins is that they are degraded by the proteasome. Ubiquitination involves activation and conjugation of the ubiquitin molecule via E1 and E2 enzymes, and subsequent ligation to the target proteins by E3 ligases. This process is reversible and can be opposed by the action of specific deubiquitinases.
3. **Reactive oxygen species (ROS) modulation** of proteins at responsive residues (e.g., reactive cysteines) in proteins can either be a reversible or irreversible post-translational modification depending on the localized intracellular concentration of ROS. Reversible modifications include inter-molecular disulphide linkages (homo- or hetero-dimerization) which cause conformational changes, sulfenylation or S-glutathionylation. Irreversible modifications such as sufinylation or sufonylation mostly occur as stress responses leading to permanent protein conformation changes. H_2O_2 mediated modifications are perhaps the most widely studied with respect to post-translational modifications of proteins.

Section: **Regulation of MCS by ubiquitination**) in response to changes in cellular environment.

Whilst some MCS, such as the mitochondria-ER-cortex anchor (MECA) in yeast, may just involve one or two tethering complexes, in many cases considerable redundancy in tethering complexes exists.[3] This suggests that altered abundance of individual tethers may not be sufficient to exert gross physical changes on MCS, depending on

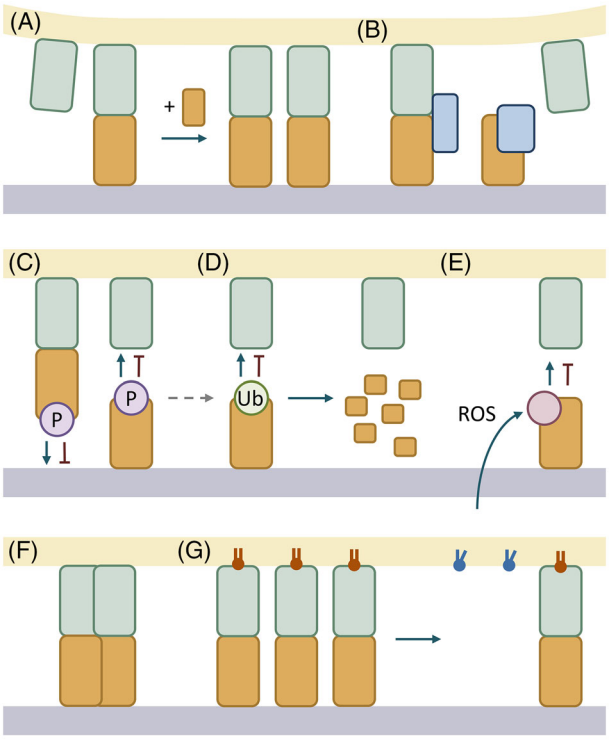

FIGURE 2 **Mechanisms to regulate organelle membrane tethering.** **(A)** Regulation of MCS formation by differential expression of a tether protein. **(B)** Altered abundance of a protein that directly interacts with a tether protein controls the assembly/disassembly of the MCS tethering complex. **(C)** Phosphorylation of a tether protein regulates MCS by altering membrane association or complex stability. Phosphorylation can also induce ubiquitination of the tether protein. **(D)** Ubiquitination of a tether protein induces its degradation, consequently reducing MCS, or alters tether protein function, leading to alterations in the MCS. **(E)** Redox regulation at MCS via ROS-mediated modifications of a tether protein. **(F)** Oligomerization of tether proteins changes the membrane tethering property. **(G)** Changes in the lipid composition of the organelle membrane alters the anchoring of a tether protein, and hence, the assembly of the MCS. The green and orange squares represent tether proteins, and the yellow and purple regions represent membranes of different organelles.

the contribution a particular protein makes to the overall tethering process. For example, at least six different tethering components contribute to the yeast plasma membrane-ER MCS.[22] In this case, to achieve significant alterations in organelle connections by changing tethering abundance may require modulating multiple proteins simultaneously and to our knowledge there are currently no clear examples of this in the literature. Rather, subtle alterations to tethering which might mediate formation of specific sub-complexes or alter flux of product transfer may occur when expression of a single tether is altered.

An example of a tissue/developmental stage-specific tether is mammalian TMEM24, an ER-anchored phosphatidylinositol transporter at plasma membrane-ER MCS (Figure 1D), which is highly expressed in the brain and in pancreatic islets. TMEM24 is expressed predominantly by neurons and expression increases with postnatal development,

suggesting enhanced TMEM24 tethering as overexpression of the protein increased both the number and length of plasma membrane-ER MCS in mature neurons and HeLa cells.[23,24] An example of a tether whose abundance is regulated in response to the cellular environment is *Saccharomyces cerevisiae* Cnm1, a molecular tether on the nuclear membrane, which interacts with Tom70 on mitochondria. Cnm1 levels are regulated by phosphatidylcholine levels, thus affecting mitochondrial clustering around the nucleus.[25] The mechanistic details of how expression of these tethers is controlled are not yet clear, but these examples provide evidence that regulating tether abundance may be a common strategy to regulate MCS. Another interesting concept is whether increasing tether abundance creates more points of contact between organelles, stabilises/enlarges pre-existing complexes or adjusts the distance between organelle membranes. Recent work suggests that the type of MCS may have an impact on the exchange events which can occur.[26,27]

Regulation of tether assembly by associated proteins

Another strategy by which altered protein expression may alter tethering is more indirect—by increasing levels of associated interaction partners which can modulate tether assembly (Figure 2B). Recently, α-synuclein, a protein strongly linked to Parkinson's disease, has been detected at mitochondria-associated ER membranes (MAM), a subdomain of the ER which controls mitochondria-ER interactions.[28] Interestingly, α-synuclein binds to VAPB, which disrupts the PTPIP51-VAPB interaction and loosens mitochondria-ER contacts. The loss of mitochondria-ER contacts disrupts IP_3 receptor-mediated delivery of calcium (Ca^{2+}) from ER stores to mitochondria and therefore alters mitochondrial ATP production.[28] In vitro binding studies indicate that α-synuclein interacts with the N-terminal MSP domain of VAPB (see Figure 1A-C). The exact function of α-synuclein is not clear, but increased levels appear to disrupt mitochondria-ER contacts by sterically interfering with the PTPIP51-VAPB tether. A second example of how altered expression of an associated protein could impact on tether assembly is the Emr1 protein in fission yeast. Emr1 interacts with components of the ER-mitochondria encounter structure (ERMES) and Emr1 levels correlate with the number of ERMES foci, suggesting a role for Emr1 in controlling assembly of the subunits of this tethering complex.[29] These examples illustrate that an altered abundance of proteins that directly interact with tether proteins can negatively or positively impact on MCS by regulating tether formation. Beyond controlling the assembly/disassembly of tethering complexes, future work may identify if tether-associated proteins can also promote formation of alternative tethering complexes for different functions.

Regulation of MCS by phosphorylation

As phosphorylation is one of the best-characterized post-translational modifications, it is likely that many, if not all, MCS complexes are in some way impacted by phosphorylation (**Box 1**). We provide here recent examples of how phosphorylation events can alter complex stability, targeting and membrane association, illustrating some of the effects that phosphorylation can have on MCS proteins (Figure 2C).

Phosphorylation of FFAT-containing proteins

The VAP protein family facilitates many ER-organelle contacts and is highly conserved between species. The ER-membrane resident family consists in mammals of VAPA, VAPB and the recently identified MOSPD1, MOSPD2, and MOSPD3.[30,31] They possess a MSP domain with which they bind to "two phenylalanines (FF) in an Acidic Tract" (FFAT) motifs, present in a variety of proteins, such as ACBD5 (peroxisomes), PTPIP51 (mitochondria) and OSBP (Golgi) (Figure 1A-C; for an overview of confirmed binding partners see[16]). VAP binding partners often reside at an opposing organelle membrane, serving as a bridge between the ER/VAP and the heterotypic membrane. Hence, altering VAP protein levels or modifying VAP by phosphorylation could impact on many binding partners, and thus MCS, simultaneously, while altering individual FFAT-containing proteins could allow for specific regulation. Below, we focus on the regulation of VAP binding by phosphorylation of the FFAT motif and how this can regulate MCS.

The multitude of diverse proteins that contain a FFAT motif signifies competitive binding to the VAP family members. Vice versa, there might also be competition between the different VAP proteins for binding to a FFAT motif-containing protein. The FFAT motif consists of the core consensus sequence ^{1}EFFDA-E^{7} flanked by acidic residues, but the residues can vary quite considerably, potentially giving proteins different binding affinities for VAP. Additionally, members of the VAP family prefer slightly different motifs: MOSPD1 and MOSPD3 favour "two phenylalanines (FF) in a Neutral Tract" (FFNT), and although MOSPD2 is more comparable to VAPA/VAPB in terms of its FFAT binding, there are some differences in the FFAT binding interface of their MSP domains.[31,32] Besides different affinities of FFAT motif-containing proteins to different VAPs, there are several regulatory mechanisms involving phosphorylation of serine/threonine residues present in the FFAT motif of various proteins that can alter VAP binding (detailed examples are given in Table 1). (1) Phosphorylation of serine/threonine residues in the acidic tract increases the overall negative charge, enhancing the interaction of the tract to the positively charged surface of the VAP MSP domain. This binding of the tract is important for facilitating the initial electrostatic interaction and phosphorylation potentially acts as a fine-tuning mechanism.[32–34] It is also possible that phosphorylation of residues in the acidic tract could convert a FFNT motif to a FFAT motif, changing its affinity for different VAP members. (2) Phosphorylation of serine/threonine in position 1 of the FFAT core can enhance the binding of the motif to the MSP domain.[35–37] (3) Phosphorylation of serine/threonine in position 4 of the FFAT core acts as an OFF-ON switch. Phosphorylation of this residue is essential for binding to the MSP domain of VAP—e.g., phosphorylation in position 4 of the STARD3 FFAT peptide altered the FFAT-VAP association from no detectable binding to a binding affinity in the micromolar range—and this motif is defined as

TABLE 1 Regulation of VAP binding by FFAT motif phosphorylation in mammalian cells

	Acidic tract (AT)						FFAT core									Effect on VAP binding	Crosstalk	Function	Ref
							1	2	3	4	5	6	7						
CERT	**S**	L	N	I	E	E	E	F	F	D	A	V	E	A	A	pS_{AT} enhances	Dephosphorylation upstream of FFAT increases pS_{AT} (PKD, CKIγ2, PP2Cε)	At Golgi-ER contacts, transfers ceramide for the synthesis of sphingomyelin	[33,110–113]
Kv2.1	**S**	M	**S**	**S**	I	D	**S**	F	I	**S**	C	A	**T**	D	F	pS_1 enhances, pS_4 essential (OFF-ON)		At plasma membrane-ER contacts, regulates Kv2 clustering	[32,35]
Kv2.2	**S**	**T**	**S**	**S**	I	D	**S**	F	**T**	**S**	C	A	**T**	D	F	pS_1 enhances, pS_4 essential (OFF-ON)		At plasma membrane-ER contacts, regulates Kv2 clustering	[32,35]
MIGA2	**S**	L	**T**	**S**	E	D	**S**	F	F	**S**	A	**T**	E	L	F	pS_1 enhances, pS_4 essential (OFF-ON)		At mitochondria-ER contacts, transfers phospholipids	[32,37]
STARD3	G	A	L	**S**	E	G	Q	F	Y	**S**	P	P	E	**S**	F	pS_{AT} enhances, pS_4 essential (OFF-ON)		At endosome-ER contacts, transfers sterol	[32]
Chlamydia IncV	E	**S**	**S**	**S**	**S**	**S**	**S**	F	H	**T**	P	P	N	**S**	D	pS_{AT} and pT_4 enhances	C-terminal hyperphosphorylation (CK2) induces pT_4	Tethers the bacterial *Chlamydia trachomatis* inclusion to host ER	[114]
ACBD4	R	D	L	D	**S**	E	V	F	C	D	**S**	L	E	Q	L	'Extended AT' enhances, pS_5 blocks (ON-OFF)		Tethers peroxisomes to the ER	[34]
ACBD5	**S**	D	**S**	D	**S**	E	V	Y	C	D	**S**	M	E	Q	F	pS_{AT} enhances, pS_5 blocks (GSK3β, ON-OFF)	AT dephosphorylation reduces pS_5	Tethers peroxisomes to the ER, important for membrane lipid transfer and motility	[34]
OSBP	D	E	D	D	E	N	E	F	F	D	A	P	E	I	I		Multisite (de)phosphorylation outside the FFAT region regulates VAP binding	At Golgi/endosome/lysosome-ER contacts, transfers cholesterol	[115]
ORP3	I	**T**	D	S	L	S	E	F	F	D	A	Q	E	V	L		Hyperphosphorylation (PKC) controls VAP	At plasma membrane-ER contacts, transfers phospholipids	[116,117]

Note: Highlighted residues have been shown to affect VAP binding, based on studies using peptides/proteins, and phosphomimetic/non-phosphorylatable and/or phosphorylated residues. Phosphorylation of light green residues enhances VAP binding; of dark green residues is essential for VAP binding; and of red residues blocks VAPB binding. Serine and threonine residues, which are potential phosphorylation sites, are shown in bold. Kinases and phosphatases linked to the mentioned phosphorylation events are underlined.

"Phospho-FFAT."[32,35,37,38] (4) Phosphorylation of serine/threonine in position 5 of the FFAT core acts, in contrast, as an ON-OFF switch: ACBD5 phosphorylated in FFAT position 5 was highly enriched in the non-bound fraction in a VAPB-pull down assay, indicating that phosphorylation blocks the binding of this residue in its hydrophobic pocket, likely via steric hindrance.[34,39] Overall, FFAT motif phosphorylation affects VAP binding in various ways—leading to an increase or decrease, an adjustment or switch—dependent on the position of the residue. A serine/threonine is also a common residue in position 7 of the FFAT motif, and phosphorylation will likely increase the binding to VAP, as this mimics the canonical glutamic acid residue.[40] Interestingly, all FFAT motifs possess a phenylalanine or tyrosine in position 2, and some in position 3, of which the tyrosine residue could be a potential target of phosphorylation, suggesting an additional regulation mechanism. Many FFAT-containing proteins possess serine/threonine residues in their FFAT motif (Table 1) and could potentially be regulated by one or more of these phosphorylation mechanisms.

One obvious knowledge gap in this field is the identification of kinases and phosphatases acting on the FFAT motifs. Recently, we showed that the kinase GSK3β phosphorylates the FFAT motif of peroxisomal membrane protein ACBD5.[34] Phosphorylation of ACBD5 in position 5 of the FFAT motif by GSK3β blocks its interaction with VAP, reducing peroxisome-ER contact size and altering peroxisome membrane dynamics.[34] Interestingly, GSK3β also negatively regulates PTPIP51-VAP-mediated mitochondria-ER contacts,[41,42] suggesting potential co-regulation between the organelle-ER contacts. Furthermore, the affinity of FFAT-containing proteins for VAPs can be dependent on a combination of multiple (non)phosphorylated residues/regions which appear to be able to crosstalk in their regulation of protein interaction and function (Table 1). Elucidating how differentially regulated FFAT motifs compete for binding with different VAP proteins to control access to the ER membrane will be a challenging task but promises to reveal novel insights into how phosphorylation of simple, short motifs has the potential to orchestrate complex cellular events.

Phosphorylation of other MCS proteins

Beyond the VAP system for ER MCS, several other phosphorylation mechanisms regulating MCS have been identified. The kinases AMPK and PDK4 promote mitochondria-ER contact formation in mammals via association with different tether complexes and enhance their stabilization, although the mechanisms are not completely clear. AMPK translocates to mitochondria-ER MCS under energy stress conditions, where it binds to and induces phosphorylation of MFN2, which acts as a mitochondria-ER tether by being present at both organelle membranes and forming dimers.[43] However, it has not been shown if MFN2 phosphorylation directly enhances the MFN2 tethering function, and hence, is accountable for the increased number of MAMs by AMPK. PDK4 associates with and stabilises the IP_3R-GRP75-VDAC1 Ca^{2+} channelling complex, stimulating tight MAM formation and Ca^{2+} transport from the ER to mitochondria.[44] Additional kinases and phosphatases associated with the IP_3R-GRP75-VDAC1 complex and other mitochondrial MCS proteins have been comprehensively reviewed elsewhere.[45]

MIRO is an adaptor protein involved in the distribution and transport of both mitochondria and peroxisomes along microtubules, and linked to the fission of the organelles.[46–49] Recently, MIRO has been identified at MCS between those two organelles and the ER.[38] MIRO recruits the lipid transport protein VPS13D to the mitochondrial and peroxisomal membrane, with VPS13D bridging to the ER membrane by binding to VAP via its FFAT motif. Moreover, phosphorylation of a conserved residue in MIRO by PLK positively regulates its targeting to mitochondria-ER contacts, and its interaction with and the integrity of the IP_3R-GRP75-VDAC1 complex.[50] Phosphorylation of the residue, which is located in the N-terminal GTPase domain of MIRO, enhances MIRO activity. Interestingly, MIRO requires an active N-GTPase domain to recruit VPS13D,[38] suggesting that phosphorylation of MIRO by PLK could also potentially regulate the MIRO-VPS13D-VAP complex.

Not all proteins span opposing organelle membranes via protein-protein interactions at MCS. The previously mentioned phosphatidylinositol transporter TMEM24 is an ER-anchored protein, which can bind directly to the negatively charged cytosolic leaflet of the plasma membrane via its positively charged C-terminal region (Figure 1D).[23,24] PKC-dependent phosphorylation of the C-terminal region, in response to oscillations in cytosolic Ca^{2+}, dissociates TMEM24 from the plasma membrane and, consequently, from plasma membrane-ER MCS. Here, this method of regulation prevents localization of a tether to its site of function which may be a more general regulatory strategy (see Section: **Regulation of MCS by altering lipid composition of organelle membranes**).

The importance of phosphorylation in the regulation of MCS has also been shown in *S. cerevisiae*. For example, phosphorylation of Vps39 reduces the formation of vacuole-mitochondria MCS, which are mediated via its interaction with vacuolar Rab GTPase Ypt7 and a putative mitochondrial binding partner,[51] while phosphorylation of membrane trafficking protein Vps53 by the AMPK homolog Snf1 leads to the formation of Golgi-mitochondria contacts.[52] Additionally, Ypk1 phosphorylates sterol-binding protein Lam2, which disrupts its association with Laf1, another sterol-binding protein, at plasma membrane-ER MCS, inhibiting retrograde sterol transport from the plasma membrane to the ER.[53]

Overall, phosphorylation of MCS components allows cells to adapt rapidly and dynamically to changing conditions. This can disrupt or promote the overall organelle tethering, but also impact on distinct functions at the organelle interface such as lipid and Ca^{2+} transfer. The next challenge is to obtain a complete map of the multitude of phosphorylation events at MCS, including the kinases/phosphates involved, and to discover their function, and under what specific conditions they occur.

Regulation of MCS by ubiquitination

Ubiquitination has the potential to specifically degrade tethering factors, to modify the affinity of tethering partners as well as to alter

the ability of proteins to mediate exchange events at MCS (**Box 1**; Figure 2D). At organelle membranes, ubiquitination-mediated degradation can fine-tune the specific protein composition of organelles by degrading individual proteins, but ubiquitinated membrane proteins can also mark entire organelles for degradation via selective autophagy. For example, the ER-associated protein degradation (ERAD) system controls specific protein levels at the ER, and the PINK1-Parkin system ubiquitinates multiple mitochondrial targets to regulate mitophagy.[54,55] In contrast, peroxisomes utilize a range of E3 ligases to differentially regulate a single protein, the import receptor PEX5, leading to altered stability, recycling or activation of pexophagy depending on how PEX5 is ubiquitinated.[56–58] Multiple E3 ligases and DUBs have been found at organelle membranes, and the distribution of over 50 ubiquitin modifying enzymes targeted to different organelles was recently collated, highlighting a potentially prominent role for ubiquitination in influencing organelle function.[59] The following section explores recent examples of ubiquitin-mediated modifications which impact on MCS.

Ubiquitin-mediated degradation leading to reduced organelle tethering

Several clear examples of ubiquitin-mediated degradation of proteins regulating organelle tethering are found at the mitochondria-ER contact site. As previously mentioned, MFN2 is a dynamin-like GTPase, which is embedded in both the outer mitochondrial and ER membrane where it can facilitate mitochondrial-ER tethering.[60] Several studies have shown that ubiquitination of MFN2 leads to regulation of ER-mitochondria contacts.[61–63] Degradation of MFN2 can be activated via ubiquitination by the E3 ligase Parkin, which, together with its activator, the protein kinase PINK1, plays a well-established role in mitophagy.[64] PINK1 and Parkin have been found to be enriched at the MAM[65] which effectively positions them to ubiquitinate MFN2 and other MCS proteins.[66] Thus, in this example, ubiquitin-mediated degradation of MFN2 leads to altered mitochondria-ER contacts.

Sigma-R1 is an ER protein which interacts with GRP78 and IP_3R at the MAM to facilitate Ca^{2+} signalling.[67] Sigma-R1 is ubiquitinated by the ERAD component and E3 ligase HRD1 and degraded by the proteasome, leading to reduced mitochondria-ER contacts.[68] As Sigma-R1 is not itself a tether, how Sigma-R1 removal impacts on mitochondria-ER contacts is not completely clear, but this may relate to its role in mediation of Ca^{2+} signalling, as mutant Sigma-R1 disrupts IP_3R-mediated delivery of Ca^{2+} from the ER to mitochondria.[69]

Overall, in mammalian cells, the mitochondria-ER interface hosts an increasingly complex set of regulatory factors which utilize ubiquitin modifications to regulate contact site dynamics either by directly degrading tethers or by altering levels of the regulators of tethering function.

In yeast, mitochondria are anchored at the plasma membrane by the MECA. MECA consists of at least two components: Num1, which binds specific phospholipids on both the mitochondrial and plasma membrane, and Mdm36.[70] A tether complex, which performs a similar function but with different components, was also recently shown to retain peroxisomes at the mother cell cortex.[71] During meiosis, mitochondria undergo extensive remodelling, disconnect from the plasma membrane and localize at the gamete nuclei. This mitochondrial detachment requires destruction of the MECA complex, and the data suggests that this is driven by phosphorylation of Num1 by the kinase Ime2, leading to Num1 degradation.[18] Specific ubiquitination was not demonstrated, but it would be interesting to identify if the ubiquitination systems involved are linked with other cell cycle-related processes, such as cyclin degradation, potentially synchronising different degradation events in the cell cycle.[72]

More generally, how cells regulate the subcellular localization of organelles during the cell cycle and how this relates to the maintenance or loss of MCS is an interesting area of study.[73] For example, in yeast, the ER also needs to detach from the plasma membrane during meiosis[74] and in mammalian cells, peroxisomes appear to align at spindle poles during mitosis[75]—both these processes may require tether remodelling. Based on the prominence of ubiquitin-mediated degradation—coupled with phosphorylation—of multiple cell cycle events,[76] it is tempting to speculate that these regulatory processes might also contribute to the control of organelle tethering during the cell cycle. However, due to the presence of multiple tethers at the majority of different MCS a significant untethering to allow large changes in organelle dynamics would presumably require simultaneous modulation of multiple tethers, likely requiring multi-level regulation.

Ubiquitin modifications leading to alterations of tethers

As well as activating degradation of tether proteins as a strategy to regulate the physical connections between organelles, ubiquitination can also serve to alter function of the protein tethers at MCS without degradation. This can allow regulation of lipid trafficking and other exchange events.

One example of this is the ubiquitin ligase MITOL, which mediates ubiquitination of MFN2 in its GTPase domain.[61] This modification does not target MFN2 for degradation but is in fact required for GTP binding, which activates MFN2 tethering function. Therefore, regulation of MFN2 stability or function seems to represent an extensively regulated control point to adjust mitochondria-ER interactions.

Another example of non-degradative ubiquitin-modifications of tether proteins is the mitochondrial-ER tether protein PTPIP51, which has been proposed to act as a transporter of phosphatidic acid from the ER to mitochondria during cardiolipin synthesis.[77] PTPIP51 has recently been shown to be ubiquitinated by MITOL. This modification did not appear to activate PTPIP51 degradation or impact VAPB interaction, so overall physical tethering is likely unaffected but instead, the authors suggested that ubiquitination at this site reduced phosphatidic acid binding.[78]

It has recently been shown that the ER-embedded E3 ubiquitin ligase RNF26, pairs with the ubiquitin conjugating enzyme UBE2J1 in the

perinuclear ER region.[79] This complex then specifically ubiquitinates the ER adaptor SQSTM1 which allows recruitment of ubiquitin binding proteins to endosomal membranes. This increases endosome-ER contacts in the perinuclear region, altering movement of secretory cargos. Therefore, in this example ubiquitination promotes assembly of endosome-ER contact sites by regulating protein-protein interactions.

In summary, ubiquitin-mediated degradation of tethering components is a clearly effective way to remove a tethering protein and thus reduce MCS. However, alternative ubiquitin modifications which do not lead directly to degradation can alter tether protein functions in different ways, leading to alterations in protein interactors or modulation of other functions of the tethers.

Regulation of MCS by redox signalling

Another type of post-translational modification involved in the potential regulation of organelle interactions is reactive oxygen species (ROS)-mediated modification of MCS proteins (**Box 1**). In this case, ROS modulation could occur via direct alterations of redox-sensitive cysteines in tether proteins (Figure 2E). Peroxisomes, the ER and mitochondria are organelles which are enriched with redox-related metabolic reactions that generate ROS, and have been proposed to form a redox hub.[80] In these hubs, ROS can act as a signalling molecule, feeding into downstream processes that regulate the metabolic fate of a cell. For example, peroxisome derived H_2O_2 was recently shown to be a major source of sulfenylation of the HEK293 cellular proteome.[81] Below we summarize emerging examples of redox regulation at MCS.

Redox regulation at mitochondria-ER MCS is an example of how inter-organelle communication can be modulated by ROS. Redox regulation at MAMs is intertwined with Ca^{2+} signalling, with many components of the Ca^{2+} signalling machinery also being ROS-sensitive proteins. For example, previous studies showed a transient increase of H_2O_2 at the mitochondria-ER interface following H_2O_2 release from mitochondrial cristae, in response to Ca^{2+} influx to mitochondria. This could potentially induce oxidation of redox-sensitive proteins at MAMs, such as IP_3R, and result in altered Ca^{2+} efflux from the ER.[82–85] Furthermore, ROS accumulation in MAMs can also alter the GRP75-mediated tethering of IP_3R and VDAC.[80,86,87] The opening of these Ca^{2+} channels can drive the upregulation of electron transport chain components, in turn leading to altered ROS generation. In line with the idea that H_2O_2 could be transferred directly between organelles at the mitochondria-ER interface, it was shown that aquaporin AQP11 is an ER resident peroxiporin (H_2O_2 channel) which partially localizes to MAMs.[88] A recent study showed that in the absence of ERO1α, an ER resident H_2O_2 generating enzyme, a compensatory mechanism operates whereby H_2O_2 generated by mitochondrial complex III enters the ER through AQP11. This process coincides with, and appears to be dependent on, increased mitochondria-ER MCS.[89] One possibility, which would be interesting to explore, is that the efflux of H_2O_2 from the mitochondria to the ER through AQP11 could be mediated by direct redox modulation of tether proteins.[80,81]

As ROS, often in the form of H_2O_2, is generated as part of numerous biochemical reactions, for example during beta-oxidation of VLCFAs in peroxisomes, linking ROS flux to the control of MCS allows the possibility to couple the extent of lipid exchange at MCS to the level of ROS being produced by a particular organelle. ROS-mediated oxidation of tethers could result in a variety of conformational changes in contact site proteins (see **Box1**), which might lead to alterations in how tethering proteins interact or the level of lipid transfer. Overall, the involvement of ROS signalling in the regulation of MCS is a relatively unexplored area of study and may prove to be more extensive than is currently appreciated.

Regulation of MCS by multiple protein modifications

Many post-translational modifications, including phosphorylation, ubiquitination and ROS-mediated modifications, can be interlinked, occurring simultaneously, or acting as priming signals for further post-translational modifications. One example is the PINK1-mediated phosphorylation of ubiquitin itself on ubiquitinated proteins, such as MFN2, leading to recruitment of Parkin and thus further enhanced ubiquitination.[90] Another example, which relates to peroxisome-lipid droplet interactions, was recently identified by the Wolfrum lab. Here, a complex sensing mechanism couples H_2O_2 generated by peroxisomal activity, with ubiquitin-mediated modification of proteins at peroxisome-lipid droplet interaction sites.[91] This suggests that lipid droplet-derived fatty acids are trafficked to peroxisomes for beta-oxidation, via lipid droplet-peroxisome MCS, resulting in elevated levels of H_2O_2. This increased H_2O_2 in peroxisomes appears to result in modulation of the ubiquitin ligase PEX2, a peroxisomal membrane protein, at multiple cysteine residues—leading to disulphide bond formation and stabilization of PEX2. Stabilized PEX2 is then able to ubiquitinate and regulate the degradation of the lipid droplet surface enzyme ATGL at lipid droplet-peroxisome MCS. ATGL, a key enzyme in lipolysis, is responsible for the liberation of free fatty acids and glycerol from triacylglycerol stores in lipid droplets, which generates peroxisomal substrates, and is escorted to lipid droplet-peroxisome MCS by PEX5.[92] Therefore, this system serves as an elegant way to control fatty acid release from lipid droplets.

Regulation of MCS by oligomerization of tether proteins

Proteins at MCS, especially lipid transfer proteins, frequently contain both structured domains, performing a wide variety of functions, as well as regions of intrinsic disorder. This allows them to act as flexible tethers between organelles, but also to act as entropic barriers preventing protein crowding and regulating membrane tethering geometry.[93] An example is mammalian OSBP, which contains a C-terminal lipid transport domain (ORD), a FFAT motif that binds VAP, a PH domain to interact with membrane lipids, and an N-terminal disordered tail (Figure 1C). When the PH domain is bound to membrane

lipids, the presence of the N-terminal tail increases the apparent surface occupancy of the domain and seems to act as an entropic shield, preventing protein crowding by excluding material around the PH domain and controlling the dynamics of membrane tethering.[94] VAPA also contains intrinsically disordered regions, which enable versatile tethering at MCS and contribute to membrane tethering plasticity and efficiency.[95]

Several tether proteins contain a coiled-coil domain (e.g., VAP, ACBD4, ACBD5, PTPIP51, OSBP, MIGA2), which can mediate di-/oligomerization of the proteins (Figures 1C and 2F). ER-resident VAPA and VAPB, for example, form homo- and heterodimers.[96] VAPA/B also contains a GXXXG dimerization motif within its transmembrane domain which can mediate self-association.[97] Furthermore, VAPA has been crystallized as a homodimer in which each MSP subunit binds to a single FFAT motif containing peptide[98] and aberrant oligomerization of VAPB has been associated with the pathophysiology of neurological disorders.[16,97] It has recently been shown that ORP2 knock-down or overexpression influences VAPA oligomerization and its interactions with SNAREs at plasma membrane-ER MCS in yeast and neurons.[99] ORP2 contains a FFAT motif and can form tetramers.[100] It delivers cholesterol to the plasma membrane in exchange for phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2). It is suggested that ORP2 promotes VAPA oligomerization by bridging the FFAT motif interacting sites in each VAPA monomer, which would be consistent with the reported VAP-FFAT crystal structure. The homo-oligomerization of peroxisomal ACBD4 and ACBD5 is also dependent on their coiled-coil domains (our unpublished observations) but the role of coiled-coils in membrane tethering is poorly understood. It is possible, that VAPB and interacting FFAT-motif protein di/oligomerization impacts on the ability of the tether proteins to form ER-MCS. Oligomerization can increase the number of membrane-interacting modules and may allow the formation of larger tether complexes, and consequently, larger MCS. An example is the clustering of Kv2 channels at the plasma membrane, which interact via a FFAT motif with VAP at the ER.[35,101] The clustering enables Kv2 channels to play a structural role in forming ER-PM junctions. Additionally, the MOSPD proteins of the VAP family also dimerize, though they do not contain a predicted coiled-coil domain or GXXXG motif.[31] The homo/heterodimerization of MOSPD1-MOSPD3 and MOSPD2-VAPA-VAPB complexes are dependent on their transmembrane regions, apparently grouping them into separate FFNT and FFAT binding complexes.

However, oligomerization could also potentially reduce the ability of a tether protein to interact with its partner proteins or other regulators. Detachment of a dimer from the membrane requires the simultaneous dissociation of its two contacts with the membrane. For OSBP it has been shown that the dwell time of its dimeric PH domain-containing region on PI(4)P-containing membranes is in the range of several minutes in contrast to a few seconds in the case of the monomeric PH domain.[94] It has been suggested that the transition from a monomeric unfolded tether to a dimeric coiled-coil tether should dramatically change the membrane tethering property of the protein both in terms of flexibility and dwell time at the membrane.[93] Overall, oligomerization of tether proteins (involving coiled-coils or other domains) appears to be an important and frequently observed feature of tether proteins, but the physiological role of this is not well understood.

Regulation of MCS by altering lipid composition of organelle membranes

As many tethering factors associate with their target organelle via interaction with membrane lipids, the lipid composition of the organelle membrane can also play a role in the assembly and organization of MCS (Figure 2G). This was vividly highlighted in a recent study which demonstrated that MCS between the ER and different organelles is dependent on ER subdomains with different lipid-ordering characteristics.[102] Many tethers are anchored at the organelle membrane using a specific lipid—if this particular lipid is no longer present then the tether will no longer connect. An example of this is the extended synaptogamin family (E-Syts), which are ER membrane proteins which connect to other organelle membranes using a C2 domain to bind to $PI(4,5)P_2$.[103] This allows the E-Syts to mediate connections to multiple organelles, with the level of $PI(4,5)P_2$ on a particular organelle membrane being an obvious control point to regulate this interaction.[104] As the lipid composition of organelle membranes is subject to homeostatic control and can be adapted in response to cellular environment,[105] this adds another level of control of MCS.

CONCLUSION

Here, we have focused on how regulation of tethering proteins can alter MCS formation. This regulation occurs at various levels and employs different mechanisms including phosphorylation and ubiquitination (Figure 2). Conceptually, MCS regulation can broadly be achieved by altering the levels or binding affinities of tether proteins at organelle-organelle interfaces. This general concept is employed at many MCS and can occur in a number of different ways. Whilst ubiquitination of tether proteins tends to lead to altered abundance, phosphorylation often leads to altered tethering protein affinity. Amongst our examples, we identify the VAP-FFAT interaction as a highly regulatable system to facilitate dynamic tethering of different organelles to the ER, discuss a role for tether regulation in controlling organelle connectedness in the cell cycle, and also highlight emerging examples of multi-level regulation with negative feedback loops at MCS. However, as this is a relatively new field, it is likely that we have only begun to scratch the surface in terms of MCS regulation. Many other types of post-translational modifications exist which may also impact on tethering proteins, including methylation, sumoylation and protein lipidation—the latter of which could conceivably allow direct modification of tether proteins by lipids as a feedback mechanism to control lipid exchange at MCS.[106] Beyond regulation of the tethers themselves, alterations in the products (e.g., lipids and Ca^{2+}) which are transferred at MCS can also influence the formation and function of MCS.[107] MCS connect numerous different biochemical pathways, in particular in lipid metabolism, where reactions are often initiated

in one organelle with subsequent steps occurring within different organelles. Therefore, MCS represent control points at which decisions on the direction of metabolic flux through a pathway may be determined (e.g., ER, mitochondria, peroxisomes, lysosomes and lipid droplets in metabolism of fatty acids). Due to this extensive interconnectedness, regulation of tethering components is unlikely to occur in isolation and presumably occurs as part of coordinated metabolic or stress response programmes. Unravelling these complex regulatory networks and assessing the contribution of different factors will be a considerable challenge for the field in the future. This will likely require the development of accessible methods to more reliably analyse multiple contacts simultaneously and to effectively track lipid transport. Indeed, attempts to analyse multiple contacts have been made using spectral imaging approaches with six fluorescently labelled organelles analysed simultaneously,[17] whilst split-fluorescence approaches have also been adapted to highlight multi-organelle interfaces.[108] These approaches may suffer from drawbacks with resolution and potential irreversibility, respectively, but have still yielded novel insights into larger organelle networks. In addition, screening approaches which attempt to systematically analyse all contact sites are already yielding many novel insights into tether regulation.[109] As well as providing a fascinating insight into cell organization, the dysregulation of MCS components in pathologies means that greater understanding of MCS regulation may reveal novel therapeutic targets, which could be beneficial to treat a variety of diseases.

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AUTHOR CONTRIBUTIONS

Joseph L. Costello and Michael Schrader conceived the project. All authors contributed to the writing of the manuscript. Suzan Kors created the figures and table.

CONFLICT OF INTEREST

The authors declare no competing financial interests.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

1. Prinz, W. A., Toulmay, A., & Balla, T. (2020). The functional universe of membrane contact sites. *Nature Reviews Molecular Cell Biology*, *21*(1), 7–24. https://doi.org/10.1038/s41580-019-0180-9
2. Cohen, S., Valm, A. M., & Lippincott-Schwartz, J. (2018). Interacting organelles. *Current Opinion in Cell Biology*, *53*, 84–91. https://doi.org/10.1016/J.CEB.2018.06.003
3. Eisenberg-Bord, M., Shai, N., Schuldiner, M., & Bohnert, M. (2016). A tether is a tether is a tether: Tethering at membrane contact sites. *Developmental Cell*, *39*(4), 395–409. https://doi.org/10.1016/J.DEVCEL.2016.10.022
4. Lackner, L. L. (2019). The expanding and unexpected functions of mitochondria contact sites. *Trends in Cell Biology*, *29*(7), 580–590. https://doi.org/10.1016/J.TCB.2019.02.009
5. Wu, H., Carvalho, P., & Voeltz, G. K. (2018). Here, there, and everywhere: The importance of ER membrane contact sites. *Science*, *361*(6401), eaan5835. https://doi.org/10.1126/SCIENCE.AAN5835
6. Liao, P. C., Yang, E. J., Borgman, T., Boldogh, I. R., Sing, C. N., Swayne, T. C., & Pon, L. A. (2022). Touch and go: Membrane contact sites between lipid droplets and other organelles. *Frontiers in Cell and Developmental Biology*, *10*, 852021. https://doi.org/10.3389/FCELL.2022.852021
7. David, Y., Castro, I. G., & Schuldiner, M. (2021). The fast and the furious: Golgi contact sites: Contact, 4, 251525642110344. https://doi.org/10.1177/25152564211034424
8. Shai, N., Schuldiner, M., & Zalckvar, E. (2016). No peroxisome is an island – Peroxisome contact sites. In *Biochimica et Biophysica Acta – Molecular Cell Research* (Vol., 1863, Issue 5, pp. 1061–1069). Elsevier B.V. https://doi.org/10.1016/j.bbamcr.2015.09.016
9. Hewlett, B., Singh, N. P., Vannier, C., & Galli, T. (2021). ER-PM contact sites – SNARING actors in emerging functions. *Frontiers in Cell and Developmental Biology*, *9*, 202. https://doi.org/10.3389/FCELL.2021.635518
10. Di Mattia, T., Tomasetto, C., & Alpy, F. (2020). Faraway, so close! Functions of Endoplasmic reticulum–Endosome contacts. *BBA – Molecular and Cell Biology of Lipids*, *1865*(1), 158490. https://doi.org/10.1016/J.BBALIP.2019.06.016
11. Silva, B. S. C., DiGiovanni, L., Kumar, R., Carmichael, R. E., Kim, P. K., & Schrader, M. (2020). Maintaining social contacts: The physiological relevance of organelle interactions. In *Biochimica et Biophysica Acta – Molecular Cell Research* (Vol., 1867, Issue 11, p. 118800). Elsevier B.V. https://doi.org/10.1016/j.bbamcr.2020.118800
12. Leal, N. S., & Martins, L. M. (2021). Mind the gap: Mitochondria and the endoplasmic reticulum in neurodegenerative diseases. *Biomedicines*, *9*(2), 227. https://doi.org/10.3390/BIOMEDICINES9020227
13. Schrader, M., Kamoshita, M., & Islinger, M. (2020). Organelle interplay – Peroxisome interactions in health and disease. *Journal of Inherited Metabolic Disease*, *43*, 71–89. https://doi.org/10.1002/jimd.12083
14. Herker, E., Vieyres, G., Beller, M., Krahmer, N., & Bohnert, M. (2021). Lipid droplet contact sites in health and disease. *Trends in Cell Biology*, *31*(5), 345–358. https://doi.org/10.1016/J.TCB.2021.01.004
15. Gil-Hernández, A., Arroyo-Campuzano, M., Simoni-Nieves, A., Zazueta, C., Gomez-Quiroz, L. E., & Silva-Palacios, A. (2021). Relevance of membrane contact sites in cancer progression. *Frontiers in Cell and Developmental Biology*, *8*, 1744. https://doi.org/10.3389/FCELL.2020.622215
16. Kors, S., Costello, J. L., & Schrader, M. (2022). VAP proteins – From organelle tethers to pathogenic host interactors and their role in neuronal disease. *Frontiers in Cell and Developmental Biology*, *10*, 895856. https://doi.org/10.3389/FCELL.2022.895856
17. Valm, A. M., Cohen, S., Legant, W. R., Melunis, J., Hershberg, U., Wait, E., Cohen, A. R., Davidson, M. W., Betzig, E., & Lippincott-Schwartz, J. (2017). Applying systems-level spectral imaging and analysis to reveal the organelle interactome. *Nature*, *546*(7656), 162–167. https://doi.org/10.1038/nature22369
18. Sawyer, E. M., Joshi, P. R., Jorgensen, V., Yunus, J., Berchowitz, L. E., & Ünal, E. (2019). Developmental regulation of an organelle tether

coordinates mitochondrial remodeling in meiosis. *The Journal of Cell Biology*, *218*(2), 559–579. https://doi.org/10.1083/jcb.201807097

19. Stoica, R., De Vos, K. J., Paillusson, S., Mueller, S., Sancho, R. M., Lau, K.-F., Vizcay-Barrena, G., Lin, W.-L., Xu, Y.-F., Lewis, J., Dickson, D. W., Petrucelli, L., Mitchell, J. C., Shaw, C. E., & Miller, C. C. J. (2014). ER-mitochondria associations are regulated by the VAPB-PTPIP51 interaction and are disrupted by ALS/FTD-associated TDP-43. *Nature Communications*, *5*, 3996. https://doi.org/10.1038/ncomms4996
20. Costello, J. L., Castro, I. G., Hacker, C., Schrader, T. A., Metz, J., Zeuschner, D., Azadi, A. S., Godinho, L. F., Costina, V., Findeisen, P., Manner, A., Islinger, M., & Schrader, M. (2017). ACBD5 and VAPB mediate membrane associations between peroxisomes and the ER. *Journal of Cell Biology*, *216*(2), 331–342. https://doi.org/10.1083/jcb.201607055
21. Hua, R., Cheng, D., Coyaud, É., Freeman, S., Di Pietro, E., Wang, Y., Vissa, A., Yip, C. M., Fairn, G. D., Braverman, N., Brumell, J. H., Trimble, W. S., Raught, B., & Kim, P. K. (2017). VAPs and ACBD5 tether peroxisomes to the ER for peroxisome maintenance and lipid homeostasis. *Journal of Cell Biology*, *216*(2), 367–377. https://doi.org/10.1083/jcb.201608128
22. Manford, A. G., Stefan, C. J., Yuan, H. L., MacGurn, J. A., & Emr, S. D. (2012). ER-to-plasma membrane tethering proteins regulate cell signaling and ER morphology. *Developmental Cell*, *23*(6), 1129–1140. https://doi.org/10.1016/J.DEVCEL.2012.11.004
23. Lees, J. A., Messa, M., Sun, E. W., Wheeler, H., Torta, F., Wenk, M. R., De Camilli, P., & Reinisch, K. M. (2017). Lipid transport by TMEM24 at ER-plasma membrane contacts regulates pulsatile insulin secretion. *Science*, 355(6326). https://doi.org/10.1126/SCIENCE.AAH6171
24. Sun, E. W., Guillén-Samander, A., Bian, X., Wu, Y., Cai, Y., Messa, M., & De Camilli, P. (2019). Lipid transporter TMEM24/C2CD2L is a Ca 2+ -regulated component of ER–plasma membrane contacts in mammalian neurons. *Proceedings of the National Academy of Sciences*, *116*(12), 5775–5784. https://doi.org/10.1073/PNAS.1820156116
25. Eisenberg-Bord, M., Zung, N., Collado, J., Drwesh, L., Fenech, E. J., Fadel, A., Dezorella, N., Bykov, Y. S., Rapaport, D., Fernandez-Busnadiego, R., & Schuldiner, M. (2021). CNM1 mediates nucleus–mitochondria contact site formation in response to phospholipid levels. *Journal of Cell Biology*, *220*(11), e202104100. https://doi.org/10.1083/JCB.202104100
26. Giacomello, M., & Pellegrini, L. (2016). The coming of age of the mitochondria–ER contact: A matter of thickness. *Cell Death, Differentiation*, *23*(9), 1417–1427. https://doi.org/10.1038/cdd.2016.52
27. Anastasia, I., Ilacqua, N., Raimondi, A., Lemieux, P., Ghandehari-Alavijeh, R., Faure, G., Mekhedov, S. L., Williams, K. J., Caicci, F., Valle, G., Giacomello, M., Quiroga, A. D., Lehner, R., Miksis, M. J., Toth, K., de Aguiar Vallim, T. Q., Koonin, E. V., Scorrano, L., & Pellegrini, L. (2021). Mitochondria-rough-ER contacts in the liver regulate systemic lipid homeostasis. *Cell Reports*, *34*(11), 108873. https://doi.org/10.1016/J.CELREP.2021.108873
28. Paillusson, S., Gomez-Suaga, P., Stoica, R., Little, D., Gissen, P., Devine, M. J., Noble, W., Hanger, D. P., & Miller, C. C. J. (2017). α-Synuclein binds to the ER–mitochondria tethering protein VAPB to disrupt Ca2+ homeostasis and mitochondrial ATP production. *Acta Neuropathologica*, *134*(1), 129–149. https://doi.org/10.1007/s00401-017-1704-z
29. Rasul, F., Zheng, F., Dong, F., He, J., Liu, L., Liu, W., Cheema, J. Y., Wei, W., & Fu, C. (2021). Emr1 regulates the number of foci of the endoplasmic reticulum-mitochondria encounter structure complex. *Nature Communications*, *12*(1), 521. https://doi.org/10.1038/s41467-020-20866-x
30. Mattia, T. D., Wilhelm, L. P., Ikhlef, S., Wendling, C., Spehner, D., Nominé, Y., Giordano, F., Mathelin, C., Drin, G., Tomasetto, C., & Alpy, F. (2018). Identification of MOSPD2, a novel scaffold for endoplasmic reticulum membrane contact sites. *EMBO Reports*, *19*(7), e45453. https://doi.org/10.15252/EMBR.201745453
31. Cabukusta, B., Berlin, I., van Elsland, D. M., Forkink, I., Spits, M., de Jong, A. W. M., Akkermans, J. J. L. L., Wijdeven, R. H. M., Janssen, G. M. C., van Veelen, P. A., & Neefjes, J. (2020). Human VAPome analysis reveals MOSPD1 and MOSPD3 as membrane contact site proteins interacting with FFAT-related FFNT motifs. *Cell Reports*, *33*(10), 108475. https://doi.org/10.1016/j.celrep.2020.108475
32. Di Mattia, T., Martinet, A., Ikhlef, S., McEwen, A. G., Nominé, Y., Wendling, C., Poussin-Courmontagne, P., Voilquin, L., Eberling, P., Ruffenach, F., Cavarelli, J., Slee, J., Levine, T. P., Drin, G., Tomasetto, C., & Alpy, F. (2020). FFAT motif phosphorylation controls formation and lipid transfer function of inter-organelle contacts. *The EMBO Journal*, *39*(23), e104369. https://doi.org/10.15252/embj.2019104369
33. Kumagai, K., Kawano-Kawada, M., & Hanada, K. (2014). Phosphoregulation of the ceramide transport protein CERT at serine 315 in the interaction with VAMP-associated protein (VAP) for inter-organelle trafficking of ceramide in mammalian cells. *Journal of Biological Chemistry*, *289*(15), 10748–10760. https://doi.org/10.1074/jbc.M113.528380
34. Kors, S., Hacker, C., Bolton, C., Maier, R., Reimann, L., Kitchener, E. J. A., Warscheid, B., Costello, J. L., & Schrader, M. (2022). Regulating peroxisome–ER contacts via the ACBD5-VAPB tether by FFAT motif phosphorylation and GSK3β. *Journal of Cell Biology*, *221*(3), e202003143. https://doi.org/10.1083/JCB.202003143
35. Kirmiz, M., Vierra, N. C., Palacio, S., & Trimmer, J. S. (2018). Identification of VAPA and VAPB as Kv2 channel-interacting proteins defining endoplasmic reticulum-plasma membrane junctions in mammalian brain neurons. *The Journal of Neuroscience*, *38*(35), 7562–7584. https://doi.org/10.1523/JNEUROSCI.0893-18.2018
36. Xu, L., Wang, X., Zhou, J., Qiu, Y., Shang, W., Liu, J. P., Wang, L., & Tong, C. (2020). Miga-mediated endoplasmic reticulum– mitochondria contact sites regulate neuronal homeostasis. *ELife*, *9*, e56584. https://doi.org/10.7554/ELIFE.56584
37. Kim, H., Lee, S., Jun, Y., & Lee, C. (2022). Structural basis for mitoguardin-2 mediated lipid transport at ER-mitochondrial membrane contact sites. *Nature Communications*, *13*(1), 1–14. https://doi.org/10.1038/s41467-022-31462-6
38. Guillén-Samander, A., Leonzino, M., Hanna, M. G., Tang, N., Shen, H., & De Camilli, P. (2021). VPS13D bridges the ER to mitochondria and peroxisomes via Miro. *Journal of Cell Biology*, *220*(5), e202010004. https://doi.org/10.1083/jcb.202010004
39. Mikitova, V., & Levine, T. P. (2012). Analysis of the key elements of FFAT-like motifs identifies new proteins that potentially bind VAP on the ER, including two AKAPs and FAPP2. *PLoS ONE*, *7*(1), e30455. https://doi.org/10.1371/journal.pone.0030455
40. Furuita, K., Jee, J. G., Fukada, H., Mishima, M., & Kojima, C. (2010). Electrostatic interaction between oxysterol-binding protein and VAMP-associated protein a revealed by NMR and mutagenesis studies. *Journal of Biological Chemistry*, *285*(17), 12961–12970. https://doi.org/10.1074/jbc.M109.082602
41. Stoica, R., Paillusson, S., Gomez-Suaga, P., Mitchell, J. C., Lau, D. H., Gray, E. H., Sancho, R. M., Vizcay-Barrena, G., De Vos, K. J., Shaw, C. E., Hanger, D. P., Noble, W., & Miller, C. C. (2016). ALS/FTD-associated FUS activates GSK-3β to disrupt the VAPB-PTPIP51 interaction and ER-mitochondria associations. *EMBO Reports*, *17*(9), 1326–1342. https://doi.org/10.15252/embr.201541726
42. Gómez-Suaga, P., Gábor Mórotz, M., Markovinovic, A., Martín-Guerrero, S. M., Preza, E., Arias, N., Mayl, K., Aabdien, A., Gesheva, V., Nishimura, A., Annibali, A., Lee, Y., Mitchell, J. C., Wray, S., Shaw, C., Noble, W., Christopher, S., Miller, C. J., … Miller, C. J. (2022). Disruption of ER-mitochondria tethering and signalling in C9orf72-associated amyotrophic lateral sclerosis and frontotemporal dementia. *Aging Cell*, *21*, e13549. https://doi.org/10.1111/ACEL.13549
43. Hu, Y., Chen, H., Zhang, L., Lin, X., Li, X., Zhuang, H., Fan, H., Meng, T., He, Z., Huang, H., Gong, Q., Zhu, D., Xu, Y., He, P., Li, L., &

Feng, D. (2021). The AMPK-MFN2 axis regulates MAM dynamics and autophagy induced by energy stresses. *Autophagy*, *17*(5), 1142. https://doi.org/10.1080/15548627.2020.1749490

44. Thoudam, T., Ha, C. M., Leem, J., Chanda, D., Park, J. S., Kim, H. J., Jeon, J. H., Choi, Y. K., Liangpunsakul, S., Huh, Y. H., Kwon, T. H., Park, K. G., Harris, R. A., Park, K. S., Rhee, H. W., & Lee, I. K. (2019). PDK4 augments ER–mitochondria contact to dampen skeletal muscle insulin signaling during obesity. *Diabetes*, *68*(3), 571–586. https://doi.org/10.2337/DB18-0363
45. Pichla, M., Sneyers, F., Stopa, K. B., Bultynck, G., & Kerkhofs, M. (2021). Dynamic control of mitochondria-associated membranes by kinases and phosphatases in health and disease. *Cellular and Molecular Life Sciences*, *78*(19), 6541–6556. https://doi.org/10.1007/S00018-021-03920-9
46. Castro, I. G., Richards, D. M., Metz, J., Costello, J. L., Passmore, J. B., Schrader, T. A., Gouveia, A., Ribeiro, D., & Schrader, M. (2018). A role for mitochondrial rho GTPase 1 (MIRO1) in motility and membrane dynamics of peroxisomes. *Traffic*, *19*(3), 229–242. https://doi.org/10.1111/TRA.12549
47. López-Doménech, G., Covill-Cooke, C., Ivankovic, D., Halff, E. F., Sheehan, D. F., Norkett, R., Birsa, N., & Kittler, J. T. (2018). Miro proteins coordinate microtubule- and actin-dependent mitochondrial transport and distribution. *The EMBO Journal*, *37*(3), 321. https://doi.org/10.15252/EMBJ.201696380
48. Okumoto, K., Ono, T., Toyama, R., Shimomura, A., Nagata, A., & Fujiki, Y. (2018). New splicing variants of mitochondrial Rho GTPase-1 (Miro1) transport peroxisomes. *The Journal of Cell Biology*, *217*(2), 619. https://doi.org/10.1083/JCB.201708122
49. Covill-Cooke, C., Toncheva, V. S., Drew, J., Birsa, N., López-Doménech, G., & Kittler, J. T. (2020). Peroxisomal fission is modulated by the mitochondrial Rho-GTPases, Miro1 and Miro2. *EMBO Reports*, *21*(2), e49865. https://doi.org/10.15252/EMBR.201949865
50. Lee, S., Lee, K. S., Huh, S., Liu, S., Lee, D. Y., Hong, S. H., Yu, K., & Lu, B. (2016). Polo kinase phosphorylates miro to control ER-mitochondria contact sites and mitochondrial Ca2+ homeostasis in neural stem cell development. *Developmental Cell*, *37*(2), 174–189. https://doi.org/10.1016/J.DEVCEL.2016.03.023
51. Hönscher, C., Mari, M., Auffarth, K., Bohnert, M., Griffith, J., Geerts, W., van der Laan, M., Cabrera, M., Reggiori, F., & Ungermann, C. (2014). Cellular metabolism regulates contact sites between vacuoles and mitochondria. *Developmental Cell*, *30*(1), 86–94. https://doi.org/10.1016/J.DEVCEL.2014.06.006
52. Wycislo, S. A., Sundag, C., Walter, S., Schuck, S., & Fröhlich, F. (2020). Phosphorylation of the GARP subunit Vps53 by Snf1 leads to the formation of a golgi – mitochondria contact site (GoMiCS) in yeast. *BioRxiv*, 2020.06.26.173864. https://doi.org/10.1101/2020.06.26.173864
53. Topolska, M., Roelants, F. M., Si, E. P., & Thorner, J. (2020). TORC2-dependent Ypk1-mediated phosphorylation of lam2/Ltc4 disrupts its association with the β-propeller protein laf1 at endoplasmic reticulum-plasma membrane contact sites in the yeast *Saccharomyces cerevisiae*. *Biomolecules*, *10*(12), 1598. https://doi.org/10.3390/BIOM10121598
54. Pickles, S., Vigié, P., & Youle, R. J. (2018). Mitophagy and quality control mechanisms in mitochondrial maintenance. *Current Biology*, *28*(4), R170–R185. https://doi.org/10.1016/J.CUB.2018.01.004
55. Christianson, J. C., & Carvalho, P. (2022). Order through destruction: How ER-associated protein degradation contributes to organelle homeostasis. *The EMBO Journal*, *41*(6), e109845. https://doi.org/10.15252/EMBJ.2021109845
56. Platta, H. W., El Magraoui, F., Schlee, D., Grunau, S., Girzalsky, W., & Erdmann, R. (2007). Ubiquitination of the peroxisomal import receptor Pex5p is required for its recycling. *Journal of Cell Biology*, *177*(2), 197–204. https://doi.org/10.1083/JCB.200611012
57. Law, K. B., Bronte-Tinkew, D., Di Pietro, E., Snowden, A., Jones, R. O., Moser, A., Brumell, J. H., Braverman, N., & Kim, P. K. (2017). The peroxisomal AAA ATPase complex prevents pexophagy and development of peroxisome biogenesis disorders. *Autophagy*, *13*(5), 868–884. https://doi.org/10.1080/15548627.2017.1291470/SUPPL_FILE/KAUP_A_1291470_SM5881.DOCX
58. Wang, W., Xia, Z. J., Farré, J. C., & Subramani, S. (2017). TRIM37, a novel E3 ligase for PEX5-mediated peroxisomal matrix protein import. *Journal of Cell Biology*, *216*(9), 2843–2858. https://doi.org/10.1083/JCB.201611170
59. Rusilowicz-Jones, E. V., Brazel, A. J., Frigenti, F., Urbé, S., & Clague, M. J. (2021). Membrane compartmentalisation of the ubiquitin system. *Seminars in Cell & Developmental Biology*, *S1084-9521*, 00292–00295. https://doi.org/10.1016/J.SEMCDB.2021.11.016
60. De Brito, O. M., & Scorrano, L. (2008). Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature*, *456*(7222), 605–610. https://doi.org/10.1038/nature07534
61. Sugiura, A., Nagashima, S., Tokuyama, T., Amo, T., Matsuki, Y., Ishido, S., Kudo, Y., McBride, H. M., Fukuda, T., Matsushita, N., Inatome, R., & Yanagi, S. (2013). MITOL regulates endoplasmic reticulum-mitochondria contacts via mitofusin2. *Molecular Cell*, *51*(1), 20–34. https://doi.org/10.1016/J.MOLCEL.2013.04.023
62. Basso, V., Marchesan, E., Peggion, C., Chakraborty, J., von Stockum, S., Giacomello, M., Ottolini, D., Debattisti, V., Caicci, F., Tasca, E., Pegoraro, V., Angelini, C., Antonini, A., Bertoli, A., Brini, M., & Ziviani, E. (2018). Regulation of ER-mitochondria contacts by Parkin via Mfn2. *Pharmacological Research*, *138*, 43–56. https://doi.org/10.1016/J.PHRS.2018.09.006
63. McLelland, G. L., Goiran, T., Yi, W., Dorval, G., Chen, C. X., Lauinger, N. D., Krahn, A. I., Valimehr, S., Rakovic, A., Rouiller, I., Durcan, T. M., Trempe, J. F., & Fon, E. A. (2018). Mfn2 ubiquitination by PINK1/parkin gates the p97-dependent release of ER from mitochondria to drive mitophagy. *ELife*, *7*, e32866. https://doi.org/10.7554/ELIFE.32866
64. Ziviani, E., Tao, R. N., & Whitworth, A. J. (2010). Drosophila Parkin requires PINK1 for mitochondrial translocation and ubiquitinates Mitofusin. *Proceedings of the National Academy of Sciences*, *107*(11), 5018–5023. https://doi.org/10.1073/PNAS.0913485107
65. Gelmetti, V., De Rosa, P., Torosantucci, L., Marini, E. S., Romagnoli, A., Di Rienzo, M., Arena, G., Vignone, D., Fimia, G. M., & Valente, E. M. (2017). PINK1 and BECN1 relocalize at mitochondria-associated membranes during mitophagy and promote ER-mitochondria tethering and autophagosome formation. *Autophagy*, *13*(4), 654–669. https://doi.org/10.1080/15548627.2016.1277309
66. Vranas, M., Lu, Y., Rasool, S., Croteau, N., Krett, J. D., Sauvé, V., Gehring, K., Fon, E. A., Durcan, T. M., & Trempe, J. F. (2022). Selective localization of Mfn2 near PINK1 enables its preferential ubiquitination by Parkin on mitochondria. *Open Biology*, *12*(1), 210255. https://doi.org/10.1098/RSOB.210255
67. Bernard-Marissal, N., Médard, J. J., Azzedine, H., & Chrast, R. (2015). Dysfunction in endoplasmic reticulum-mitochondria crosstalk underlies SIGMAR1 loss of function mediated motor neuron degeneration. *Brain*, *138*(4), 875–890. https://doi.org/10.1093/BRAIN/AWV008
68. Zhou, Z., Torres, M., Sha, H., Halbrook, C. J., van den Bergh, F., Reinert, R. B., Yamada, T., Wang, S., Luo, Y., Hunter, A. H., Wang, C., Sanderson, T. H., Liu, M., Taylor, A., Sesaki, H., Lyssiotis, C. A., Wu, J., Kersten, S., Beard, D. A., & Qi, L. (2020). Endoplasmic reticulum-associated degradation regulates mitochondrial dynamics in brown adipocytes. *Science*, *54*(6486). https://doi.org/10.1126/SCIENCE.AAY2494
69. Martín-Guerrero, S. M., Markovinovic, A., Mórotz, G. M., Salam, S., Noble, W., & Miller, C. C. J. (2022). Targeting ER-mitochondria signaling as a therapeutic target for frontotemporal dementia and related amyotrophic lateral sclerosis. *Frontiers in Cell and Developmental Biology*, *10*, 915931. https://doi.org/10.3389/FCELL.2022.915931

70. Ping, H. A., Kraft, L. M., Chen, W. T., Nilles, A. E., & Lackner, L. L. (2016). Num1 anchors mitochondria to the plasma membrane via two domains with different lipid binding specificities. *Journal of Cell Biology*, *213*(5), 513–524. https://doi.org/10.1083/JCB.201511021
71. Hulmes, G. E., Hutchinson, J. D., Dahan, N., Nuttall, J. M., Allwood, E. G., Ayscough, K. R., & Hettema, E. H. (2020). The Pex3-Inp1 complex tethers yeast peroxisomes to the plasma membrane. *Journal of Cell Biology*, *219*(10), e201906021. https://doi.org/10.1083/JCB.201906021/152119
72. Eisenberg, A. R., Higdon, A., Keskin, A., Hodapp, S., Jovanovic, M., & Brar, G. A. (2018). Precise post-translational tuning occurs for most protein complex components during meiosis. *Cell Reports*, *25*(13), 3603-3617.e2.e2. https://doi.org/10.1016/J.CELREP.2018.12.008
73. Carlton, J. G., Jones, H., & Eggert, U. S. (2020). Membrane and organelle dynamics during cell division. *Nature Reviews Molecular Cell Biology*, *21*(3), 151–166. https://doi.org/10.1038/s41580-019-0208-1
74. Otto, G. M., Cheunkarndee, T., Leslie, J. M., & Brar, G. A. (2021). Programmed cortical er collapse drives selective er degradation and inheritance in yeast meiosis. *Journal of Cell Biology*, 220(12). https://doi.org/10.1083/JCB.202108105
75. Kredel, S., Oswald, F., Nienhaus, K., Deuschle, K., Röcker, C., Wolff, M., Heilker, R., Nienhaus, G. U., & Wiedenmann, J. (2009). mRuby, a bright monomeric red fluorescent protein for labeling of subcellular structures. *PLOS ONE*, *4*(2), e4391. https://doi.org/10.1371/JOURNAL.PONE.0004391
76. Zou, T., Lin, Z., Bae, K.-H., & Deak, P. (2021). The involvement of ubiquitination machinery in cell cycle regulation and cancer progression. *International Journal of Molecular Sciences*, *22*(11), 5754. https://doi.org/10.3390/IJMS22115754
77. Yeo, H. K., Park, T. H., Kim, H. Y., Jang, H., Lee, J., Hwang, G.-S., Ryu, S. E., Park, S. H., Song, H. K., Seung Ban, H., Yoon, H.-J., & Byung Lee, I. (2021). Phospholipid transfer function of PTPIP51 at mitochondria-associated ER membranes. *EMBO Reports*, *22*(6), e51323. https://doi.org/10.15252/EMBR.202051323
78. Ito, N., Takahashi, T., Shiiba, I., Nagashima, S., Inatome, R., & Yanagi, S. (2022). MITOL regulates phosphatidic acid-binding activity of RMDN3/PTPIP51. *The Journal of Biochemistry*, *171*(5), 529–541. https://doi.org/10.1093/JB/MVAB153
79. Cremer, T., Jongsma, M. L. M., Trulsson, F., Vertegaal, A. C. O., Neefjes, J., & Berlin, I. (2021). The ER-embedded UBE2J1/RNF26 ubiquitylation complex exerts spatiotemporal control over the endolysosomal pathway. *Cell Reports*, *34*(3), 108659. https://doi.org/10.1016/J.CELREP.2020.108659
80. Yoboue, E. D., Sitia, R., & Simmen, T. (2018). Redox crosstalk at endoplasmic reticulum (ER) membrane contact sites (MCS) uses toxic waste to deliver messages. *Cell Death, & Disease*, *9*(3), 1–14. https://doi.org/10.1038/s41419-017-0033-4
81. Lismont, C., Revenco, I., Li, H., Costa, C. F., Lenaerts, L., Hussein, M. A. F., De Bie, J., Knoops, B., Van Veldhoven, P. P., Derua, R., & Fransen, M. (2022). Peroxisome-derived hydrogen peroxide modulates the sulfenylation profiles of key redox signaling proteins in Flp-In T-REx 293 cells. *Frontiers in Cell and Developmental Biology*, *10*, 888873. https://doi.org/10.3389/FCELL.2022.888873
82. Booth, D. M., Enyedi, B., Geiszt, M., Várnai, P., & Hajnóczky, G. (2016). Redox nanodomains are induced by and control calcium signaling at the ER-mitochondrial interface. *Molecular Cell*, *63*(2), 240–248. https://doi.org/10.1016/J.MOLCEL.2016.05.040
83. Gottschalk, B., Klec, C., Waldeck-Weiermair, M., Malli, R., & Graier, W. F. (2018). Intracellular Ca2+ release decelerates mitochondrial cristae dynamics within the junctions to the endoplasmic reticulum. *Pflugers Archiv European Journal of Physiology*, *470*(8), 1193–1203. https://doi.org/10.1007/S00424-018-2133-0
84. Joseph, S. K., Young, M. P., Alzayady, K., Yule, D. I., Ali, M., Booth, D. M., & Hajnóczky, G. (2018). Redox regulation of type-I inositol trisphosphate receptors in intact mammalian cells. *Journal of Biological Chemistry*, *293*(45), 17464–17476. https://doi.org/10.1074/JBC.RA118.005624
85. Booth, D. M., Várnai, P., Joseph, S. K., & Hajnóczky, G. (2021). Oxidative bursts of single mitochondria mediate retrograde signaling toward the ER. *Molecular Cell*, *81*(18), 3866-3876.e2.e2. https://doi.org/10.1016/J.MOLCEL.2021.07.014
86. Tiwary, S., Nandwani, A., Khan, R., & Datta, M. (2021). GRP75 mediates endoplasmic reticulum–mitochondria coupling during palmitate-induced pancreatic β-cell apoptosis. *Journal of Biological Chemistry*, 101368(6). https://doi.org/10.1016/J.JBC.2021.101368
87. Yuan, M., Gong, M., He, J., Xie, B., Zhang, Z., Meng, L., Tse, G., Zhao, Y., Bao, Q., Zhang, Y., Yuan, M., Liu, X., Luo, C., Wang, F., Li, G., & Liu, T. (2022). IP3R1/GRP75/VDAC1 complex mediates endoplasmic reticulum stress-mitochondrial oxidative stress in diabetic atrial remodeling. *Redox Biology*, *52*, 102289. https://doi.org/10.1016/J.REDOX.2022.102289
88. Bestetti, S., Galli, M., Sorrentino, I., Pinton, P., Rimessi, A., Sitia, R., & Medraño-Fernandez, I. (2020). Human aquaporin-11 guarantees efficient transport of H2O2 across the endoplasmic reticulum membrane. *Redox Biology*, *28*, 101326. https://doi.org/10.1016/J.REDOX.2019.101326
89. Sorrentino, I., Galli, M., Medraño-Fernandez, I., & Sitia, R. (2022). Transfer of H2O2 from Mitochondria to the endoplasmic reticulum via Aquaporin-11. *Redox Biology*, *55*, 102410. https://doi.org/10.1016/J.REDOX.2022.102410
90. McLelland, G. L., & Fon, E. A. (2018). MFN2 retrotranslocation boosts mitophagy by uncoupling mitochondria from the ER. *Autophagy*, *14*(9), 1658–1660. https://doi.org/10.1080/15548627.2018.1505154
91. Ding, L., Sun, W., Balaz, M., He, A., Klug, M., Wieland, S., Caiazzo, R., Raverdy, V., Pattou, F., Lefebvre, P., Lodhi, I. J., Staels, B., Heim, M., & Wolfrum, C. (2021). Peroxisomal β-oxidation acts as a sensor for intracellular fatty acids and regulates lipolysis. *Nature Metabolism*, *3*(12), 1648–1661. https://doi.org/10.1038/s42255-021-00489-2
92. Kong, J., Ji, Y., Jeon, Y. G., Han, J. S., Han, K. H., Lee, J. H., Lee, G., Jang, H., Choe, S. S., Baes, M., & Kim, J. B. (2020). Spatiotemporal contact between peroxisomes and lipid droplets regulates fasting-induced lipolysis via PEX5. *Nature Communications*, *11*(1), 1–16. https://doi.org/10.1038/s41467-019-14176-0
93. Jamecna, D., & Antonny, B. (2021). Intrinsically disordered protein regions at membrane contact sites. *BBA – Molecular and Cell Biology of Lipids*, *1866*(11), 159020. https://doi.org/10.1016/J.BBALIP.2021.159020
94. Jamecna, D., Polidori, J., Mesmin, B., Dezi, M., Levy, D., Bigay, J., & Antonny, B. (2019). An intrinsically disordered region in OSBP acts as an entropic barrier to control protein dynamics and orientation at membrane contact sites. *Developmental Cell*, *49*(2), 220-234.e8.e8. https://doi.org/10.1016/J.DEVCEL.2019.02.021
95. Subra, M., Dezi, M., Bigay, J., Lacas-Gervais, S., Cicco, A. D., Araújo, A. R. D., Abélanet, S., Fleuriot, L., Debayle, D., Gautier, R., Patel, A., Roussi, F., Antonny, B., Lévy, D., & Mesmin, B. (2022). VAP-A intrinsically disordered regions enable versatile tethering at membrane contact sites. *BioRxiv*, 2022.05.13.491839. https://doi.org/10.1101/2022.05.13.491839
96. Nishimura, Y., Hayashi, M., Inada, H., & Tanaka, T. (1999). Molecular cloning and characterization of mammalian homologues of vesicle-associated membrane protein-associated (VAMP-associated) proteins. *Biochemical and Biophysical Research Communications*, *254*(1), 21–26. https://doi.org/10.1006/bbrc.1998.9876
97. Kim, S. H., Leal, S. S., Halevy, D. B., Gomes, C. M., & Lev, S. (2010). Structural requirements for VAP-B oligomerization and their implication in amyotrophic lateral sclerosis-associated VAP-B(P56S) neurotoxicity. *Journal of Biological Chemistry*, *285*(18), 13839–13849. https://doi.org/10.1074/jbc.M109.097345

98. Kaiser, S. E., Brickner, J. H., Reilein, A. R., Fenn, T. D., Walter, P., & Brunger, A. T. (2005). Structural basis of FFAT motif-mediated ER targeting. *Structure*, *13*(7), 1035–1045. https://doi.org/10.1016/j.str.2005.04.010
99. Weber-Boyvat, M., Trimbuch, T., Shah, S., Jäntti, J., Olkkonen, V. M., & Rosenmund, C. (2021). ORP/Osh mediate cross-talk between ER-plasma membrane contact site components and plasma membrane SNAREs. *Cellular and Molecular Life Sciences*, *78*(4), 1689–1708. https://doi.org/10.1007/S00018-020-03604-W
100. Wang, H., Ma, Q., Qi, Y., Dong, J., Du, X., Rae, J., Wang, J., Wu, W. F., Brown, A. J., Parton, R. G., Wu, J. W., & Yang, H. (2019). ORP2 delivers cholesterol to the plasma membrane in exchange for phosphatidylinositol 4, 5-bisphosphate (PI(4,5)P2). *Molecular Cell*, *73*(3), 458–473.e7. https://doi.org/10.1016/J.MOLCEL.2018.11.014
101. Johnson, B., Leek, A. N., Solé, L., Maverick, E. E., Levine, T. P., & Tamkun, M. M. (2018). Kv2 potassium channels form endoplasmic reticulum/plasma membrane junctions via interaction with VAPA and VAPB. *Proceedings of the National Academy of Sciences*, *115*(31), E7331–E7340. https://doi.org/10.1073/pnas.1805757115
102. King, C., Sengupta, P., Seo, A. Y., & Lippincott-Schwartz, J. (2020). ER membranes exhibit phase behavior at sites of organelle contact. *Proceedings of the National Academy of Sciences*, *117*(13), 7225–7235. https://doi.org/10.1073/PNAS.1910854117
103. Xiao, J., Luo, J., Hu, A., Xiao, T., Li, M., Kong, Z., Jiang, L., Zhou, Z., Liao, Y., Xie, C., Chu, B., Miao, H., Li, B., Shi, X., & Song, B.-L. (2019). Cholesterol transport through the peroxisome-ER membrane contacts tethered by PI(4,5)P2 and extended synaptotagmins. *Science China. Life Sciences*, *62*(9), 1117–1135. https://doi.org/10.1007/s11427-019-9569-9
104. Lee, E., Vanneste, S., Pérez-Sancho, J., Benitez-Fuente, F., Strelau, M., Macho, A. P., Botella, M. A., Friml, J., & Rosado, A. (2019). Ionic stress enhances ER–PM connectivity via phosphoinositide-associated SYT1 contact site expansion in *Arabidopsis*. *Proceedings of the National Academy of Sciences*, *116*(4), 1420–1429. https://doi.org/10.1073/PNAS.1818099116
105. Ernst, R., Ballweg, S., & Levental, I. (2018). Cellular mechanisms of physicochemical membrane homeostasis. *Current Opinion in Cell Biology*, *53*, 44–51. https://doi.org/10.1016/J.CEB.2018.04.013
106. Chen, B., Sun, Y., Niu, J., Jarugumilli, G. K., & Wu, X. (2018). Protein lipidation in cell signaling and diseases: Function, regulation, and therapeutic opportunities. *Cell Chemical Biology*, *25*(7), 817–831. https://doi.org/10.1016/J.CHEMBIOL.2018.05.003
107. Zhang, Y., Ge, J., Bian, X., & Kumar, A. (2022). Quantitative models of lipid transfer and membrane contact formation: *Contact*, *5*, 251525642210960. https://doi.org/10.1177/25152564221096024
108. Vallese, F., Catoni, C., Cieri, D., Barazzuol, L., Ramirez, O., Calore, V., Bonora, M., Giamogante, F., Pinton, P., Brini, M., & Calì, T. (2020). An expanded palette of improved SPLICS reporters detects multiple organelle contacts in vitro and in vivo. *Nature Communications*, *11*(1), 6069. https://doi.org/10.1038/s41467-020-19892-6
109. Shai, N., Yifrach, E., van Roermund, C. W. T., Cohen, N., Bibi, C., IJlst, L., Cavellini, L., Meurisse, J., Schuster, R., Zada, L., Mari, M. C., Reggiori, F. M., Hughes, A. L., Escobar-Henriques, M., Cohen, M. M., Waterham, H. R., Wanders, R. J. A., Schuldiner, M., & Zalckvar, E. (2018). Systematic mapping of contact sites reveals tethers and a function for the peroxisome-mitochondria contact. *Nature Communications*, *9*(1), 1761. https://doi.org/10.1038/s41467-018-03957-8
110. Fugmann, T., Hausser, A., Schöffler, P., Schmid, S., Pfizenmaier, K., & Olayioye, M. A. (2007). Regulation of secretory transport by protein kinase D–mediated phosphorylation of the ceramide transfer protein. *The Journal of Cell Biology*, *178*(1), 15. https://doi.org/10.1083/JCB.200612017
111. Saito, S., Matsui, H., Kawano, M., Kumagai, K., Tomishige, N., Hanada, K., Echigo, S., Tamura, S., & Kobayashi, T. (2008). Protein phosphatase 2C*ε* is an endoplasmic reticulum integral membrane protein that dephosphorylates the ceramide transport protein CERT to enhance its association with organelle membranes. *Journal of Biological Chemistry*, *283*(10), 6584–6593. https://doi.org/10.1074/jbc.M707691200
112. Tomishige, N., Kumagai, K., Kusuda, J., Nishijima, M., & Hanada, K. (2009). Casein kinase I*γ*2 down-regulates trafficking of ceramide in the synthesis of sphingomyelin. *Molecular Biology of the Cell*, *20*(1), 348. https://doi.org/10.1091/MBC.E08-07-0669
113. Shimasaki, K., Kumagai, K., Sakai, S., Yamaji, T., & Hanada, K. (2022). Hyperosmotic stress induces phosphorylation of CERT and enhances its tethering throughout the endoplasmic reticulum. *International Journal of Molecular Sciences*, *23*(7), 4025. https://doi.org/10.3390/IJMS23074025
114. Ende, R. J., Murray, R. L., D'Spain, S. K., Coppens, I., & Derré, I. (2022). Phosphoregulation accommodates Type III secretion and assembly of a tether of ER-Chlamydia inclusion membrane contact sites. *ELife*, *11*, https://doi.org/10.7554/ELIFE.74535
115. Goto, A., Liu, X., Robinson, C.-A., & Ridgway, N. D. (2012). Multisite phosphorylation of oxysterol-binding protein regulates sterol binding and activation of sphingomyelin synthesis. *Molecular Biology of the Cell*, *23*(18), 3624–3635. https://doi.org/10.1091/mbc.E12-04-0283
116. Weber-Boyvat, M., Kentala, H., Lilja, J., Vihervaara, T., Hanninen, R., Zhou, Y., Peränen, J., Nyman, T. A., Ivaska, J., & Olkkonen, V. M. (2015). OSBP-related protein 3 (ORP3) coupling with VAMP-associated protein A regulates R-Ras activity. *Experimental Cell Research*, *331*(2), 278–291. https://doi.org/10.1016/j.yexcr.2014.10.019
117. Gulyás, G., Sohn, M., Kim, Y. J., Várnai, P., & Balla, T. (2020). ORP3 phosphorylation regulates phosphatidylinositol 4-phosphate and Ca2+ dynamics at plasma membrane-ER contact sites. *Journal of Cell Science*, *133*(6), jcs237388. https://doi.org/10.1242/JCS.237388

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