Title

Endoplasmic Reticulum Architecture and Inter-Organelle Communication in Metabolic Health and Disease.

Journal

Cold Spring Harbor perspectives in biology, 15(2)

Authors

Arruda, Ana Paula

Parlakgül, Güneş

Publication Date

2023-02-01

DOI

10.1101/cshperspect.a041261

Peer reviewed

Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

Endoplasmic Reticulum Architecture and Inter-Organelle Communication in Metabolic Health and Disease

Ana Paula Arruda[1,2] and Güneş Parlakgül[1,3]

[1]Department of Nutritional Sciences and Toxicology, University of California Berkeley, Berkeley, California 94720, USA

[2]Chan Zuckerberg Biohub, San Francisco, California 94158, USA

[3]Sabri Ülker Center for Metabolic Research and Department of Molecular Metabolism, Harvard T.H. Chan School of Public Health, Boston, Massachusetts 02115, USA

Correspondence: aarruda@berkeley.edu

The endoplasmic reticulum (ER) is a key organelle involved in the regulation of lipid and glucose metabolism, proteostasis, Ca^{2+} signaling, and detoxification. The structural organization of the ER is very dynamic and complex, with distinct subdomains such as the nuclear envelope and the peripheral ER organized into ER sheets and tubules. ER also forms physical contact sites with all other cellular organelles and with the plasma membrane. Both form and function of the ER are highly adaptive, with a potent capacity to respond to transient changes in environmental cues such as nutritional fluctuations. However, under obesity-induced chronic stress, the ER fails to adapt, leading to ER dysfunction and the development of metabolic pathologies such as insulin resistance and fatty liver disease. Here, we discuss how the remodeling of ER structure and contact sites with other organelles results in diversification of metabolic function and how perturbations to this structural flexibility by chronic overnutrition contribute to ER dysfunction and metabolic pathologies in obesity.

Metabolic adaptation in response to oscillations in nutrient availability is essential to maintain homeostasis and health. Throughout evolution, a diverse and robust array of adaptive mechanisms have been selected to cope with acute changes in nutritional states. The endoplasmic reticulum (ER) is a key platform supporting these mechanisms. In addition to mediating nutrient sensing, ER is involved in a variety of biological processes such as protein synthesis, folding and secretion, glucose homeostasis, and Ca^{2+} signaling. Importantly, the ER is the main site of lipid biosynthesis, including synthesis of sterols, glycerophospholipids, sphingolipids, and the formation of lipid droplets (LDs) (Fu et al. 2012b; Walther et al. 2017). The ER hosts elegant and robust adaptive pathways that maintain cellular and systemic homeostasis. For example, under stress conditions, the ER activates the unfolded protein response (UPR) to reestablish ER homeostasis by reducing protein synthesis, increasing expres-

Editors: Susan Ferro-Novick, Tom A. Rapoport, and Randy Schekman
Additional Perspectives on The Endoplasmic Reticulum available at www.cshperspectives.org

 doi: 10.1101/cshperspect.a041261
Cite this article as *Cold Spring Harb Perspect Biol* 2023;15:a041261

sion of ER chaperones, promoting membrane expansion, and facilitating protein disposal (Schröder and Kaufman 2005; Hotamisligil 2010; Walter and Ron 2011). ER membrane expansion has also been shown to restore ER health independent of UPR signaling (Schuck et al. 2009). Protective mechanisms have also evolved to sense and maintain ER membrane lipid levels. For example, SREBP2, a transcription factor localized to the ER membrane, responds to alterations in ER sterol content by regulating the transcription of genes involved in cholesterol homeostasis (Goldstein et al. 2006). Although all these adaptive mechanisms work efficiently when ER faces stress transiently, such as during daily cycles of fasting and feeding, they fail when the stress or the increased functional demand becomes chronic, such as in the case of obesity. The resulting ER dysfunction in multiple tissues underlies the development of a cluster of obesity-associated diseases such as type 2 diabetes, cardiovascular disease, and fatty liver disease, which represent major public health problems (Özcan et al. 2004; Rutkowski et al. 2008; Erbay et al. 2009; Gregor et al. 2009).

The mechanisms involved in the development of ER dysfunction in obesity are complex and multifactorial. For example, obesity leads to aberrant and insufficient activation of the UPR, which directly impacts glucose and lipid metabolism (Özcan et al. 2004; Wang et al. 2009; Yang et al. 2015). Obesity also leads to alterations in the activity of key ER-resident transcription factors, such as SREBPs and NFE2L1, as well as defective Ca^{2+} homeostasis (Kammoun et al. 2009; Arruda and Hotamisligil 2015; Bartelt et al. 2018). Excellent reviews covering these aspects of ER dysfunction in obesity are available, and therefore will not be discussed in depth in this article (Eizirik et al. 2008; Hotamisligil 2010; Fu et al. 2012b; Arruda and Hotamisligil 2015; Han and Kaufman 2016; Lemmer et al. 2021; Marciniak et al. 2021). Here, we discuss emerging data demonstrating that the ER responds to stress and fluctuations in metabolic demand by remodeling its structural organization and its ability to form contact sites with other organelles. We also discuss how changes in the spatial organization of the ER affect metabolic function, and how the lack of this structural flexibility is a key problem associated with ER dysfunction in obesity.

COMPARTMENTALIZATION OF ER FUNCTION THROUGH DISTINCT STRUCTURAL SUBDOMAINS

The ER is formed by a continuous network of membranes that comprise distinct subdomains: the perinuclear ER and the peripheral ER. The peripheral ER presents itself as a network of tubules devoid of ribosomes (smooth ER) as well as parallel stacked flat sheets, which is often, but not always, characterized by the presence of ribosomes (rough ER) (Shibata et al. 2010; Chen et al. 2013; Goyal and Blackstone 2013; Westrate et al. 2015). More recently, it has also been shown that the tubular ER can be organized as dense ER matrices (Nixon-Abell et al. 2016). The ER also forms physical contact sites with every other organelle in the cell in addition to the plasma membrane (PM) (Phillips and Voeltz 2016; Cohen et al. 2018; Wu et al. 2018). Although the presence and basic functions of ER have been conserved across species, the evolution has introduced a diverse complexity to its spatiotemporal organization, which correlates with the functional demands of the cells. For example, cells with high protein secretory capacity, such as pancreatic acinar cells, which produce and secrete digestive enzymes, contain a high level of stacked rough ER sheets (Fig. 1) (Palade and Siekevitz 1956; Terasaki et al. 2013). This is also the case for intestinal goblet cells, which secrete glycoproteins that make the intestinal mucus. Conversely, cells specialized in the synthesis and secretion of steroid hormones or lipids such as Leydig cells and adipocytes, contain predominantly smooth ER tubules. These cell types perform mainly one predominant function such as protein or lipid synthesis and maintain a more static structural state of the ER in its native tissue environment (Fig. 1) (Mori and Christensen 1980). In contrast, cells such as hepatocytes or enterocytes have pleiotropic functions, including high rates of protein and lipid synthesis and secretion, depending on metabolic demand. Additionally, hepatocytes and

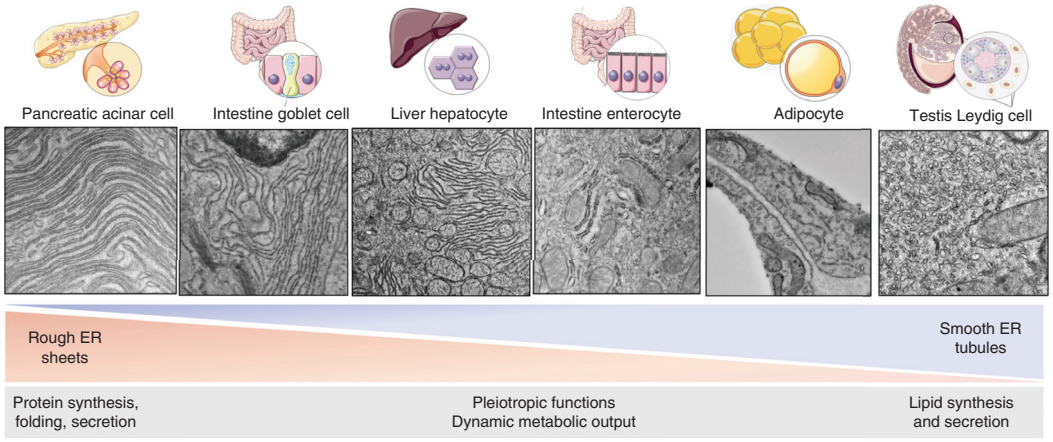

Figure 1. Distinct endoplasmic reticulum (ER) architecture across specialized cell types. Transmission electron microscopy images of specialized cells in their native tissue environment. (From *left* to *right*) Pancreatic acinar cells, intestinal Goblet cells, hepatocytes, enterocytes, adipocytes from white adipose tissue, and Leydig cells from testis. The images are derived from lean healthy mouse tissues and were acquired by authors Dr. Arruda and Dr. Parlakgül. (The cartoons representing the tissues were adapted from images from Servier Medical Art [smart.servier.com], which are licensed under a Creative Commons Attribution 3.0 Unported License.)

enterocytes are subjected to drastic metabolic transitions in response to nutritional fluctuations, such as cycles of fasting and feeding (Fu et al. 2012b; Rui 2014; Ko et al. 2020). Accordingly, these cells contain highly heterogenous and dynamic ER structures, which, presumably, support their pleiotropic functionality and metabolic flexibility (Fig. 1).

The shape of ER subdomains is determined by its interaction with the cytoskeleton and by the presence of transmembrane proteins that sculpt the ER membrane (Terasaki et al. 1986; Shibata et al. 2010; Goyal and Blackstone 2013; Joensuu et al. 2014; Lu et al. 2020; Zheng et al. 2022). In the next section, we will briefly introduce the main players involved in the regulation of peripheral ER architecture and discuss the evidence showing how the shape of ER relates to its metabolic function.

The Structure and Function of ER Sheets

ER sheets consist of two flat membranes separated by a constant luminal space and restricted in the edges by curved membranes. It is decorated by membrane-bound ribosomes and is often stacked in a parallel organization connected by a twisted membrane surface (Shibata et al. 2010; Terasaki et al. 2013). The space between the two parallel ER membranes is maintained by the oligomerization of a protein called Climp-63, a single-pass transmembrane protein that contains a large coiled-coil domain located in the ER lumen and a microtubule-binding domain located in its cytosolic side (Fig. 2) (Klopfenstein et al. 1998; Vedrenne et al. 2005; Shibata et al. 2010). Overexpression of Climp-63 in multiple cell lines, yeast, and in liver tissue leads to proliferation of ER sheets at the expense of ER tubules (Shibata et al. 2010; Parlakgül et al. 2022). Deletion of Climp-63 results in narrowing of the ER lumen but it does not abolish ER sheets (Shibata et al. 2010; Shen et al. 2019). Other proteins involved in ER sheet formation and stabilization include RRBP1 (or p180) and kinectin (KTN1) (Ogawa-Goto et al. 2007; Shibata et al. 2010). RRBP1 is an ER transmembrane protein that contains a small intraluminal domain and a large cytosolic domain that has been proposed to regulate the flatness of ER sheets. Recently it has also been shown that Climp-63, RRBP1, and KTN1 influence the shape and distribution of ER sheets by interacting with distinct subsets of cytoskeletal microtubules (Zheng et al. 2022). Therefore, cy-

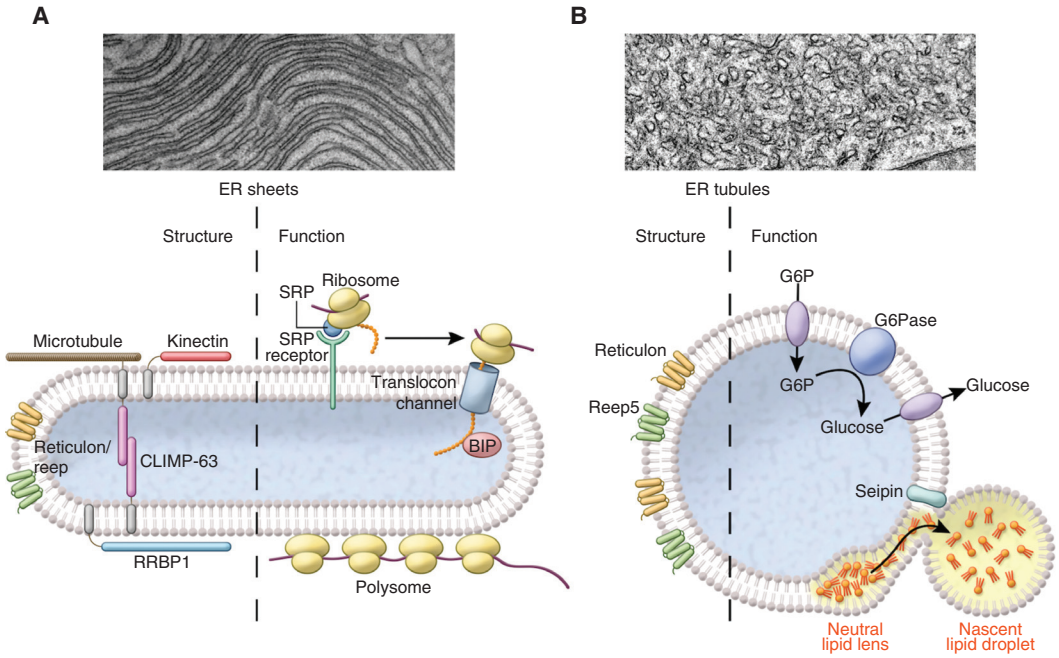

Figure 2. Structure and function of peripheral endoplasmic reticulum (ER) sheets and ER tubules. (*A*) (*Left* side) Membrane-shaping proteins such as Climp-63, p180 (RRBP1), and kinectin regulate the formation and stabilization of ER sheets. (*Right* side) ER sheets are the preferential site for membrane-bound polysome and translocon complex localization. Therefore, protein cotranslation and translocation preferentially occurs at this ER subdomain. First, the signal recognition particle (SRP) binds to a ribosome containing a nascent polypeptide. Next, the SRP-ribosome-nascent polypeptide complex interacts with the SRP receptor in the ER membrane. The nascent polypeptide chain is imported into the ER lumen through the translocon channel (SEC61 and associated proteins), where chaperones like BIP assist in protein folding. (*B*) (*Left* side) The high curvature of the tubular ER membranes is maintained by proteins containing the reticulon homology domain (RHD), such as reticulons and REEP5. (*Right* side) The ER tubules are enriched in proteins involved in lipid metabolism and are the main site of LD formation and budding, where seipin protein is localized. Another protein enriched in ER tubules is the glucose-6-phosphatase (G6Pase), an enzyme involved in the conversion of glucose-6-phosphate (G6P) to glucose. The catalytic domain of G6Pase is located in the ER lumen. Therefore, this reaction requires G6P to be imported from the cytosol to the ER lumen and then glucose to be exported from the ER lumen to the cytosol by a transporter. Hydrolysis of G6P is the last step of glucose production from gluconeogenesis and glycogenolysis.

toskeleton–ER interactions are essential for the determination of the shape and dynamics of ER sheets.

Functionally, ER sheets are the preferential site of protein synthesis, folding, and post-translation modification (Sabatini and Blobel 1970; Kreibich et al. 1978; Amar-Costesec et al. 1989; Voeltz et al. 2002; Shibata et al. 2006). Indeed, as mentioned earlier, cells specialized in protein synthesis and secretion, such as acinar cells and Goblet cells, are enriched in rough ER sheets and almost devoid of ER tubules (Fig. 1). There are at least two main characteristics of ER sheets that seem to facilitate protein synthesis, translocation and folding. First, their flat surface accommodates large, membrane-bound polysomes where nascent polypeptides are synthesized (Shibata et al. 2010; Terasaki et al. 2013). In fact, ER sheets are enriched with the translocon complex formed by the Sec61 channel, the signal recognition particle (SRP) receptor and its associated proteins, responsible for the translocation of nascent polypeptides from translating ribosomes into or across the ER membrane (Park and Rapoport 2012; Reid and Nicchitta 2015). Second, ER sheets have a large luminal volume-

to-surface ratio compared to ER tubules, which may optimize processes occurring in the ER lumen, such as protein folding (Schuck et al. 2009; Terasaki et al. 2013; Westrate et al. 2015). Of note, although evidence points to ER sheets as the preferential site of protein synthesis and folding, this division of tasks is not absolute and protein synthesis may also occur in tubular ER, albeit to a lesser degree.

The Structure and Function of ER Tubules

The main players involved in shaping ER tubules are the reticulons (Rtns) and the receptor expression-enhancing protein 5 (REEP5 or DP1) (Voeltz et al. 2006; Shibata et al. 2010; di Sano et al. 2012; Wang et al. 2021). These proteins share a common, evolutionarily conserved reticulon homology domain (RHD), which forms two short hairpins that incompletely span the two leaflets of the ER membrane, displacing more of the outer leaflet than the inner, which generates membrane curvature (Fig. 2) (Voeltz et al. 2006; Shibata et al. 2008). Contrary to ER sheets, the curved membrane of ER tubules seems not to accommodate large polyribosomes, and therefore presents a smooth surface. Overexpression of reticulon 4 isoform a (Rtn4a) or REEP5 induces proliferation of ER tubules at the expense of ER sheets. On the other hand, down-regulation of these proteins leads to the conversion of ER tubules into ER sheets (Voeltz et al. 2006; Shibata et al. 2010). The formation of the tubular ER network also depends on small GTPases called atlastins (ATLs), which mediate tubule fusion and the formation of three-way junctions. Lastly, both the morphology and dynamics (shrinkage and expansion) of tubular ER are supported by its interaction with the cytoskeleton. In fact, depolymerization of microtubules collapse the tubular ER network (Terasaki et al. 1986; Waterman-Storer et al. 1995; Shibata et al. 2008).

Functionally, the tubular ER has been shown to play a key role in lipid metabolism. For example, the tubular ER is enriched in proteins involved in LD formation (Fig. 2) (Jacquier et al. 2011; Choudhary et al. 2015; Walther et al. 2017). The process of LD formation starts with the accumulation of neutral lipids within the tubular ER membrane bilayer. When the accumulation of neutral lipids reaches a threshold, phase separation occurs, leading to the nucleation of a lipid lens, which grows and buds to the cytosol as an LD (Wilfling et al. 2013; Walther et al. 2017; Olzmann and Carvalho 2019). The proper formation of LD largely depends on seipin, an integral membrane protein predominantly located at ER tubule–LD contacts (Salo et al. 2016; Wang et al. 2016; Cao et al. 2019). The high curvature of ER tubules is shown to facilitate the formation of LDs by locally disturbing the bilayer order (Jacquier et al. 2011; Kassan et al. 2013). Additionally, it reduces the energy barrier required for neutral lipid condensation (Santinho et al. 2020). In fact, genetic manipulations that drive ER tubule proliferation such as overexpression of REEP5 or Rtn4 result in increased LD biogenesis (Santinho et al. 2020).

In addition to enzymes involved in lipid metabolism, proteomic analysis of tubular ER isolated from yeast showed that this subdomain is enriched in proteins involved in vesicle-based trafficking, organelle contacts, and ER signaling (Wang et al. 2017). In hepatocytes, tubular ER is also enriched in enzymes involved in glucose metabolism, such as glucose 6-phosphatase, which catalyzes the conversion of glucose-6-phosphate to glucose, the last step of gluconeogenesis (Fig. 2) (Garfield and Cardell 1979; Csala et al. 2006). Although it is currently unknown whether and, if so, how the shape of tubular ER facilitates these processes, the fact that these enzymes are enriched in tubular ER suggests a structure–function relationship.

Structure and Metabolic Function of ER Membrane Contact Sites

Another property of the ER, especially the tubular ER, is its ability to form membrane contact sites (MCSs) with every other organelle and the PM (Phillips and Voeltz 2016; Cohen et al. 2018). MCSs are regions of close proximity between organelles (around 15–30 nm) maintained by tethering proteins or by protein–lipid interactions. Through these physical interactions, ER can act as a conduit, allowing organelles to exchange ions, lipids, and metabolites to execute coopera-

tive metabolic processes across and beyond organelle borders, without disturbing the entire cytosol. ER MCSs have been shown to be involved in many cellular functions and this is a rapidly expanding field.

For example, some of the enzymes participating in biochemical pathways that require the synthesis or transport of hydrophobic molecules are hosted in different organelles, resulting in the splitting of metabolic processes (see details in Fig. 3). This is the case for enzymes involved in phospholipid synthesis (Vance 1990), steroid hormone synthesis (Issop et al. 2015; Prasad et al. 2015), or coenzyme Q synthesis pathways, whose enzymes are located at ER and mitochondria interfaces known as MAMs (mitochondria-associated membranes) (Eisenberg-Bord et al. 2019; Subramanian et al. 2019). The presence of MCSs facilitate the shuttling of molecules between these organelles and by doing so, regulate the activity of these metabolic pathways.

The ER MCSs also enable the transfer of ions such as Ca^{2+} between organelles. For example, MAMs facilitate Ca^{2+} flux from ER to mitochondria, which is known to regulate mitochondrial oxidative function, redox balance, and apoptosis (Fig. 3). Ca^{2+} transport also occurs at MCSs between the ER and PM through the store-operated calcium entry (SOCE), a mechanism involved in the control of ER and cytosolic Ca^{2+} levels. ER and endosomes also exchange Ca^{2+} through contact sites, although the functional significance of this process is unclear (Rizzuto et al. 1998; Scorrano et al. 2003; Arruda and Hotamisligil 2015; Phillips and Voeltz 2016).

The formation of ER MCSs has also an impact on the morphology and dynamics of other organelles (Fig. 3). For example, in COS-7 (a monkey kidney cell line) and other cells, mitochondrial and endosomal fission occur at sites of contacts with the ER (Friedman et al. 2011; Rowland et al. 2014). Interestingly, an inverse relationship is also possible, wherein the interaction between the ER and the lysosomes or late endosomes regulates ER morphology through the ability of these organelles to pull or shrink tubular ER membranes (Lu et al. 2020; Spits et al. 2021).

Interestingly, the MCSs between ER and other organelles are regulated not only by the abundance and localization of membrane tethering proteins but also by the general shape of the ER. An example of that is seen in the reduced frequency of MAMs when ER sheet formation is artificially enhanced through Climp-63 overexpression in COS-7 cells (Lewis et al. 2016) and hepatocytes in vivo (Parlakgül et al. 2022). On the other hand, down-regulation of Rtn4 in mouse embryonic fibroblasts (MEFs) leads to decreased ER tubulation and a marked decrease in ER-PM contact sites (Jozsef et al. 2014). These findings suggest that the ratio between ER sheets and tubules affects the number of MCSs and impacts biological processes coordinated by them.

IMPACT OF STARVATION ON ER STRUCTURE AND FUNCTION

As outlined above, ER is a central platform of metabolic regulation, and its complex structural organization is directly linked to the diversity and robustness of its metabolic capacity. Alterations in metabolic states promoted by changes in nutrient availability impact ER function and its adaptive capacity, at least in part by inducing the remodeling of its spatial organization. This is the case, for example, of when cells face nutrient starvation. Starvation is characterized by a state of extreme fasting, which cause cells to rewire their metabolism to decrease energy-demanding processes such as protein synthesis and to optimize the use of endogenous substrates through the activation of processes such as autophagy. During prolonged starvation, substrates are eventually depleted, and the cells enter a state of stress and, ultimately, death.

In cell lines, starvation has been shown to alter ER structure and MCSs in multiple ways. For example, starvation induces narrowing of ER-mitochondria contact sites (MAMs), which in some cases results in increased mitochondrial oxidative capacity but in others leads to overflow of Ca^{2+} from ER to the mitochondria, mitochondrial Ca^{2+} overload, and apoptosis (Csordás et al. 2006; Cieri et al. 2018). Increased MAMs during starvation has also been shown to impact the regulation of autophagy, as the formation autophagosome occurs, at least in part, at MAMs (Hamasaki et al. 2013; Bosc et al. 2020). Starva-

Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

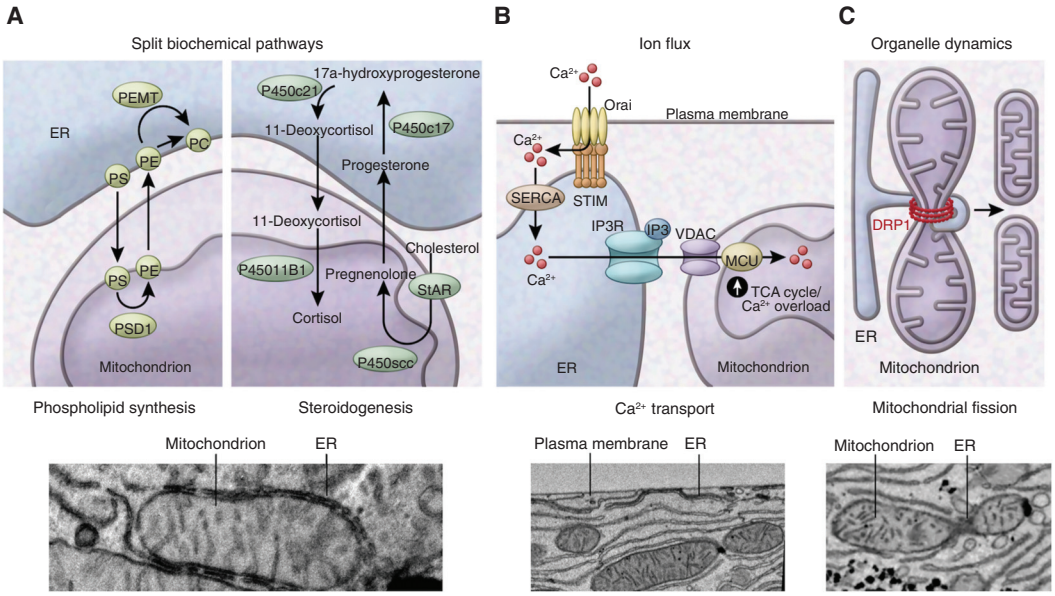

Figure 3. Functions of endoplasmic reticulum (ER) membrane contact sites. (*A*) Examples of split biochemical pathways for which enzymes are located in different organelles requiring trafficking of metabolites through inter-organelle contact sites. Phospholipid synthesis: The phosphatidylserine (PS) present in the ER membrane is shuttled to the inner mitochondria membrane, where it is decarboxylated by the PS decarboxylase (PSD1) generating phosphatidylethanolamine (PE). Mitochondrial PE can be then shuttled back to the ER and be converted to phosphatidylcholine (PC) through the phosphatidylethanolamine *N*-methyltransferase (PEMT) (Vance 1990). Steroidogenesis: Steroid hormones originate from cholesterol and require the shuttling of intermediates across the ER and mitochondria (Miller 2007). Cholesterol gets into the inner mitochondrial membrane through the steroidogenic acute regulatory protein (StAR), which is shown to be located at the mitochondria-associated membranes (MAMs) (Prasad et al. 2015). In the mitochondria, the cholesterol side-chain cleavage enzyme (P450scc) catalyzes the conversion of cholesterol into pregnenolone. Next, pregnenolone is converted to progesterone by the enzyme 3β-hydroxysteroid dehydrogenase (3βHSD). In the next step, 17α-hydroxylation of progesterone occurs, which is catalyzed by the enzyme CYP17A1 in the ER. Steroid 21-hydrolase (P450c21) then converts 17α-hydroxyprogesterone into 11-deoxycorticosterone and 11-dexycortisol, respectively. Last, 11β-hydroxylase (P450c11β1) completes the synthesis of cortisol in the mitochondria. Alterations in proteins such as ATAD3, which stabilize MAMs in steroidogenic cells, have been shown to result in decreased hormone-stimulated steroid hormone synthesis (Issop et al. 2015). (*B*) Ca^{2+} flux at the MAMs: Ca^{2+} transport from ER to mitochondria occurs through the IP3R, located in the ER membrane, the mitochondrial anion transporter (VDAC), located at the outer mitochondrial membrane, and the MCU, located in the inner mitochondrial membrane. In the mitochondria, Ca^{2+} regulates the TCA cycle dehydrogenases, thus modulating the rate of NADH production and ATP synthesis. Excess Ca^{2+} flux from ER to mitochondria leads to mitochondrial Ca^{2+} overload and apoptosis. ER Ca^{2+} levels are also regulated through the store-operated Ca^{2+} entry (SOCE) at ER-PM contact sites. SOCE is mediated by the physical coupling between STIM, an ER protein, and Orai, a Ca^{2+} channel located at the PM, which allows Ca^{2+} entry from the extracellular compartment to the cytosol, and then into the ER through SERCA. (*C*) Regulation of organelle fission at ER contact sites: Sites of physical interactions between ER and mitochondria are shown to recruit key proteins involved in mitochondria fission such as DRP1. At the MAMs, DRP1 homo-oligomerizes, forming rings that help the ER tubule to constrict the mitochondria, leading to mitochondria fission.

tion also impacts the formation of ER contact sites with lysosomes, which is critical for the dynamics of the ER tubular network. Short-term starvation of COS-7 cells for a few hours leads to accumulation of lysosomes in the perinuclear regions of the cell with a reduction in ER tubules in the periphery of the cell. Prolonged starvation of 24 h, however, leads to redistribution of lysosomes in the entire cell, which increases the ER tubular network (Lu et al. 2020). Therefore, by changing the intracellular position of lysosomes, a key nutrient-sensing organelle, cells can rapidly alter ER architecture in response to nutritional challenges. Lastly, nutrient starvation also leads to remodeling of the ER through the induction of ER-phagy, a specific type of autophagy where portions of ER are targeted to degradation for substrate recycling. ER-phagy is mediated by specific receptors, and, depending on the receptor triggering the process, this results in degradation of ER sheets or ER tubules, thus influencing the general organization of the ER (Hübner and Dikic 2020).

An interesting question is whether the architectural alterations of the ER induced by starvation in cell culture models are also part of the adaptive mechanisms to fasting in specialized metabolic tissues such as the liver. In cell culture, starvation is modeled by maintaining the cells in media with low levels of nutrients in the absence of serum. However, when considering the physiologic response of a mammalian organism to the lack of nutrient intake, each tissue responds very differently. For example, in the hepatocytes, fasting leads to increased uptake of lipids from the circulation, which originates from increased lipolysis in the adipose tissue. Also, during fasting, hepatocytes rewire their glucose metabolism to enhance glucose production through glycogenolysis and gluconeogenesis (Petersen et al. 2017). Fasting also induces ketone body production through fatty acid oxidation (Kersten et al. 1999; Rui 2014; Trefts et al. 2017). Therefore, fasting in hepatocytes is not equivalent to a lack of nutrient input, as modeled in cell culture, but rather to a change in the type of nutrients being used, stored, and exported. In fact, earlier ultrastructural work has shown that fasting leads to changes in hepatocyte ER organization such as the induction of tubular ER proliferation (Striffler et al. 1981) and alterations in ER contacts sites with mitochondria and peroxisomes (Sood et al. 2014; Ilacqua et al. 2022). However, a detailed analysis of the effects of fasting/feeding cycles on the structural organization of the ER and its functional implications in a tissue-specific context is lacking and is an interesting area of future research.

IMPACT OF OBESITY ON ER STRUCTURE AND FUNCTION

Although the complex regulation of ER architecture on the tissue level in response to fasting/feeding cycles is not yet well understood, there is evidence that disrupted ER architecture and MCSs is an important component of ER dysfunction in obesity-linked chronic overnutrition. Likely considering their critical metabolic role, most studies in this area have focused on hepatocytes in the liver; thus, our discussion has an emphasis on this tissue.

The liver plays a central role in coordinating whole body homeostasis and secretes a high number of proteins, lipids, lipoproteins, and glucose into circulation depending on the systemic metabolic demand. However, in obesity, overconsumption of lipids, carbohydrates, and calories promotes chronic stress to liver cells, resulting in altered hormonal responses such as insulin resistance and dysregulated glucose and lipid metabolism. Over time, imbalanced liver energy metabolism drives the development of nonalcoholic fatty liver disease (NAFLD), a pathological condition characterized by excessive hepatic lipid accumulation (steatosis), uncontrolled glucose production, and increased glycogen storage (Unger et al. 2010; Loomba et al. 2021). This excessive nutrient accumulation in the liver not only disturbs metabolic networks but also poses a significant physical challenge to the hepatocyte subcellular organization.

Impact of Obesity in General ER Architectural Organization

Detailed 3D ultrastructural analysis of mouse hepatic ER in its native tissue environment has

shown that in the fed state, hepatic ER comprises a mixture of parallel organized ER sheets and a network of ER tubules, with an ER sheet-to-tubule ratio of ~53% (Fig. 4) (Parlakgül et al. 2022). However, hepatic ER of mice with genetic obesity (leptin-deficient mice, *ob/ob*) undergoes a marked remodeling, characterized by the loss of parallel organized rough ER sheets and increased abundance of smooth ER tubules, which causes a decrease in the ER sheet-to-tubule ratio to ~23% (Fig. 4) (Parlakgül et al. 2022). This obesity-linked decrease in ER sheets is correlated with decreased expression of the ER sheet-shaping proteins such as Climp-63 and p180, and increased expression of proteins that shape the tubular ER (e.g., Rtn4 and REEP5) (Parlakgül et al. 2022). Increased Rtn4 expression has also been reported in livers from high-fat diet (HFD)-induced obese mice (Zhang et al. 2020). Whether alterations in the abundance of the ER-shaping proteins in the liver are the primary cause or the consequence of the shifts in ER organization in obesity remains to be determined.

Remodeling of the ER structure in obesity could be also related to an aberrant lipid composition of the ER membrane. Obesity leads to alterations in the ratio and saturation levels of the two most abundant membrane phospholipids, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) (Fu et al. 2011). Additionally, obesity leads to accumulation of lipids such as 1,2 diacylglycerol (DAG) (Lyu et al. 2020) and cholesterol (Bashiri et al. 2016) in the ER, which could alter fluidity and curvature of the lipid bilayers. Although the impact of ER membrane lipid composition on ER shape is not well understood, there are clues that it may have an important influence. For example, in Chinese Hamster Ovarian (CHO) cells, palmitate treatment induces saturation of PC in the ER membrane, which is associated with compromised ER structure and integrity (Borradaile et al.

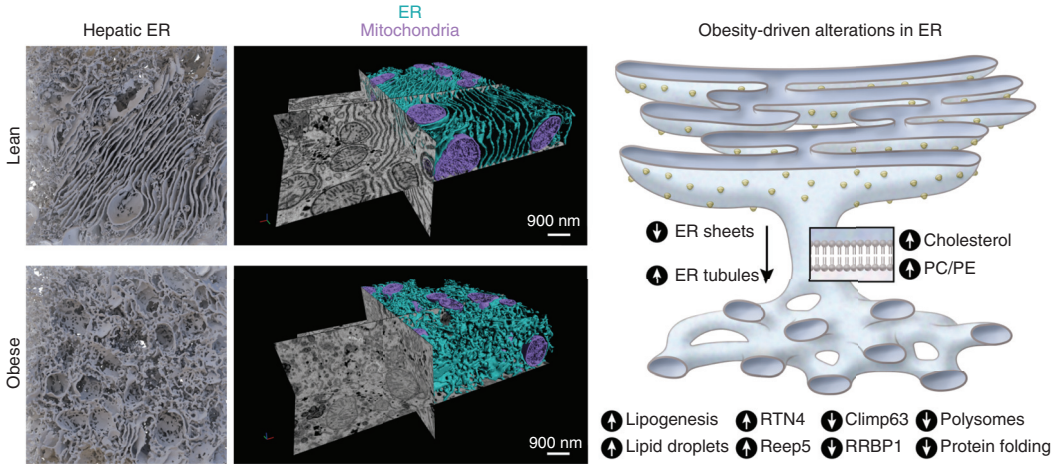

Figure 4. Obesity leads to loss of parallel endoplasmic reticulum (ER) sheets and predominance of ER tubules in the liver. (*Left* panel) Three-dimensional (3D) reconstruction of segmented ER morphology from livers derived from lean (*upper* panel) and obese (*ob/ob*) (*lower* panel) mice ($1000 \times 1000 \times 400$ pixels—$8 \times 8 \times 3.2$ μm^3). (*Middle* panel) Reconstruction of hepatic ER and mitochondria segmentation on raw FIB-SEM images of lean (*upper* panel) and obese (*ob/ob*) mice (*lower* panel). (*Right* panel) The cartoon depicts the transition of ER subdomains promoted by obesity. Obesity leads to decreased parallel organized rough ER sheets and increased smooth ER tubules, resulting in a decreased ER sheet-to-tubule ratio. These alterations are accompanied by changes in the expression of ER-shaping proteins. While Climp-63 and RRBP1 (p180) levels are decreased, the expression levels of Reticulon 4 (Rtn4) and REEP5 are increased. Accordingly, obesity leads to decreased membrane-bound-polysome content. On the other hand, de novo lipogenesis and lipid droplet accumulation are increased in obesity. (The *left* and *middle* images shown in this figure are reprinted from Parlakgül et al. 2022; with permission from the authors through an agreement with Springer Nature 2022.)

2006). In oocytes, depletion of DAGs in ER membranes results in enrichment of ER sheet-like structures, whereas increasing DAGs induces tubular ER formation (Ulloa et al. 2019). Alterations in ER membrane lipid composition also affect the function and localization of ER membrane proteins, including those that actively shape ER. For example, an increased PC/PE ratio and cholesterol in ER membrane leads to malfunction of enzymes embedded in the ER membrane such as the sarco/ER Ca^{2+} ATPase (SERCA) (Li et al. 2004; Fu et al. 2011). Additionally, perturbations of ER membrane lipid composition and saturation have also been shown to trigger the activation of key members of the UPR such as serine/threonine-protein kinase/endoribonuclease (IRE1) (Volmer et al. 2013; Halbleib et al. 2017). Unraveling the relationship between membrane lipid composition and ER remodeling in obesity remains an interesting and open area of investigation.

The functional and metabolic consequences of ER remodeling in obesity is just beginning to be understood. The decrease in rough ER sheets in livers of obese mice is accompanied by decreased ER protein synthesis, decreased binding of polysomes to ER membrane, and reduced translation rates (Fu et al. 2012a). In addition, protein folding in the ER is compromised in obesity, which leads to activation of the UPR (Özcan et al. 2004; Malhi and Kaufman 2011; Yang et al. 2015). Interestingly, in hepatocytes of obese mice, increasing ER sheet formation by exogenous overexpression of Climp-63 increases ER folding capacity and relocation of chaperone GRP78 to rough ER sheets. This results in lower markers of stress such as CHOP and ATF4. Gain-of-function of Climp-63 also improves hepatic insulin sensitivity, systemic glucose metabolism, and lipid accumulation in the liver (Parlakgül et al. 2022). Similarly, loss-of-function of Rtn4b, which drives formation and stabilization of ER tubules, protects mice from HFD-induced obesity and increases liver insulin sensitivity (Zhang et al. 2020). More recently, it has been shown that deletion of RHBDL4, an ER-resident rhomboid protease that interacts with Climp-63, leads to disturbed ER sheets, hepatocyte stress, and development of liver steatosis (Lastun et al. 2021). Hence, alterations in ER architecture have an important impact on ER dysfunction in obesity, highlighting the potential of shaping the ER as a therapeutic measure to treat fatty liver disease and diabetes.

Impact of Obesity on ER Inter-Organelle Contact Sites

In addition to alterations in the ER sheet-to-tubule ratio, obesity leads to aberrant formation of ER MCS with other organelles. For example, hepatocytes from obese mice display chronic enrichment in ER contact sites with the mitochondria through MAMs (Arruda et al. 2014). Also, hepatic MAM content positively correlates with the severity of fatty liver disease in humans (Feriod et al. 2017) and is increased in obesity in other tissues such as skeletal muscle as well (Thoudam et al. 2019). Protein expression analysis of MAMs isolated from livers of lean and obese mice show that both the quantity and relative protein composition of MAMs are altered in this condition. For example, obesity leads to increased expression of IP3R, the channel that releases Ca^{2+} from ER to cytosol/mitochondria, and PACS-2, a MAM-tethering protein (Arruda et al. 2014). Increased levels of IP3R and PACS-2 at MAMs have also been shown in a model of fatty liver disease caused by the deletion of mitofusin 2 (Mfn-2) (Hernández-Alvarez et al. 2019).

Ca^{2+} imaging experiments showed that increased levels of IP3R in the MAMs of hepatocytes isolated from obese mice lead to excess Ca^{2+} flux from ER to mitochondria. This results in mitochondrial Ca^{2+} overload, increased reactive oxygen species (ROS) production, and the activation of inflammatory signaling such as the phosphorylation of the c-Jun amino-terminal kinase (JNK) (Fig. 5) (Arruda et al. 2014). Increased ROS and JNK activity are well known mediators of insulin resistance in hepatocytes (Hirosumi et al. 2002; Ayer et al. 2021). In fact, liver-specific down-regulation of PACS-2 and IP3R1 in obese mice decreased ER-to-mitochondria calcium flux and JNK phosphorylation, resulting in improved mitochondrial function, insulin sensitivity, and systemic glucose metabo-

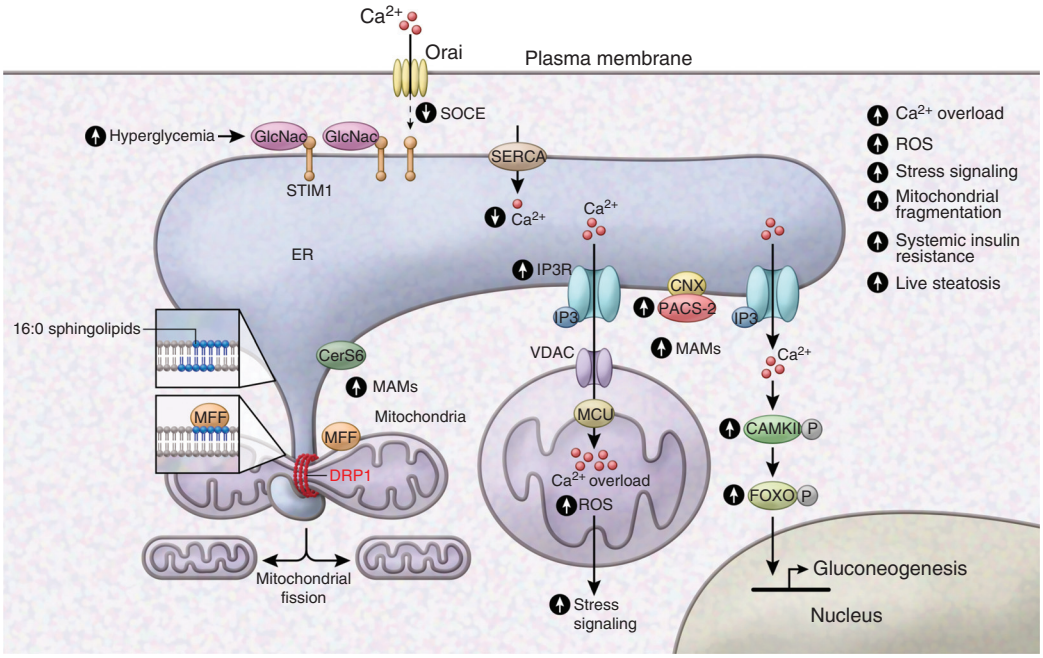

Figure 5. Obesity-driven alterations in endoplasmic reticulum (ER) contact sites in the liver. Obesity leads to chronic enrichment in mitochondria-associated membranes (MAMs) and increased expression of MAM proteins such as the inositol trisphosphate receptor isoform 1 (IP3R1) and phosphofurin acidic cluster sorting protein 2 (PACS-2). Increased MAMs and IP3Rs contributes to lower ER Ca^{2+} levels and higher mitochondrial Ca^{2+}, which leads to higher ROS production and mitochondrial dysfunction. Increased IP3R1 activity in obesity also leads to increased cytosolic Ca^{2+} and phosphorylation of CaMKII, which in turn phosphorylates FOXO, with a direct impact on gluconeogenesis. Obesity leads to alterations in sphingolipid composition of the MAMs, driven by the enrichment of ceramide synthase CerS6 at these contact sites. Increased $C_{16:0}$ sphingolipids binding to the mitochondrial fission factor (Mff) leads to the recruitment of DRP1 to the mitochondria triggering mitochondrial fragmentation and dysfunction. Down-regulation of CerS6 in the liver protects from obesity and insulin resistance. Obesity also affects processes at the ER-PM contact sites, such as store-operated Ca^{2+} entry (SOCE). In obesity, SOCE is dysregulated due to defective STIM1 translocation, caused, at least in part, by aberrant STIM1 *O*-GlycNAcylation, a post-translational modification involving the addition of *O*-linked *N*-acetylglucosamine (GlcNac) to serine and threonine amino acids.

lism (Fig. 5) (Arruda et al. 2014). Down-regulation of IP3R in livers of obese mice has also been shown to decrease hepatic gluconeogenesis by inhibiting the activation of CaMKII and the transcription factor CRTC2 (Wang et al. 2012; Ozcan et al. 2013). In another study, hepatic deletion of HCLS1-Associated Protein X-1 (HAX-1), a protein that interacts and positively regulates IP3R1, led to decreased Ca^{2+} flux from ER to mitochondria, increased mitochondria respiration, and protection against diet-induced liver steatosis (Alogaili et al. 2020). Therefore, restoring MAM abundance and Ca^{2+} signaling in hepatocytes of obese mice results in positive outcomes for systemic glucose and lipid metabolism and holds the potential to be used as a target for treating fatty liver disease.

In addition to alterations in Ca^{2+} signaling, chronic exposure of mice to HFD also leads to alterations in lipid signaling at MAMs. For example, HFD induces the expression of the enzyme ceramide synthase 6 (CerS6), which drives the accumulation of 16:0 ceramides in hepatocyte MAMs. Mass spectrometry-based approaches have demonstrated that ceramides at MAMs are able to bind to the protein mitochon-

drial fission factor (Mff), inducing mitochondria fission. Conversely, deletion of CerS6 in the liver of mice exposed to HFD protects against fatty acid–induced mitochondrial fragmentation and ameliorates hepatic steatosis (Hammerschmidt et al. 2019). Altogether, these data show that alterations of MAMs in obesity affect both ER Ca^{2+} and lipid signaling, highlighting the role of MAMs a major hub of metabolic alterations in this condition (Fig. 5).

The precise cause of increased MAM formation in obesity is still unclear. On the one hand, increased MAMs could result from up-regulation of proteins shown to stabilize the MAMs, such as PACS-2 and other tethering proteins. However, it is also possible that increased MAMs result from the phenotypic switch to dominance of ER tubules observed in hepatocytes from obese mice (Parlakgül et al. 2022). Indeed, in cell lines, overexpression of Rtns leads to increased ER tubules and ER-mitochondria contacts (Reali et al. 2015). Additionally, it was also shown that changes in saturation of phospholipids such as PC and PE impact MAM content and dynamics. As obesity leads to increased saturation of phospholipids in the ER membrane, this mechanism could have relevance for obesity-induced alterations in MAMs. Notably, although many studies describe an increase in MAM content in the liver and muscle in obesity, it is also shown that obesity resulted in decreased MAM content in these tissues (Tubbs et al. 2014, 2018). This discrepancy may be associated with the sensitivity, specificity, and spatial resolution of the methods used by the different studies and/or nutritional status of the animals. Regardless, there is general agreement that obesity is associated with aberrant MAM content and function.

In addition to MAMs, obesity also impacts ER function at ER-PM MCSs by altering SOCE (Wilson et al. 2015; Arruda et al. 2017). SOCE is mediated by the physical coupling of the ER protein STIM and the PM calcium channel Orai induced by depletion of luminal ER Ca^{2+} levels. The physical association of STIM and Orai allows Ca^{2+} entry from the extracellular compartment to the cytosol, and then into the ER through SERCA. This process is important to replenish ER Ca^{2+} concentration, which is necessary for proper chaperone activity and protein folding in the ER lumen. SOCE is impaired in the livers of obese mice, which is partially driven by enhanced STIM1 *O*-GlcNAcylation, a post-translational modification caused by the addition of *O*-linked *N*-acetylglucosamine (GlcNac) to serine and threonine amino acids. Increased STIM1 *O*-GlcNAcylation leads to defective STIM1 translocation to the ER-PM interface and decreased Ca^{2+} import. Overexpression of STIM1 in livers of obese mice leads to increased STIM1 translocation and cortical ER-PM MCS, resulting in improved SOCE and systemic glucose tolerance (Fig. 5; Arruda et al. 2017). Defects in SOCE have also been observed in mouse models of diabetes, implying SOCE in β-cell health and insulin secretion capacity (Kono et al. 2018).

In addition to regulating Ca^{2+} signaling, ER-PM MCS also controls the trafficking of cellular lipids such as cholesterol (Sandhu et al. 2018) and phosphatidylinositol (Lees et al. 2017; Bian et al. 2018). Therefore, alterations in the capacity of ER to form PM contacts in obesity may have relevance beyond changes in Ca^{2+} signaling. In summary, the loss of the structural flexibility of the ER in obesity leads to miscommunication of organelles and to dysregulation of organelle function, eventually leading to the development of cellular and systemic pathologies such as insulin resistance and fatty liver disease.

CONCLUDING REMARKS

ER is a heterogenous and dynamic organelle, and its various metabolic functions are compartmentalized in distinct subdomains. Through establishing and terminating ER contact sites with other organelles and/or rearranging the relative abundance of its subdomains, ER can regulate the diversity and activity of metabolic signaling to maximize cellular function. In this article, we discussed evidence showing that oscillations in nutrient availability lead to structural remodeling of the ER and the disruption of this flexibility leads to ER dysfunction in obesity-linked metabolic diseases. However, there remain key questions to be answered: What are the detailed mechanisms through which changes in ER shape translate into alterations of metabolic function? What

are the molecular mechanisms involved in the remodeling of ER structural domains in obesity? Does it involve hormonal signaling? How do changes in ER structure and contact sites integrate with well-known mechanisms of ER dysfunction, such as UPR or autophagy? Addressing these issues will be required to better understand the ER structure–function relationship and to translate our understanding of ER structural dynamics into therapeutic interventions for metabolic diseases.

ACKNOWLEDGMENTS

We are grateful to Kathryn Claiborn, Alexander Bartelt, Scott Widenmaier, Hatoon Baazim, and Zhe Cao for their critical reading and comments. We are thankful to Bruce Worden for assistance with the illustrations. A.P.A. is a Chan Zuckerberg Biohub investigator. We apologize to any authors whose important contributions were not cited due to space limitations.

REFERENCES

Alogaili F, Chinnarasu S, Jaeschke A, Kranias EG, Hui DY. 2020. Hepatic HAX-1 inactivation prevents metabolic diseases by enhancing mitochondrial activity and bile salt export. *J Biol Chem* **295:** 4631–4646. doi:10.1074/jbc.RA119.012361

Amar-Costesec A, Dublet B, Beaufay H. 1989. Translocation and proteolytic processing of nascent secretory polypeptide chains: two functions associated with the ribosomal domain of the endoplasmic reticulum. *Biol Cell* **65:** 99–108. doi:10.1111/j.1768-322X.1989.tb00778.x

Arruda AP, Hotamisligil GS. 2015. Calcium homeostasis and organelle function in the pathogenesis of obesity and diabetes. *Cell Metab* **22:** 381–397. doi:10.1016/j.cmet.2015.06.010

Arruda AP, Pers BM, Parlakgül G, Güney E, Inouye K, Hotamisligil GS. 2014. Chronic enrichment of hepatic endoplasmic reticulum-mitochondria contact leads to mitochondrial dysfunction in obesity. *Nat Med* **20:** 1427–1435. doi:10.1038/nm.3735

Arruda AP, Pers BM, Parlakgul G, Güney E, Goh T, Cagampan E, Lee GY, Goncalves RL, Hotamisligil GS. 2017. Defective STIM-mediated store operated Ca^{2+} entry in hepatocytes leads to metabolic dysfunction in obesity. *eLife* **6:** e29968. doi:10.7554/eLife.29968

Ayer A, Fazakerley DJ, James DE, Stocker R. 2022. The role of mitochondrial reactive oxygen species in insulin resistance. *Free Radic Biol Med* **179:** 339–362. doi:10.1016/j.freeradbiomed.2021.11.007

Bartelt A, Widenmaier SB, Schlein C, Johann K, Goncalves RLS, Eguchi K, Fischer AW, Parlakgül G, Snyder NA, Nguyen TB, et al. 2018. Brown adipose tissue thermogenic adaptation requires Nrf1-mediated proteasomal activity. *Nat Med* **24:** 292–303. doi:10.1038/nm.4481

Bashiri A, Nesan D, Tavallaee G, Sue-Chue-Lam I, Chien K, Maguire GF, Naples M, Zhang J, Magomedova L, Adeli K, et al. 2016. Cellular cholesterol accumulation modulates high fat high sucrose (HFHS) diet-induced ER stress and hepatic inflammasome activation in the development of non-alcoholic steatohepatitis. *Biochim Biophys Acta* **1861:** 594–605. doi:10.1016/j.bbalip.2016.04.005

Bian X, Saheki Y, de Camilli P. 2018. Ca^{2+} releases E-Syt1 autoinhibition to couple ER-plasma membrane tethering with lipid transport. *EMBO J* **37:** 219–234. doi:10.15252/embj.201797359

Borradaile NM, Han X, Harp JD, Gale SE, Ory DS, Schaffer JE. 2006. Disruption of endoplasmic reticulum structure and integrity in lipotoxic cell death. *J Lipid Res* **47:** 2726–2737. doi:10.1194/jlr.M600299-JLR200

Bosc C, Broin N, Fanjul M, Saland E, Farge T, Courdy C, Batut A, Masoud R, Larrue C, Skuli S, et al. 2020. Autophagy regulates fatty acid availability for oxidative phosphorylation through mitochondria-endoplasmic reticulum contact sites. *Nat Commun* **11:** 4056. doi:10.1038/s41467-020-17882-2

Cao Z, Hao Y, Fung CW, Lee YY, Wang P, Li X, Xie K, Lam WJ, Qiu Y, Tang BZ, et al. 2019. Dietary fatty acids promote lipid droplet diversity through seipin enrichment in an ER subdomain. *Nat Commun* **10:** 2902. doi:10.1038/s41467-019-10835-4

Chen S, Novick P, Ferro-Novick S. 2013. ER structure and function. *Curr Opin Cell Biol* **25:** 428–433. doi:10.1016/j.ceb.2013.02.006

Choudhary V, Ojha N, Golden A, Prinz WA. 2015. A conserved family of proteins facilitates nascent lipid droplet budding from the ER. *J Cell Biol* **211:** 261–271. doi:10.1083/jcb.201505067

Cieri D, Vicario M, Giacomello M, Vallese F, Filadi R, Wagner T, Pozzan T, Pizzo P, Scorrano L, Brini M, et al. 2018. SPLICS: a split green fluorescent protein-based contact site sensor for narrow and wide heterotypic organelle juxtaposition. *Cell Death Differ* **25:** 1131–1145. doi:10.1038/s41418-017-0033-z

Cohen S, Valm AM, Lippincott-Schwartz J. 2018. Interacting organelles. *Curr Opin Cell Biol* **53:** 84–91. doi:10.1016/j.ceb.2018.06.003

Csala M, Bánhegyi G, Benedetti A. 2006. Endoplasmic reticulum: a metabolic compartment. *FEBS Lett* **580:** 2160–2165. doi:10.1016/j.febslet.2006.03.050

Csordás G, Renken C, Várnai P, Walter L, Weaver D, Buttle KF, Balla T, Mannella CA, Hajnóczky G. 2006. Structural and functional features and significance of the physical linkage between ER and mitochondria. *J Cell Biol* **174:** 915–921. doi:10.1083/jcb.200604016

di Sano F, Bernardoni P, Piacentini M. 2012. The reticulons: guardians of the structure and function of the endoplasmic reticulum. *Exp Cell Res* **318:** 1201–1207. doi:10.1016/j.yexcr.2012.03.002

Eisenberg-Bord M, Tsui HS, Antunes D, Fernández-del-Río L, Bradley MC, Dunn CD, Nguyen TPT, Rapaport D, Clarke CF, Schuldiner M. 2019. The endoplasmic reticulum-mitochondria encounter structure complex coordi-

nates coenzyme Q biosynthesis. *Contact (Thousand Oaks)* **2:** 251525641882540.

Eizirik DL, Cardozo AK, Cnop M. 2008. The role for endoplasmic reticulum stress in diabetes mellitus. *Endocr Rev* **29:** 42–61. doi:10.1210/er.2007-0015

Erbay E, Babaev VR, Mayers JR, Makowski L, Charles KN, Snitow ME, Fazio S, Wiest MM, Watkins SM, Linton MF, et al. 2009. Reducing endoplasmic reticulum stress through a macrophage lipid chaperone alleviates atherosclerosis. *Nat Med* **15:** 1383–1391. doi:10.1038/nm.2067

Feriod CN, Gustavo Oliveira A, Guerra MT, Nguyen L, Mitchell Richards K, Jurczak MJ, Ruan HB, Paulo Camporez J, Yang X, Shulman GI, et al. 2017. Hepatic inositol 1,4,5 trisphosphate receptor type 1 mediates fatty liver. *Hepatol Commun* **1:** 23–35. doi:10.1002/hep4.1012

Friedman JR, Lackner LL, West M, DiBenedetto JR, Nunnari J, Voeltz GK. 2011. ER tubules mark sites of mitochondrial division. *Science* **334:** 358–362. doi:10.1126/science.1207385

Fu S, Yang L, Li P, Hofmann O, Dicker L, Hide W, Lin X, Watkins SM, Ivanov AR, Hotamisligil GS. 2011. Aberrant lipid metabolism disrupts calcium homeostasis causing liver endoplasmic reticulum stress in obesity. *Nature* **473:** 528–531. doi:10.1038/nature09968

Fu S, Fan J, Blanco J, Gimenez-Cassina A, Danial NN, Watkins SM, Hotamisligil GS. 2012a. Polysome profiling in liver identifies dynamic regulation of endoplasmic reticulum translatome by obesity and fasting. *PLoS Genet* **8:** e1002902. doi:10.1371/journal.pgen.1002902

Fu S, Watkins SM, Hotamisligil GS. 2012b. The role of endoplasmic reticulum in hepatic lipid homeostasis and stress signaling. *Cell Metab* **15:** 623–634. doi:10.1016/j.cmet.2012.03.007

Garfield SA, Cardell RR. 1979. Hepatic glucose-6-phosphatase activities and correlated ultrastructural alterations in hepatocytes of diabetic rats. *Diabetes* **28:** 664–679. doi:10.2337/diab.28.7.664

Goldstein JL, DeBose-Boyd RA, Brown MS. 2006. Protein sensors for membrane sterols. *Cell* **124:** 35–46. doi:10.1016/j.cell.2005.12.022

Goyal U, Blackstone C. 2013. Untangling the web: mechanisms underlying ER network formation. *Biochim Biophys Acta* **1833:** 2492–2498. doi:10.1016/j.bbamcr.2013.04.009

Gregor MF, Yang L, Fabbrini E, Mohammed BS, Eagon JC, Hotamisligil GS, Klein S. 2009. Endoplasmic reticulum stress is reduced in tissues of obese subjects after weight loss. *Diabetes* **58:** 693–700. doi:10.2337/db08-1220

Halbleib K, Pesek K, Covino R, Hofbauer HF, Wunnicke D, Hänelt I, Hummer G, Ernst R. 2017. Activation of the unfolded protein response by lipid bilayer stress. *Mol Cell* **67:** 673–684.e8. doi:10.1016/j.molcel.2017.06.012

Hamasaki M, Furuta N, Matsuda A, Nezu A, Yamamoto A, Fujita N, Oomori H, Noda T, Haraguchi T, Hiraoka Y, et al. 2013. Autophagosomes form at ER-mitochondria contact sites. *Nature* **495:** 389–393. doi:10.1038/nature11910

Hammerschmidt P, Ostkotte D, Nolte H, Gerl MJ, Jais A, Brunner HL, Sprenger HG, Awazawa M, Nicholls HT, Turpin-Nolan SM, et al. 2019. CerS6-derived sphingolipids interact with Mff and promote mitochondrial fragmentation in obesity. *Cell* **177:** 1536–1552.e23. doi:10.1016/j.cell.2019.05.008

Han J, Kaufman RJ. 2016. The role of ER stress in lipid metabolism and lipotoxicity. *J Lipid Res* **57:** 1329–1338. doi:10.1194/jlr.R067595

Hernández-Alvarez MI, Sebastián D, Vives S, Ivanova S, Bartoccioni P, Kakimoto P, Plana N, Veiga SR, Hernández V, Vasconcelos N, et al. 2019. Deficient endoplasmic reticulum-mitochondrial phosphatidylserine transfer causes liver disease. *Cell* **177:** 881–895.e17. doi:10.1016/j.cell.2019.04.010

Hirosumi J, Tuncman G, Chang L, Görgün CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS. 2002. A central role for JNK in obesity and insulin resistance. *Nature* **420:** 333–336. doi:10.1038/nature01137

Hotamisligil GS. 2010. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell* **140:** 900–917. doi:10.1016/j.cell.2010.02.034

Hübner CA, Dikic I. 2020. ER-phagy and human diseases. *Cell Death Differ* **27:** 833–842. doi:10.1038/s41418-019-0444-0

Ilacqua N, Anastasia I, Raimondi A, Lemieux P, de Aguiar Vallim TQ, Toth K, Koonin E v., Pellegrini L. 2022. A three-organelle complex made by wrappER contacts with peroxisomes and mitochondria responds to liver lipid flux changes. *J Cell Sci* **135**. doi:10.1242/jcs.259091

Issop L, Fan J, Lee S, Rone MB, Basu K, Mui J, Papadopoulos V. 2015. Mitochondria-associated membrane formation in hormone-stimulated Leydig cell steroidogenesis: role of ATAD3. *Endocrinology* **156:** 334–345. doi:10.1210/en.2014-1503

Jacquier N, Choudhary V, Mari M, Toulmay A, Reggiori F, Schneiter R. 2011. Lipid droplets are functionally connected to the endoplasmic reticulum in *Saccharomyces cerevisiae*. *J Cell Sci* **124:** 2424–2437. doi:10.1242/jcs.076836

Joensuu M, Belevich I, Rämö O, Nevzorov I, Vihinen H, Puhka M, Witkos TM, Lowe M, Vartiainen MK, Jokitalo E. 2014. ER sheet persistence is coupled to myosin 1c-regulated dynamic actin filament arrays. *Mol Biol Cell* **25:** 1111–1126. doi:10.1091/mbc.e13-12-0712

Jozsef L, Tashiro K, Kuo A, Park E, Skoura A, Albinsson S, Rivera-Molina F, Harrison KD, Iwakiri Y, Toomre D, et al. 2014. Reticulon 4 is necessary for endoplasmic reticulum tubulation, STIM1-Orai1 coupling, and store-operated calcium entry. *J Biol Chem* **289:** 9380–9395. doi:10.1074/jbc.M114.548602

Kammoun HL, Chabanon H, Hainault I, Luquet S, Magnan C, Koike T, Ferré P, Foufelle F. 2009. GRP78 expression inhibits insulin and ER stress-induced SREBP-1c activation and reduces hepatic steatosis in mice. *J Clin Invest* **119:** 1201–1215. doi:10.1172/JCI37007

Kassan A, Herms A, Fernández-Vidal A, Bosch M, Schieber NL, Reddy BJN, Fajardo A, Gelabert-Baldrich M, Tebar F, Enrich C, et al. 2013. Acyl-CoA synthetase 3 promotes lipid droplet biogenesis in ER microdomains. *J Cell Biol* **203:** 985–1001. doi:10.1083/jcb.201305142

Kersten S, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B, Wahli W. 1999. Peroxisome proliferator–activated receptor α mediates the adaptive response to fasting. *J Clin Invest* **103:** 1489–1498. doi:10.1172/JCI6223

Klopfenstein DRC, Kappeler F, Hauri HP. 1998. A novel direct interaction of endoplasmic reticulum with micro-

tubules. *EMBO J* **17:** 6168–6177. doi:10.1093/emboj/17.21.6168

Ko CW, Qu J, Black DD, Tso P. 2020. Regulation of intestinal lipid metabolism: current concepts and relevance to disease. *Nat Rev Gastroenterol Hepatol* **17:** 169–183. doi:10.1038/s41575-019-0250-7

Kono T, Tong X, Taleb S, Bone RN, Iida H, Lee CC, Sohn P, Gilon P, Roe MW, Evans-Molina C. 2018. Impaired store-operated calcium entry and STIM1 loss lead to reduced insulin secretion and increased endoplasmic reticulum stress in the diabetic β-cell. *Diabetes* **67:** 2293–2304. doi:10.2337/db17-1351

Kreibich G, Ulrich BL, Sabatini DD. 1978. Proteins of rough microsomal membranes related to ribosome binding. I: Identification of ribophorins I and II, membrane proteins characteristics of rough microsomes. *J Cell Biol* **77:** 464–487. doi:10.1083/jcb.77.2.464

Lastun VL, Levet C, Freeman M. 2022. The mammalian rhomboid protein RHBDL4 protects against endoplasmic reticulum stress by regulating the morphology and distribution of ER sheets. *J Biol Chem* **298:** 101935. doi:10.1016/j.jbc.2022.101935

Lees JA, Messa M, Sun EW, Wheeler H, Torta F, Wenk MR, de Camilli P, Reinisch KM. 2017. Lipid transport by TMEM24 at ER-plasma membrane contacts regulates pulsatile insulin secretion. *Science* **355:** eaah6171. doi:10.1126/science.aah6171

Lemmer IL, Willemsen N, Hilal N, Bartelt A. 2021. A guide to understanding endoplasmic reticulum stress in metabolic disorders. *Mol Metab* **47:** 101169. doi:10.1016/j.molmet.2021.101169

Lewis SC, Uchiyama LF, Nunnari J. 2016. ER-mitochondria contacts couple mtDNA synthesis with mitochondrial division in human cells. *Science* **353:** aaf5549. doi:10.1126/science.aaf5549

Li Y, Ge M, Ciani L, Kuriakose G, Westover EJ, Dura M, Covey DF, Freed JH, Maxfield FR, Lytton J, et al. 2004. Enrichment of endoplasmic reticulum with cholesterol inhibits sarcoplasmic-endoplasmic reticulum calcium ATPase-2b activity in parallel with increased order of membrane lipids: implications for depletion of endoplasmic reticulum calcium stores and apoptosis in cholesterol-loaded macrophages. *J Biol Chem* **279:** 37030–37039. doi:10.1074/jbc.M405195200

Loomba R, Friedman SL, Shulman GI. 2021. Mechanisms and disease consequences of nonalcoholic fatty liver disease. *Cell* **184:** 2537–2564. doi:10.1016/j.cell.2021.04.015

Lu M, van Tartwijk FW, Lin JQ, Nijenhuis W, Parutto P, Fantham M, Christensen CN, Avezov E, Holt CE, Tunnacliffe A, et al. 2020. The structure and global distribution of the endoplasmic reticulum network are actively regulated by lysosomes. *Sci Adv* **6:** eabc7209. doi:10.1126/sciadv.abc7209

Lyu K, Zhang Y, Zhang D, Kahn M, ter Horst KW, Rodrigues MRS, Gaspar RC, Hirabara SM, Luukkonen PK, Lee S, et al. 2020. A membrane-bound diacylglycerol species induces PKCε-mediated hepatic insulin resistance. *Cell Metab* **32:** 654–664.e5. doi:10.1016/j.cmet.2020.08.001

Malhi H, Kaufman RJ. 2011. Endoplasmic reticulum stress in liver disease. *J Hepatol* **54:** 795–809. doi:10.1016/j.jhep.2010.11.005

Marciniak SJ, Chambers JE, Ron D. 2022. Pharmacological targeting of endoplasmic reticulum stress in disease. *Nat Rev Drug Discov* **21:** 115–140. doi:10.1038/s41573-021-00320-3

Miller WL. 2007. Steroidogenic acute regulatory protein (StAR), a novel mitochondrial cholesterol transporter. *Biochim Biophys Acta* **1771:** 663–676. doi:10.1016/j.bbalip.2007.02.012

Mori H, Christensen AK. 1980. Morphometric analysis of Leydig cells in the normal rat testis. *J Cell Biol* **84:** 340–354. doi:10.1083/jcb.84.2.340

Nixon-Abell J, Obara CJ, Weigel A V, Li D, Legant WR, Xu CS, Pasolli HA, Harvey K, Hess HF, Betzig E, et al. 2016. Increased spatiotemporal resolution reveals highly dynamic dense tubular matrices in the peripheral ER. *Science* **354:** aaf3928. doi:10.1126/science.aaf3928

Ogawa-Goto K, Tanaka K, Ueno T, Tanaka K, Kurata T, Sata T, Irie S. 2007. P180 is involved in the interaction between the endoplasmic reticulum and microtubules through a novel microtubule-binding and bundling domain. *Mol Biol Cell* **18:** 3741–3751. doi:10.1091/mbc.e06-12-1125

Olzmann JA, Carvalho P. 2019. Dynamics and functions of lipid droplets. *Nat Rev Mol Cell Biol* **20:** 137–155. doi:10.1038/s41580-018-0085-z

Özcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Özdelen E, Tuncman G, Görgün C, Glimcher LH, Hotamisligil GS. 2004. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* **306:** 457–461. doi:10.1126/science.1103160

Ozcan L, Cristina De Souza J, Harari AA, Backs J, Olson EN, Tabas I. 2013. Activation of calcium/calmodulin-dependent protein kinase II in obesity mediates suppression of hepatic insulin signaling. *Cell Metab* **18:** 803–815. doi:10.1016/j.cmet.2013.10.011

Palade GE, Siekevitz P. 1956. Pancreatic microsomes; an integrated morphological and biochemical study. *J Biophys Biochem Cytol* **2:** 671–690. doi:10.1083/jcb.2.6.671

Park E, Rapoport TA. 2012. Mechanisms of Sec61/SecY-mediated protein translocation across membranes. *Annu Rev Biophys* **41:** 21–40. doi:10.1146/annurev-biophys-050511-102312

Parlakgül G, Arruda AP, Pang S, Cagampan E, Min N, Güney E, Lee GY, Inouye K, Hess HF, Xu CS, et al. 2022. Regulation of liver subcellular architecture controls metabolic homeostasis. *Nature* **603:** 736–742. doi:10.1038/s41586-022-04488-5

Petersen MC, Vatner DF, Shulman GI. 2017. Regulation of hepatic glucose metabolism in health and disease. *Nat Rev Endocrinol* **13:** 572–587. doi:10.1038/nrendo.2017.80

Phillips MJ, Voeltz GK. 2016. Structure and function of ER membrane contact sites with other organelles. *Nat Rev Mol Cell Biol* **17:** 69–82. doi:10.1038/nrm.2015.8

Prasad M, Kaur J, Pawlak KJ, Bose M, Whittal RM, Bose HS. 2015. Mitochondria-associated endoplasmic reticulum membrane (MAM) regulates steroidogenic activity via steroidogenic acute regulatory protein (StAR)-voltage-dependent anion channel 2 (VDAC2) interaction. *J Biol Chem* **290:** 2604–2616. doi:10.1074/jbc.M114.605808

Reali V, Mehdawy B, Nardacci R, Filomeni G, Risuglia A, Rossin F, Antonioli M, Marsella C, Fimia GM, Piacentini M, et al. 2015. Reticulon protein-1C is a key component of

Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

MAMs. *Biochim Biophys Acta* **1853:** 733–745. doi:10.1016/j.bbamcr.2014.12.031

Reid DW, Nicchitta CV. 2015. Diversity and selectivity in mRNA translation on the endoplasmic reticulum. *Nat Rev Mol Cell Biol* **16:** 221–231. doi:10.1038/nrm3958

Rizzuto R, Pinton P, Carrington W, Fay FS, Fogarty KE, Lifshitz LM, Tuft RA, Pozzan T. 1998. Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca^{2+} responses. *Science* **280:** 1763–1766. doi:10.1126/science.280.5370.1763

Rowland AA, Chitwood PJ, Phillips MJ, Voeltz GK. 2014. ER contact sites define the position and timing of endosome fission. *Cell* **159:** 1027–1041. doi:10.1016/j.cell.2014.10.023

Rui L. 2014. Energy metabolism in the liver. *Compr Physiol* **4:** 177–197. doi:10.1002/cphy.c130024

Rutkowski DT, Wu J, Back SH, Callaghan MU, Ferris SP, Iqbal J, Clark R, Miao H, Hassler JR, Fornek J, et al. 2008. UPR pathways combine to prevent hepatic steatosis caused by ER stress-mediated suppression of transcriptional master regulators. *Dev Cell* **15:** 829–840. doi:10.1016/j.devcel.2008.10.015

Sabatini DD, Blobel G. 1970. Controlled proteolysis of nascent polypeptides in rat liver cell fractions. II: Location of the polypeptides in rough microsomes. *J Cell Biol* **45:** 146–157. doi:10.1083/jcb.45.1.146

Salo VT, Belevich I, Li S, Karhinen L, Vihinen H, Vigouroux C, Magré J, Thiele C, Hölttä-Vuori M, Jokitalo E, et al. 2016. Seipin regulates ER–lipid droplet contacts and cargo delivery. *EMBO J* **35:** 2699–2716. doi:10.15252/embj.201695170

Sandhu J, Li S, Fairall L, Pfisterer SG, Gurnett JE, Xiao X, Weston TA, Vashi D, Ferrari A, Orozco JL, et al. 2018. Aster proteins facilitate nonvesicular plasma membrane to ER cholesterol transport in mammalian cells. *Cell* **175:** 514–529.e20. doi:10.1016/j.cell.2018.08.033

Santinho A, Salo VT, Chorlay A, Li S, Zhou X, Omrane M, Ikonen E, Thiam AR. 2020. Membrane curvature catalyzes lipid droplet assembly. *Curr Biol* **30:** 2481–2494.e6. doi:10.1016/j.cub.2020.04.066

Schröder M, Kaufman RJ. 2005. The mammalian unfolded protein response. *Annu Rev Biochem* **74:** 739–789. doi:10.1146/annurev.biochem.73.011303.074134

Schuck S, Prinz WA, Thorn KS, Voss C, Walter P. 2009. Membrane expansion alleviates endoplasmic reticulum stress independently of the unfolded protein response. *J Cell Biol* **187:** 525–536. doi:10.1083/jcb.200907074

Scorrano L, Oakes SA, Opferman JT, Cheng EH, Sorcinelli MD, Pozzan T, Korsmeyer SJ. 2003. BAX and BAK regulation of endoplasmic reticulum Ca^{2+}: a control point for apoptosis. *Science* **300:** 135–139. doi:10.1126/science.1081208

Shen B, Zheng P, Qian N, Chen Q, Zhou X, Hu J, Chen J, Teng J. 2019. Calumenin-1 interacts with Climp63 to cooperatively determine the luminal width and distribution of endoplasmic reticulum sheets. *iScience* **22:** 70–80. doi:10.1016/j.isci.2019.10.067

Shibata Y, Voeltz GK, Rapoport TA. 2006. Rough sheets and smooth tubules. *Cell* **126:** 435–439. doi:10.1016/j.cell.2006.07.019

Shibata Y, Voss C, Rist JM, Hu J, Rapoport TA, Prinz WA, Voeltz GK. 2008. The reticulon and DP1/Yop1p proteins form immobile oligomers in the tubular endoplasmic reticulum. *J Biol Chem* **283:** 18892–18904. doi:10.1074/jbc.M800986200

Shibata Y, Shemesh T, Prinz WA, Palazzo AF, Kozlov MM, Rapoport TA. 2010. Mechanisms determining the morphology of the peripheral ER. *Cell* **143:** 774–788. doi:10.1016/j.cell.2010.11.007

Sood A, Jeyaraju DV, Prudent J, Caron A, Lemieux P, McBride HM, Laplante M, Tóth K, Pellegrini L. 2014. A mitofusin-2-dependent inactivating cleavage of Opa1 links changes in mitochondria *cristae* and ER contacts in the postprandial liver. *Proc Natl Acad Sci* **111:** 16017–16022. doi:10.1073/pnas.1408061111

Spits M, Heesterbeek IT, Voortman LM, Akermans JJ, Wijdeven RH, Cabukusta B, Neefjes J. 2021. Mobile late endosomes modulate peripheral endoplasmic reticulum network architecture. *EMBO Rep* **22:** e50815. doi:10.15252/embr.202050815

Striffler JS, Cardell EL, Cardell RR. 1981. Effects of glucagon on hepatic glycogen and smooth endoplasmic reticulum. *Am J Anat* **160:** 363–379. doi:10.1002/aja.1001600402

Subramanian K, Jochem A, Le Vasseur M, Lewis S, Paulson BR, Reddy TR, Russell JD, Coon JJ, Pagliarini DJ, Nunnari J. 2019. Coenzyme Q biosynthetic proteins assemble in a substrate-dependent manner into domains at ER-mitochondria contacts. *J Cell Biol* **218:** 1353–1369. doi:10.1083/jcb.201808044

Terasaki M, Chen LB, Fujiwara K. 1986. Microtubules and the endoplasmic reticulum are highly interdependent structures. *J Cell Biol* **103:** 1557–1568. doi:10.1083/jcb.103.4.1557

Terasaki M, Shemesh T, Kasthuri N, Klemm RW, Schalek R, Hayworth KJ, Hand AR, Yankova M, Huber G, Lichtman JW, et al. 2013. Stacked endoplasmic reticulum sheets are connected by helicoidal membrane motifs. *Cell* **154:** 285–296. doi:10.1016/j.cell.2013.06.031

Thoudam T, Ha CM, Leem J, Chanda D, Park JS, Kim HJ, Jeon JH, Choi YK, Liangpunsakul S, Huh YH, et al. 2019. PDK4 augments ER-mitochondria contact to dampen skeletal muscle insulin signaling during obesity. *Diabetes* **68:** 571–586. doi:10.2337/db18-0363

Trefts E, Gannon M, Wasserman DH. 2017. The liver. *Curr Biol* **27:** R1147–R1151. doi:10.1016/j.cub.2017.09.019

Tubbs E, Theurey P, Vial G, Bendridi N, Bravard A, Chauvin MA, Ji-Cao J, Zoulim F, Bartosch B, Ovize M, et al. 2014. Mitochondria-associated endoplasmic reticulum membrane (MAM) integrity is required for insulin signaling and is implicated in hepatic insulin resistance. *Diabetes* **63:** 3279–3294. doi:10.2337/db13-1751

Tubbs E, Chanon S, Robert M, Bendridi N, Bidaux G, Chauvin MA, Ji-Cao J, Durand C, Gauvrit-Ramette D, Vidal H, et al. 2018. Disruption of mitochondria-associated endoplasmic reticulum membrane (MAM) integrity contributes to muscle insulin resistance in mice and humans. *Diabetes* **67:** 636–650. doi:10.2337/db17-0316

Ulloa G, Hamati F, Dick A, Fitzgerald J, Mantell J, Verkade P, Collinson L, Arkill K, Larijani B, Poccia D. 2019. Lipid species affect morphology of endoplasmic reticu-

Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

lum: a sea urchin oocyte model of reversible manipulation. *J Lipid Res* **60:** 1880–1891. doi:10.1194/jlr.RA119000210

Unger RH, Clark GO, Scherer PE, Orci L. 2010. Lipid homeostasis, lipotoxicity and the metabolic syndrome. *Biochim Biophys Acta* **1801:** 209–214. doi:10.1016/j.bbalip.2009.10.006

Vance JE. 1990. Phospholipid synthesis in a membrane fraction associated with mitochondria. *J Biol Chem* **265:** 7248–7256. doi:10.1016/S0021-9258(19)39106-9

Vedrenne C, Klopfenstein DR, Hauri HP. 2005. Phosphorylation controls CLIMP-63-mediated anchoring of the endoplasmic reticulum to microtubules. *Mol Biol Cell* **16:** 1928–1937. doi:10.1091/mbc.e04-07-0554

Voeltz GK, Rolls MM, Rapoport TA. 2002. Structural organization of the endoplasmic reticulum. *EMBO Rep* **3:** 944–950. doi:10.1093/embo-reports/kvf202

Voeltz GK, Prinz WA, Shibata Y, Rist JM, Rapoport TA. 2006. A class of membrane proteins shaping the tubular endoplasmic reticulum. *Cell* **124:** 573–586. doi:10.1016/j.cell.2005.11.047

Volmer R, van der Ploeg K, Ron D. 2013. Membrane lipid saturation activates endoplasmic reticulum unfolded protein response transducers through their transmembrane domains. *Proc Natl Acad Sci* **110:** 4628–4633. doi:10.1073/pnas.1217611110

Walter P, Ron D. 2011. The unfolded protein response: from stress pathway to homeostatic regulation. *Science* **334:** 1081–1086. doi:10.1126/science.1209038

Walther TC, Chung J, Farese RV. 2017. Lipid droplet biogenesis. *Annu Rev Cell Dev Biol* **33:** 491–510. doi:10.1146/annurev-cellbio-100616-060608

Wang Y, Vera L, Fischer WH, Montminy M. 2009. The CREB coactivator CRTC2 links hepatic ER stress and fasting gluconeogenesis. *Nature* **460:** 534–537. doi:10.1038/nature08111

Wang Y, Li G, Goode J, Paz JC, Ouyang K, Screaton R, Fischer WH, Chen J, Tabas I, Montminy M. 2012. Inositol-1,4,5-trisphosphate receptor regulates hepatic gluconeogenesis in fasting and diabetes. *Nature* **485:** 128–132. doi:10.1038/nature10988

Wang H, Becuwe M, Housden BE, Chitraju C, Porras AJ, Graham MM, Liu XN, Thiam AR, Savage DB, Agarwal AK, et al. 2016. Seipin is required for converting nascent to mature lipid droplets. *eLife* **5:** e16582. doi:10.7554/eLife.16582

Wang X, Li S, Wang H, Shui W, Hu J. 2017. Quantitative proteomics reveal proteins enriched in tubular endoplasmic reticulum of *Saccharomyces cerevisiae*. *eLife* **6:** e23816. doi:10.7554/eLife.23816

Wang N, Clark LD, Gao Y, Kozlov MM, Shemesh T, Rapoport TA. 2021. Mechanism of membrane-curvature generation by ER-tubule shaping proteins. *Nat Commun* **12:** 568. doi:10.1038/s41467-020-20625-y

Waterman-Storer CM, Gregory J, Parsons SF, Salmon ED. 1995. Membrane/microtubule tip attachment complexes (TACs) allow the assembly dynamics of plus ends to push and pull membranes into tubulovesicular networks in interphase *Xenopus* egg extracts. *J Cell Biol* **130:** 1161–1169. doi:10.1083/jcb.130.5.1161

Westrate LM, Lee JE, Prinz WA, Voeltz GK. 2015. Form follows function: the importance of endoplasmic reticulum shape. *Annu Rev Biochem* **84:** 791–811. doi:10.1146/annurev-biochem-072711-163501

Wilfling F, Wang H, Haas JT, Krahmer N, Gould TJ, Uchida A, Cheng JX, Graham M, Christiano R, Fröhlich F, et al. 2013. Triacylglycerol synthesis enzymes mediate lipid droplet growth by relocalizing from the ER to lipid droplets. *Dev Cell* **24:** 384–399. doi:10.1016/j.devcel.2013.01.013

Wilson CH, Ali ES, Scrimgeour N, Martin AM, Hua J, Tallis GA, Rychkov GY, Barritt GJ. 2015. Steatosis inhibits liver cell store-operated Ca^{2+} entry and reduces ER Ca^{2+} through a protein kinase C-dependent mechanism. *Biochem J* **466:** 379–390. doi:10.1042/BJ20140881

Wu H, Carvalho P, Voeltz GK. 2018. Here, there, and everywhere: the importance of ER membrane contact sites. *Science* **361:** eaan5835. doi:10.1126/science.aan5835

Yang L, Calay ES, Fan J, Arduini A, Kunz RC, Gygi SP, Yalcin A, Fu S, Hotamisligil GS. 2015. METABOLISM. *S*-nitrosylation links obesity-associated inflammation to endoplasmic reticulum dysfunction. *Science* **349:** 500–506. doi:10.1126/science.aaa0079

Zhang S, Guo F, Yu M, Yang X, Yao Z, Li Q, Wei Z, Feng K, Zeng P, Zhao D, et al. 2020. Reduced Nogo expression inhibits diet-induced metabolic disorders by regulating ChREBP and insulin activity. *J Hepatol* **73:** 1482–1495. doi:10.1016/j.jhep.2020.07.034

Zheng P, Obara CJ, Szczesna E, Nixon-Abell J, Mahalingan KK, Roll-Mecak A, Lippincott-Schwartz J, Blackstone C. 2022. ER proteins decipher the tubulin code to regulate organelle distribution. *Nature* **601:** 132–138. doi:10.1038/s41586-021-04204-9

Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org