Biochemical Society Transactions (2022) **50** 1389–1402
https://doi.org/10.1042/BST20220519

Review Article

Fundamental roles for inter-organelle communication in aging

Eric K. F. Donahue, Elizabeth M. Ruark and Kristopher Burkewitz

Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN, U.S.A.

Correspondence: Kristopher Burkewitz (kristopher.burkewitz@vanderbilt.edu)

Advances in public health have nearly doubled life expectancy over the last century, but this demographic shift has also changed the landscape of human illness. Today, chronic and age-dependent diseases dominate the leading causes of morbidity and mortality worldwide. Targeting the underlying molecular, genetic and cell biological drivers of the aging process itself appears to be an increasingly viable strategy for developing therapeutics against these diseases of aging. Towards this end, one of the most exciting developments in cell biology over the last decade is the explosion of research into organelle contact sites and related mechanisms of inter-organelle communication. Identification of the molecular mediators of inter-organelle tethering and signaling is now allowing the field to investigate the consequences of aberrant organelle interactions, which frequently seem to correlate with age-onset pathophysiology. This review introduces the major cellular roles for inter-organelle interactions, including the regulation of organelle morphology, the transfer of ions, lipids and other metabolites, and the formation of hubs for nutrient and stress signaling. We explore how these interactions are disrupted in aging and present findings that modulation of inter-organelle communication is a promising avenue for promoting longevity. Through this review, we propose that the maintenance of inter-organelle interactions is a pillar of healthy aging. Learning how to target the cellular mechanisms for sensing and controlling inter-organelle communication is a key next hurdle for geroscience.

Introduction

Over the course of evolution, cellular pathways and processes have become highly compartmentalized. Cell biologists have spent years mapping the molecular constituents, metabolic pathways, and cellular roles of each of these compartments, typically organelles enclosed by membrane barriers. These cataloging approaches provide simple and foundational models of how cells distribute tasks among their many organelles but rely upon isolating and purifying each compartment to study it discretely from its neighbors. Today, fueled by a combination of enabling technologies and recent discoveries in cell biology, the field is now circling back to understand how the many compartments of cells are *connected*. Through these efforts, new levels of complexity and logic are emerging to explain how interactions between organelles control cell and organismal homeostasis.

Compartmentalization of metabolic processes within organelles enables cells to concentrate substrates and insulate these pathways from undesirable inputs or external interference. Compartmentalization also brings new vulnerabilities, however. Because each organelle network performs unique roles in the cell, they must function cohesively to perform the complete set of tasks required for life. This community structure increases complexity and creates new dependencies, as organelles must efficiently share metabolic substrates and coordinate functional states with distal partners. As appreciation grows for how interconnected this web of organelles really is, indeed we are finding more signs that miscommunication between these compartments is an important source of disease etiology and progression [1]. This interdependency raises fundamental questions in aging and

Received: 20 July 2022
Revised: 27 September 2022
Accepted: 17 October 2022

Version of Record published:
28 October 2022

Biochemical Society Transactions (2022) **50** 1389–1402
https://doi.org/10.1042/BST20220519

stress biology relating to how aberrant connections between organelles may act as mechanisms of aging and/or therapeutic targets for promoting healthspan and protecting against specific age-onset diseases.

This review focuses primarily on inter-organelle communication associated with organelle contact sites and how these processes relate to aging biology. However, the breadth of cellular processes that involve functional or signaling coordination between organelles independently of contact sites is both fascinating and staggering [2], and we will include some examples that are proving foundational to the aging field. We also limit discussion of organelle-localized sensors activating retrograde signaling and transcriptional responses in the nucleus — some of the earliest and best understood examples of inter-organelle communication. These organelle stress response pathways are indeed critical in aging biology and well-covered in other recent reviews. [3–5] Finally, many intriguing connections are emerging involving inter-organelle communication and specific age-onset pathophysiologies [6], but here we strive to take more of a geroscience perspective on how inter-organelle communication may serve as a fundamental driver of the aging process itself.

Overview of inter-organelle interactions

Given the immense complexity of the organelle interactome, we will discuss an overview of the established roles and purposes of these interactions and highlight examples with special relevance to aging biology. Broadly these roles include the coordination of organelle dynamics and cellular distributions, flux of ions and metabolites, lipid and membrane transfer, and integration of signal transduction pathways. Several recent in-depth reviews cover more of the specific genetic and molecular mediators of organelle crosstalk in various contexts [6–10].

Structural interactions

Organelles physically interact as a means to spatially coordinate organelle morphology and dynamics [11,12]. The endoplasmic reticulum (ER) plays an especially central role in these dynamics, as ER tubules mediate the fission of endosomes [13,14], mitochondria [15,16] and biomolecular condensates [17]. The role of ER in controlling mitochondrial dynamics is currently the best understood and most complex example of these organelle structural interactions. ER tubules form relatively stable associations with mitochondrial membranes. At a subset of these contact sites, mitochondrial constrictions occur and eventually develop into complete fission events through a mechanism that involves ER-assisted recruitment of core fission and actin-remodeling machineries [15,16,18] (Figure 1A). Evidence is emerging to suggest that ER-mitochondrial contacts are not only facilitating fission, but mitochondrial fusion as well, thus playing a role in coordinating the balance between these opposing processes [19]. Given the many links between aberrant mitochondrial morphology and age-related diseases [20], exploring the role of these contact sites as mitochondrial dynamics 'hotspots' is an exciting avenue moving forward.

Importantly, ER influence over mitochondrial homeostasis extends deeper than the outer mitochondrial membrane. Some of the more stable ER-mitochondrial contact sites correlate with internal mitochondrial functions and properties, including membrane potential [19,21,22]. Sites of ER-directed mitochondrial fission are also spatially and functionally associated with mtDNA replication [16,21–25], thereby linking the ER to control of mitochondrial genome segregation and copy number control (Figure 1A). Through these contacts, the ER is thus a key mediator of mitochondrial 'structure-function,' and with analogous roles for the ER in mediating fission and distribution of other non-mitochondrial organelle networks just beginning to emerge, it will be exciting to learn if the ER is as deeply involved in their functional outputs as well. As the most expansive and inter-connected organelle network of the cell, the ER is certainly well-positioned to function as a primary sensor and coordinator of organelle dynamics.

Because every organelle network is constantly responding to unique and fluctuating functional demands, the availability of cellular resources must be similarly flexible. The logic is straightforward then for each organelle to have an input into mitochondria, the central bioenergetic and metabolic hub of the cell, as a way to coordinate the availability of energy and metabolic substrates. Consistent with this logic, diverse organelles beyond the ER play roles in controlling mitochondrial dynamics. Lysosomes also form contacts with mitochondria for bi-directional regulation of organelle fission and distribution through a mechanism centering on the Rab7 GTPase [26] (Figure 1B). More recently, studies have shown a role for vesicles from the trans-Golgi network in mitochondrial dynamics [27]. On these vesicles, ADP-ribosylation factor 1 (Arf1) and PI(4)KIIIβ convert phosphatidylinositol to PI(4)P, which is required for membrane remodeling in the late steps of mitochondrial fission (Figure 1C). Thus, ER, lysosomes and Golgi networks all seem to play roles in controlling mitochondrial

Biochemical Society Transactions (2022) **50** 1389–1402
https://doi.org/10.1042/BST20220519

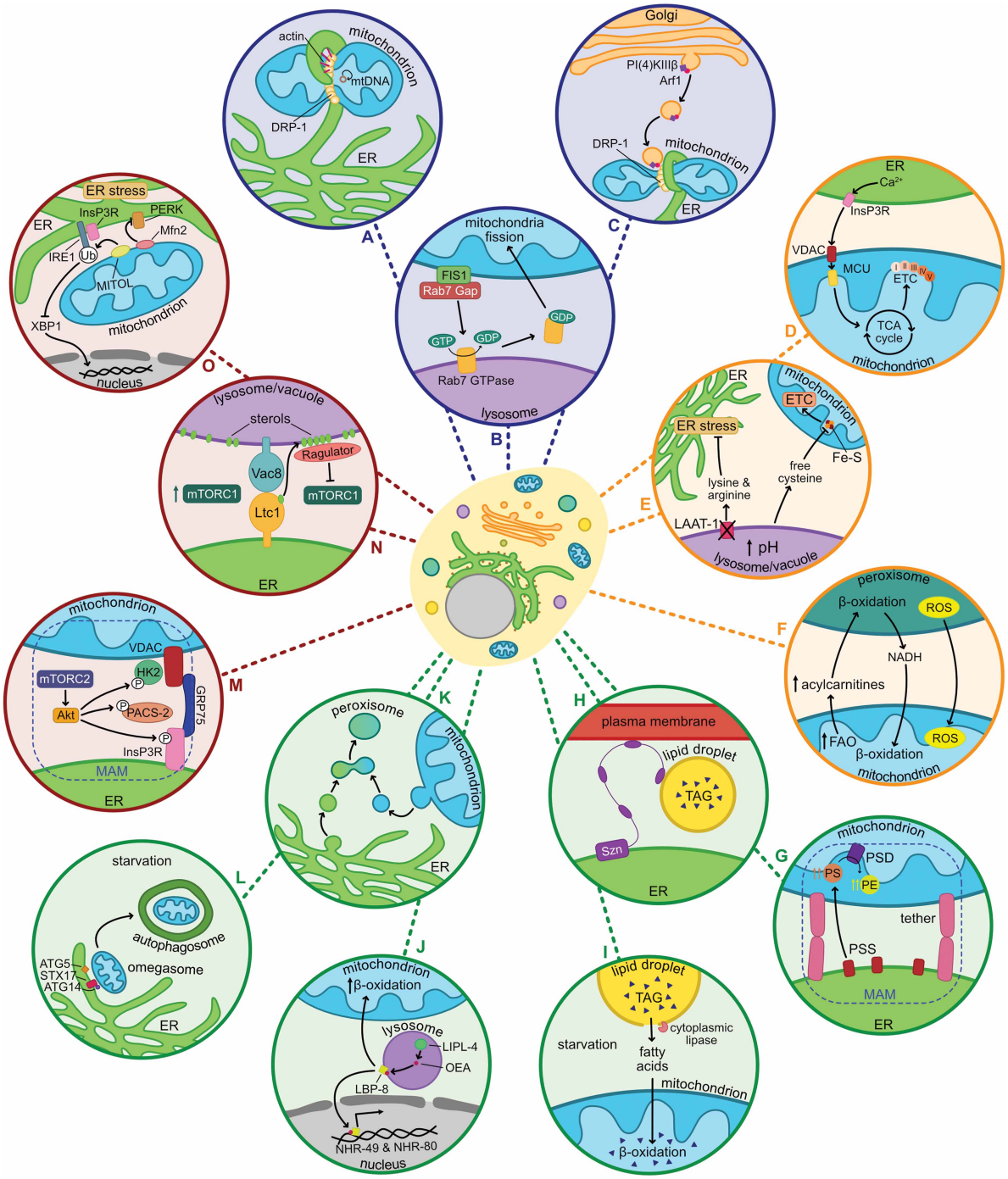

Figure 1. Inter-organelle interactions are critical for homeostatic maintenance. Part 1 of 2

Organelles interact with each other via structural interactions (blue circles, **A**–**C**), transfer of ions and other metabolites (orange circles, **D**–**F**), transport of lipids (green circles, **G**–**L**), and by coordinating nutrient and stress signals (red circles, **M**–**O**). (**A**) ER tubules are associated with sites of mtDNA replication and recruitment of DRP-1 for mitochondrial fission. (**B**) Lysosomal Rab7 GTPase regulates contact formation with mitochondria, where a Rab7 GAP completes the cycle by reciprocally activating Rab7 GTP hydrolysis. (**C**) Golgi vesicles containing membrane remodeling proteins localize at mitochondria during late stages of fission. (**D**) ER-mitochondrial calcium flux promotes mitochondrial bioenergetics and ATP production. (**E**) Increasing vacuolar pH leads to ER and mitochondrial dysfunction via defects in amino acid storage. (**F**) Cell redox and fatty acid oxidation crosstalk occur between peroxisomes and mitochondria. (**G**) MAMs provide an interface for non-vesicular lipid transfer. (**H**) Snazarus promotes triacylglyceride storage in lipid droplets at the PM. (**I**) Lipid droplets supply fuel mitochondrial

Biochemical Society Transactions (2022) **50** 1389–1402
https://doi.org/10.1042/BST20220519

Figure 1. Inter-organelle interactions are critical for homeostatic maintenance. Part 2 of 2

β-oxidation through fatty acid release by lipases. (**J**) Lysosomal lipase LIPL-4 releases oleoylethanolamide (OEA), which is ferried by chaperone LBP-8 to activate transcription via NHR-49 and NHR-80, thus modulating mitochondrial metabolism and promoting longevity. (**K**) Peroxisomes form as a hybrid of ER-mitochondrial components. (**L**) STX17 recruits ATG14 to ER-mitochondrial contact sites and, with ATG5, initiates the formation of autophagosomes. (**M**) Growth factor driven mTORC2 localization to MAMs activates Akt signaling to regulate tethering via GRP75, promote HK2 localization to VDAC, and control InsP3R-mediated calcium release. (**N**) Vacuolar membrane sterol content regulates activation of mTOR signaling at Ltc1-Vac8 contacts. (**O**) MITOL ubiquitylation of IRE1 prevents XBP1 mRNA splicing and Mfn2 represses the phosphorylation activity of PERK in response to ER stress at ER-mitochondria contacts. ADP-ribosylation factor 1 (Arf1), autophagy-related 5 (ATG5), autophagy-related 14 (ATG14), dynamin-related protein 1 (DRP-1), endoplasmic reticulum (ER), electron transport chain (ETC), fatty acid oxidation (FAO), mitochondrial fission 1 (FIS1), glucose-regulated protein 75 (GRP75), hexokinase 2 (HK2), inositol triphosphate receptor (InsP3R), lipid binding protein 8 (LBP-8), lipase like 4 (LIPL-4), mitochondria-associated ER membrane (MAM), mTOR complex 1 (mTORC1), mTOR complex 2 (mTORC2), nicotinamide adenine dinucleotide (NADH), nuclear hormone receptor-49/80 (NHR-49/80), phosphofurin acidic cluster sorting protein 2 (PACS-2), phosphatidylethanolamine (PE), phosphatidylinositol 4-kinase IIIβ (PI(4)KIIIβ), plasma membrane (PM), phosphatidylserine (PS), phosphatidylserine decarboxylase (PSD), phosphatidylserine synthase (PSS), reactive oxygen species (ROS), syntaxin 17 (STX17), Snazarus (Szn), triacylglycerides (TAG), tricarboxylic acid (TCA) cycle, vacuolar protein 8 (Vac8), X-box binding protein (XBP1).

fission. How many organelles need to cooperate to execute a single fission event is not consistently clear, but one recent study demonstrated that over 80% of mitochondrial fission sites have four-way structural interactions with the ER, lysosomes, and Golgi-derived vesicles [27]. Intriguingly, spatially distinct regions of the mitochondrion may be differentially targeted for certain types of fission events [21]. Fission events in the peripheral regions of tubular mitochondria are linked more strongly to lysosomal contacts and lead to mitophagy, whereas midzone fission events are mediated by the ER. Consistent with the association of ER contacts with replicating nucleoids, these ER-mediated midzone fission events lead to mitochondrial biogenesis [21]. Overall, the increasing number of distinct molecular and organelle players that are important for maintaining mitochondrial dynamics may begin to explain why aberrant mitochondrial morphology is so consistently associated with age-onset disease. Given the apparent sensitivity of mitochondrial shape to the fitness and inputs of so many distal organelles, changes in fission/fusion processes may represent an early warning sign that healthy inter-organelle interactions are beginning to break down.

Transfer of ions and polar metabolites

Ions and polar metabolites are transported between organelles for multiple purposes, including both signaling and fueling metabolic pathways with essential substrates. Reaching back nearly three decades now, the 'quasi-synaptic' connections formed between the ER and mitochondria and their utility as a mechanism to funnel calcium are perhaps the best-studied example of inter-organelle communication [28–30]. The more recent molecular identification of the mitochondrial calcium uniporter complex [31–34] and various tethers [35,36] has greatly enhanced genetic tractability to probe questions in this area. The release of ER calcium into the mitochondrial matrix potently stimulates ATP production by coordinately activating several key metabolic enzymes controlling the mitochondrial uptake of fuel and reducing equivalents, the TCA cycle, and the electron transport chain [37]. The transfer of calcium from the ER to mitochondria thus acts as a rheostat-like control for cells to tune mitochondrial bioenergetic tone, but is also stimulated in specific contexts to provide acute bioenergetic support for recovery [38,39]. For example, ER stress promotes ER-mitochondrial coupling, potentially through mechanisms involving calcium leakage from the ER. After recruitment of mitochondria, ER calcium released by the inositol triphosphate receptor (InsP3R) flows directly into the matrix to acutely activate respiration, and this burst of ATP production is important for the resolution and recovery from perturbation to the ER [39] (Figure 1D). Both too little and too much ER-mitochondrial coupling increases cell dysfunction and death upon ER stress, which is notably a chronic feature of aging [39–41]. Thus, cells must keep the number and activities of these contact sites under tight regulatory control for a healthy aging process, and the mechanisms cells use to sense and regulate the amount of these contacts remain a fundamentally important question as the field moves forward. Dysregulation of ER-mitochondrial contacts and/or calcium signaling is now extensively implicated in diverse age-onset pathophysiologies and diseases, including cancer, metabolic disease and neurodegeneration [6,40,42–44]. Despite increasingly ubiquitous correlations with disease, however, more direct

roles of ER-mitochondrial contacts and communication in driving cell and organismal aging at foundational levels remain less thoroughly explored. Recent links between lifespan and ER-mitochondrial communication have emerged from experiments in both worms and flies when contact sites or inter-organelle calcium flux are modulated [45,46], altogether suggesting that ER-mitochondrial miscommunication may not simply be a common proximal step in disease pathogenesis, but an underlying driver of age-dependent dysfunction.

A compartmentalized architecture requires cells to connect metabolic modules between organelles [9], and many of the emerging links between organelle communication and the aging process arise from these metabolic connections. Pioneering work in yeast models revealed that mitochondrial declines during aging are at least partly the result of failures in another distal organelle, the vacuole/lysosome [47]. In replicatively aged cells, vacuoles lose the ability to maintain low pH as a relatively early step in the aging process. Surprisingly, the loss of canonical degradative functions of the vacuole is not the trigger for mitochondrial dysfunction; rather, failure in vacuolar compartmentalization of amino acids is the cause. In particular, free cysteine serves as a mitochondrial toxin in aging cells by interfering with iron and redox homeostasis when vacuolar storage fails and cysteine levels increase in other compartments [47,48] (Figure 1E). The damage triggered by defects in vacuolar/lysosomal compartmentalization is not limited to mitochondria either. Although the mechanism of amino acid toxicity appears quite different, failures in lysosomal amino acid storage are also recently linked to stress and dysfunction in the ER [49] (Figure 1E). These results perhaps suggest that early, age-dependent defects in the lysosomal compartment initiate a much broader cascade of inter-compartmental metabolic dysfunction.

While the vacuole-mitochondrial interaction in yeast typifies how a failure in one organelle can instigate dysfunction in another, there are also contrasting scenarios where different compartments provide metabolic resiliency. For example, peroxisomes and mitochondria can function in redundant or complementary roles in regulating cellular redox control and fatty acid oxidation [50–52]. Supporting this concept, simultaneous inhibition of mitochondrial fission and fusion factors extends lifespan in *C. elegans* through a mechanism that depends entirely upon a functional peroxisomal network [53]. Metabolic profiling of these long-lived, mitochondrial 'adynamic' worms revealed that fatty acid oxidation capacities and very long- and long-chain acylcarnitines are both elevated, suggesting that peroxisomal networks confer metabolic adaptability when mitochondria lose their morphological plasticity [53] (Figure 1F). How mitochondrial and peroxisome networks signal and coordinate this adaptation is not yet clear, but both networks are major players in cellular redox balance and considerable crosstalk can occur via reactive oxygen species (ROS) signaling and oxidative modifications [52]. Furthermore, many of the calcium transport machineries of the ER and mitochondria are redox sensitive [54], suggesting that local ROS production could serve to convey the functional status of several key metabolic hubs. Given the many complex links between redox biology and aging, the use of reactive oxygen species as a line of communication between metabolic organelles is likely an exciting area of future investigation.

Lipid and membrane transfer

Inter-organelle interactions are critical for effectively distributing hydrophobic lipid and membrane components between compartments and throughout the cell. While membrane fusion events necessary for the vesicular traffic of the endomembrane system promote sharing of membrane lipids, there have long been clues suggesting that non-vesicular modes of lipid transport are physiologically important. Such examples include neurons, in which many subcellular compartments are distant from the Golgi complex [55], and mitochondrial networks with membranes that do not fuse with the endomembrane system. In fact, Vance's pioneering work to understand how ER and mitochondria coordinate phospholipid biosynthesis was the first discovery of specific cellular functions occurring at organelle contact sites [56]. Today, we appreciate that the combination of lipid transfer proteins (LTPs) with the close proximity of membranes at organelle contact sites does facilitate a fusion-independent, high-volume and precisely targeted flux of lipids between organelles [57,58]. Recent examples have demonstrated that cells coordinate both pathways of lipid transport, i.e. vesicular trafficking and non-vesicular LTP-mediated flux [58–61], but how cells manage these lipid dynamics at a broader systems level is a complex and important question moving forward. These mechanisms represent the essential pathways by which cells regulate the varied compositions of organelle membranes, and modulation of lipid compositions is an emerging node for the regulation of lifespan [62].

Mitochondria-associated ER membranes (MAMs) serve as a classic example of an inter-organelle pathway mediating membrane homeostasis. MAMs are contact sites between ER and mitochondria that are so tightly linked that they can be biochemically fractionated and purified [56], and at these sites the subsequent enzymes

Biochemical Society Transactions (2022) **50** 1389–1402
https://doi.org/10.1042/BST20220519

of a lipid biosynthetic pathway are split between each organelle. Phosphatidylserine synthase is enriched in the ER at MAMs and promotes efficient transfer of new phosphatidylserine from the ER to the mitochondrial membrane [58,63]. After phosphatidylserine enters the mitochondrial membrane, phosphatidylserine decarboxylase converts it into phosphatidylethanolamine [63–65] (Figure 1G). Demonstrating the potential for cells to tune lipid trafficking by regulating contact site formation, genetic enrichment of phosphatidylserine synthase at MAMs or increased tethering between ER and mitochondria enhances the flux of phosphatidylserine to mitochondria [58,63]. Recent studies have revealed more nuanced dynamics of this spatial organization by showing that altered metabolic demands can promote ER-targeting of phosphatidylserine decarboxylase, which was previously thought to be purely mitochondrial [66]. The fates of the phosphatidylethanolamine produced in each compartment seem to be unique [66,67], adding another layer of regulation for cells to control membrane lipid distributions. Intriguingly, phosphatidylethanolamine levels decline in aged eukaryotes, and both exogenous administration and enhanced production through phosphatidylserine decarboxylase overexpression enhances autophagy and extends lifespan in yeast and Drosophila [68].

While the example above focuses on lipids involved in membrane composition, organelle interactions also regulate neutral lipids involved in energy homeostasis [69]. Lipid droplets store triacylglycerol and sterol esters, and a number of enzymes exploit the unique properties of lipid droplets and their monolayer membrane to both sense and regulate the amount and composition of lipids stored within [70]. A recent burst of research has revealed that lipid droplets are functionally and spatially heterogeneous, and excitingly, modulating specific subpopulations of lipid droplets prolongs organismal lifespan [71–73]. Specifically, some lipid droplets are spatially segregated to the cell periphery, where they form contacts with plasma membrane and are linked with lipid trafficking mechanisms distinct from those found in perinuclear lipid droplets. Sorting nexin Snazarus is enriched at the lipid droplet-plasma membrane contact, and expanding this peripheral lipid droplet pool through Snazarus overexpression enhances starvation resistance and lifespan in Drosophila [72] (Figure 1H). While the mechanisms of lifespan extension in this model remain unclear, enriching this peripheral lipid droplet pool is sufficient to shift the lipidome composition towards unsaturated species correlated with longevity [62,72]. Given the fundamental links between lifespan and cell/organismal energy homeostasis [62,74,75], the mechanisms by which cells sense, store and mobilize lipid fuels is an active area of research that will likely become even more fertile for identifying therapeutic targets relevant to aging biology.

During fluctuations in nutrient conditions, cells spatially remodel their interior compartments in ways that facilitate the flux of lipid substrates between lipid droplets, sites of oxidation, i.e. peroxisomes and mitochondria, and sites of membrane synthesis [76,77]. Metabolic plasticity depends upon efficient and precise mobilization of lipids, which ensures that substrate is available for either membrane synthesis or energy production at the right time and place in the cell [78]. By spatially linking lipid droplets with specific target organelles during periods of high metabolic demand, cells also avoid the lipotoxic effects of free fatty acids in the cytosol. Towards this end, mitochondria can directly associate with lipid droplets through multiple mechanisms, tending to involve conserved perilipin proteins [69,79,80]. Intriguingly, this association with lipid droplets defines a unique, functional subpopulation of mitochondria [79]. While tempting to expect that these droplet-associated mitochondria are simply poised for delivery and β-oxidation of fatty acids, instead this mitochondrial subpopulation is supporting lipid droplet expansion via local, pyruvate-fueled ATP production for triacylglyceride synthesis [79]. Notably, these studies were performed in adipose cells, and how interactions between lipid droplets and mitochondria affect mitochondrial specialization may be specific to cell type and context. During fasting or nutrient scarcity, cells can also mobilize fatty acid fuels rapidly and in bulk via lipophagy, and in some cases lipid droplet-lysosomal contacts facilitate the direct delivery of storage lipids to lysosomal lipases without requiring the autophagosome intermediates [81]. In contrast, other studies reveal that cytoplasmic lipases are responsible for release and delivery of fatty acids to mitochondria during periods of acute nutrient restriction, while autophagic machineries instead recycle organelle membranes to refill lipid droplets with substrate [76] (Figure 1I). A final example of inter-organelle lipid transfer illustrates how rewiring these lipid flux pathways can link directly to longevity. Specifically, the genetic activation of a lysosomal lipase, LIPL-4, extends lifespan in *C. elegans* by releasing lipid signals that trigger both a transcriptional response via nuclear hormone receptors and a shift in mitochondrial metabolic functions [82,83] (Figure 1J). In this case, oleoylethanolamine is the lipid signal and the specificity of the signaling is mediated in part by cytosolic lipid chaperones. Overall, this wide variety of cellular strategies to orient and facilitate lipid mobilization highlights an exciting frontier in understanding the contextual importance of remodeling organelle interactions to adapt to stress and metabolic fluctuations.

Finally, one of the most basic and vital roles for inter-organelle interactions is the de novo generation of organelles. Relating back to the central role of the ER in mediating structural dynamics between organelle networks, it is also a nexus of membrane biogenesis. Lipid droplets are a key example of this process, born solely from ER tubules [84–86]. De novo lipid droplet formation begins between the ER membrane bilayer with formation of an oil lens by local triacylglycerol synthesis [70]. As the lens fills with triacylglycerols, it buds outwards from the ER membrane and recruits specific lipid droplet-associated proteins before release into the cytoplasm, generating the unique monolayer membrane described above. Other organelles require more complex coordination. Peroxisomes arise from two compartments, the ER and mitochondria [87] (Figure 1K), while autophagosome biogenesis is linked to several organelles, likely depending on the spatial or environmental context [7,88,89]. The ER is a primary contributor to autophagosome initiation and maturation, and ER contact sites with other organelles appear to be hotspots for initiating autophagosome formation. For example, starvation causes ER-resident mediators of autophagy formation, syntaxin-17 (STX17) and autophagy-related 14 (ATG14), to concentrate at MAMs, where they recruit autophagy-related 5 (ATG5) and initiate formation of the omegasome, the membrane responsible for autophagosome formation [89,90] (Figure 1L). Intriguingly, autophagosomes formed at ER-mitochondrial contacts seem to recruit the necessary machineries for mitochondrial turnover via mitophagy [91]. Golgi vesicles, endosomes, and lipid droplets have all been shown to regulate autophagosome initiation or maturation, suggesting cells may promote accurate autophagic targeting by using the same organelle to scaffold the autophagosome [90]. Given the strong associations between autophagy, metabolism, and age-related disease, understanding the role of inter-organelle communication in mediating subcellular turnover will be an exciting area of investigation moving forward.

Organelle interfaces as signaling hubs

Finally, we increasingly understand that organelle contact sites serve as central hubs for signal transduction pathways. Nutrient-signaling pathways possess the deepest ties to the aging process, and from these pathways the mechanistic target of rapamycin (mTOR) is emerging as a major reader and regulator of inter-organelle communication. The first direct link between mTOR and contact sites was the finding that mTORC2 presence at ER-mitochondrial contact sites is dynamic and dependent on growth factor signaling [92]. Furthermore, mTORC2-Akt signaling coordinates contact site functions by phosphorylating distinct mediators of ER-mitochondrial tethering, signaling and metabolism (Figure 1M). While it remains technically challenging to differentiate mTORC2 activity at the ER-mitochondrial contact site from its function at other cell locales, this model provides an example of a mechanism to couple metabolic sensing with local regulation of contact site activities. Similarly, pharmacological studies with rapamycin suggested that mTORC1 function may also be linked to the positioning and coupling of ER and mitochondria, though it is not yet clear if mTORC1 directly regulates contact site residents [93].

Of course, the organelles most canonically linked to mTOR regulation are lysosomes, and a series of studies between yeast and mammalian models have linked contact site-dependent regulation of vacuolar/lysosomal membrane compositions to the activation state of mTOR. In yeast, sterol transport proteins, specifically Ltc1 and Ltc3/4, act as tethers at membrane contact sites between ER-vacuole and ER-plasma membrane, respectively [94]. In an example of cells exploiting contact sites to spatially segregate signaling pathways, Ltc1 at the ER-vacuole regulates mTORC1 activation, while Ltc3/4 at the plasma membrane contact is linked instead to mTORC2. At each of these contact sites, the sterol transport function of these proteins promotes the local formation of sterol-enriched membrane microdomains that are important for concentrating mTOR signaling partners, including components of the Ragulator complex upstream of mTORC1 [94]. In the case of Ltc1, which interacts with vacuole membrane protein Vac8 at ER-vacuole contacts, these vacuolar microdomains play a critical role in driving recruitment of GTPase activating proteins to ultimately inactivate the Ragulator complex and reduce mTORC1 activation [94] (Figure 1N). Sterols are also linked to mTORC signaling in mammalian cells, where oxysterol binding protein serves the lipid transfer role analogous to Ltc1, and VAMP-associated protein (VAP)-A/VAP-B act to tether ER and lysosomes [95]. In mammalian cells, high levels of lysosomal cholesterol are sufficient to activate mTORC1. In the context of Niemann-Pick lysosomal storage disease, where lysosomal cholesterol levels are chronically elevated, mTORC1 is constitutively hyperactive. Furthermore, the hyperactive mTORC1 signaling in this context plays a central role in promoting mitochondrial dysfunction and neurodegenerative phenotypes associated with Niemann-Pick disease [95,96]. Given how tightly linked mTOR, mitochondrial dysfunction, and neurodegeneration are with aging, these results suggest an intriguing model

Biochemical Society Transactions (2022) **50** 1389–1402
https://doi.org/10.1042/BST20220519

where aberrant inter-organelle sterol trafficking disrupts core nutrient-signaling pathways to exacerbate the cascade of cellular pathophysiologies correlated with aging [97].

Similar to nutrient signaling, stress response pathways are important for a number of pro-longevity interventions, and organelle contact sites are also emerging as hubs for launching and integrating these responses. In addition to the InsP3R-mediated links between ER stress and mitochondria described above, canonical mediators of the ER stress response, termed the unfolded protein response (UPR), also localize to ER-mitochondrial contact sites. Inositol-requiring enzyme (IRE)1 is a dominant player in the UPR, normally responding to unfolded proteins in the ER lumen by oligomerizing and activating splicing and/or digestion of ER-associated mRNAs [3]. Independent of its enzymatic activity, however, IRE1 promotes mitochondrial bioenergetics by acting as a scaffold for the InsP3R at ER-mitochondrial contacts. Localization at ER-mitochondrial contacts also allows IRE1 to receive inputs back from mitochondria. Specifically, mitochondrial ubiquitin ligase MITOL ubiquitylates IRE1 at contact sites, attenuating UPR activation by preventing IRE1-mediated splicing of XBP1 mRNA [98] (Figure 1O). This crosstalk is physiologically important in contexts of unresolvable or excessive ER stress, upon which cells switch from a survival response to actively inducing apoptosis. ER stress promotes apoptosis partly by overloading mitochondria with calcium, and it thus it appears that IRE1 presence at the ER-mitochondrial contact sites is a key mechanism to control this survival switch. A second major UPR mediator, PKR-like endoplasmic reticulum kinase (PERK), is also linked to this survival switch [99,100]. Also found at contact sites, PERK strengthens ER-mitochondrial tethering by mechanisms that are not yet clear, but in doing so propagates apoptosis-associated redox signaling between organelles [101]. The extent to which the ER responds to mitochondrial stress is less clear, but in one example, disruption of mitochondrial dynamics via Mitofusin 2 ablation triggers widespread ER disruption and enhanced UPR activation [99] (Figure 1O). The ER response in this context appears maladaptive, as inhibition of PERK in Mitofusin 2-depleted cells surprisingly rescues some aspects of mitochondrial dysfunction. Lastly, there are no reports of the third major UPR branch, activating transcription factor (ATF)-6, at contact sites, but a recent study in *C. elegans* revealed a link between *atf-6* and lifespan through mechanisms involving mitochondria [46]. Specifically, aging animals appear to possess hyperactivated UPR signaling through ATF-6, which leads to impaired ER calcium release through the InsP3R and downstream defects in mitochondrial function. Taken together, these stress response pathways illustrate how organelle contact sites can act both as avenues for stressed organelles to receive support or transmit damage to neighbors. Understanding how to target the signaling at contact sites and shift the balance towards beneficial outcomes will be an exciting pursuit in geroscience going forward.

Conclusions and future directions

Our perspective on physiology is intrinsically linked to anatomy. At the subcellular level, however, organelle anatomy is complicated by the fact that very little is fixed in place. Organelles are motile and their morphologies and distributions within the cell are highly dynamic. Thus, the mechanisms by which these organelle compartments signal and connect to each other are similarly dynamic. The discoveries of diverse molecular signaling and tethering mechanisms linking organelles together is therefore opening entirely new perspectives and frontiers in cell physiology. As the manipulation of inter-organelle interactions grows increasingly tractable, the field is shifting towards understanding the cellular and organismal roles of these interactions. The observation that a growing proportion of chronic, age-related diseases features signs of aberrant inter-organelle communication compels a deeper look into whether these lines of communication might play a more fundamental role in the aging process.

The number and nature of inter-organelle interactions are highly diverse, but a few key themes emerge (Figure 1). First, in multiple cases organelle morphology and dynamics rely on specific interactions with other organelles. Age-related deficiencies in one network can therefore drive improper shape, positioning and quality control of another at this structural level. Because the ER appears to direct fission of multiple other organelles, it may play an especially important role in maintaining organelle architecture during aging. Second, organelle interactions enable the precise and efficient transfer of ions and polar metabolites. In cases such as ER-mitochondrial calcium flux, the ions serve as a mechanism for one organelle to recruit and regulate the metabolic functions of another. Alternatively, age-onset defects in lysosomal amino acid storage drive declines in multiple other organelles, revealing the importance of metabolite targeting and compartmentalization during aging. Third, organelle contact sites mediate precise, high-volume flux of hydrophobic lipids and membranes. By coupling lipid droplets with different partners, cells can control the pathways and fates of their lipid fuels.

Artificially rewiring lipid storage and release is proving to extend lifespan in multiple contexts, which is exciting considering that modulation of energy metabolism is central to multiple conserved longevity paradigms. Finally, inter-organelle contact sites provide local hubs for integration of signaling pathways. Local nutrient flux at organelle contact sites fine-tune the activation of nutrient sensors, such as mTORC1. And in examples involving ER and mitochondria, ER stress sensors directly regulate the mediators of inter-organelle signaling, providing a pathway to promote functional resilience in cells by enabling a compromised organelle to receive support.

Inter-organelle interactions appear to play roles in the aging process that are perhaps as broad as traditional "hallmarks of aging" [97] (Figure 2). In this current framework, interconnectivity between the hallmarks is a central theme. For example, age-dependent damage at the molecular level (i.e. the genome, proteome and the networks that maintain them) is the primary instigator of declines that grow in scale, from mitochondria to cells, tissues, and ultimately systemic physiology [97]. Consistent with this theme of damage crossing scales, organelle communication plays a key role in how dysfunction is transmitted within and between cells. For instance, the ER is a key link between molecular damage and mitochondrial function, as proteostasis defects or metabolic dysfunction in the ER leads to remodeling of its contacts and signaling with mitochondria [40,46,102] (Figure 2A,B). Mitochondrial function is also highly dependent on lysosomes, not only due to lysosomal roles in mitophagic turnover, but also because age-onset impairment of lysosomal amino acid compartmentalization causes pronounced toxicity to mitochondria [47,48]. Similarly, lysosomal homeostasis depends on contacts with ER and mitochondria, and age-related declines in lysosomal processes can cascade into

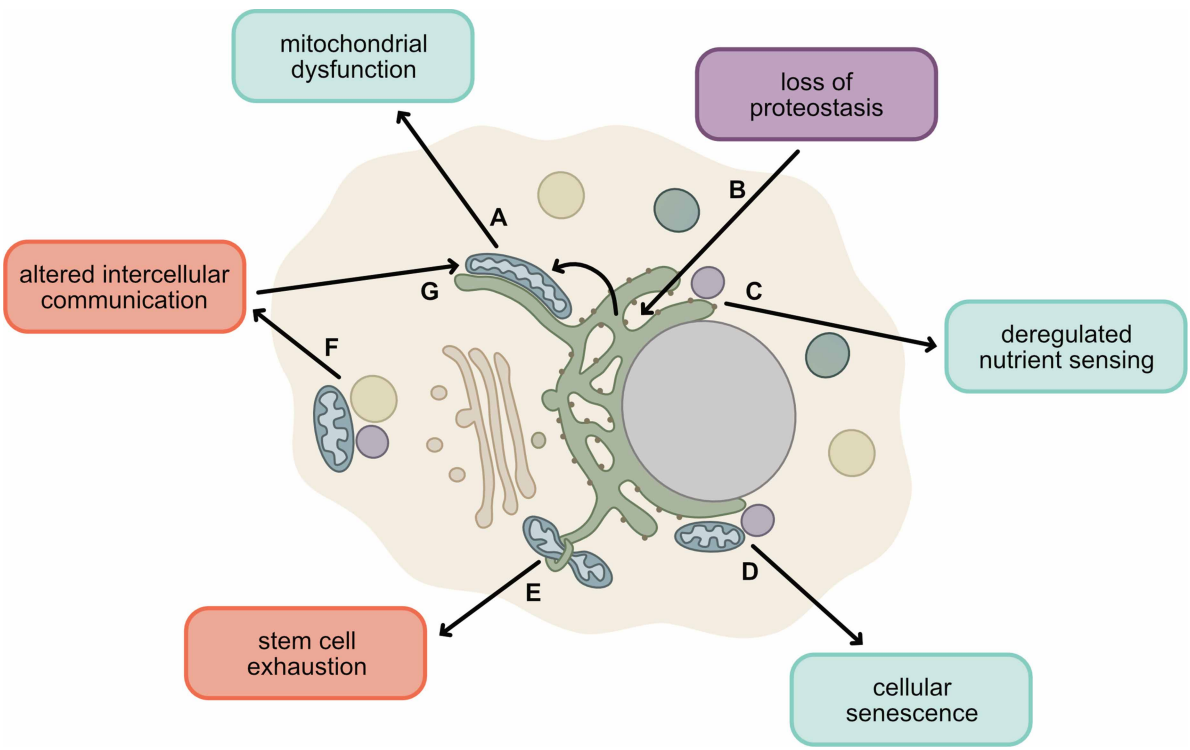

Figure 2. Altered inter-organelle interactions are integrated with diverse aging hallmarks.
(**A** and **B**) Proteostasis failures cause ER stress and activate the UPR^{ER}, which enhances Ca^{2+} flux into mitochondria at ER-mitochondrial contact sites and promotes mitochondrial dysfunction when unresolved. (**C**) Age-onset decline of lysosomal function leads to impaired nutrient signaling through mTOR and its downstream effectors. (**D**) Dysfunction of both ER and lysosomes is transmitted to the mitochondrial network, which is a key determinant of cellular senescence. (**E**) ER tubules are important regulators of mitochondrial dynamics, which in turn control stem cell maintenance and fate decisions. (**F**) Dysfunction at contacts between mitochondria, lipid droplets, and lysosomes impinge on cell nonautonomous signaling pathways. (**G**) Systemic metabolic stress and altered signaling impacts ER-mitochondrial contact site formation and communication.

Biochemical Society Transactions (2022) **50** 1389–1402
https://doi.org/10.1042/BST20220519

impaired nutrient signaling through direct links to mTOR [26,94–96] (Figure 2C). Both mitochondrial and lysosomal/autophagosomal function are also intimately linked to another hallmark, cell senescence [103,104]. Given the key roles of inter-organelle contacts in maintaining mitochondrial and lysosomal health, studies into the roles of inter-organelle communication in cell senescence are an intriguing future pursuit (Figure 2D). Similarly, mitochondrial fission/fusion dynamics and function are important determinants for stem cell fate decisions and proliferative capacities, leading to intriguing suggestions that age-related alterations in ER-mitochondrial sites may underlie stem cell exhaustion [105] (Figure 2E). Experimental links between altered organelle communication and systemic pathology are also emerging. Altered trafficking of lipids along the chain from lipid droplets to lysosomes and mitochondria can promote altered intercellular lipid signaling and cell nonautonomous impacts on aging [76,83,106,107] (Figure 2F). Similarly, intercellular propagation of ER stress signals within the liver promotes ER dysfunction and insulin resistance [108]. In obesity, excess nutrients and cell nonautonomous signaling promote chronic hepatic ER stress, which triggers a vicious cycle of aberrant MAM formation, mitochondrial dysfunction and systemic metabolic defects [40] (Figure 2G).

Moving forward, many exciting challenges await both in our basic understanding of how cells sense and regulate the dynamics of communication between organelle pairs and how these dynamics impact the aging process. The microscopy needed for quantitative analyses of contact site dynamics continues to evolve and propel the field forward. Trade-offs between spatial and temporal resolution and the limited ability to visualize multiple cell structures have traditionally limited advancement, but these barriers continue to fall [109–111]. One of the key hurdles is our limited ability to simultaneously visualize both a physical contact site and aspects of its function or composition. Towards this end, emerging mass spectrometry imaging technologies will also begin to provide additional information on the molecular mediators and metabolic activity at these tiny organelle interfaces [112,113]. Already, the ability to subclassify individual organelles based on their contact site-partners [21,79] and/or location in the cell [72] has revealed unexpected functional heterogeneity within an organelle network. As we continue to identify the mediators of this intra-organelle heterogeneity, new avenues open for precision-targeting of maladaptive cell biological and metabolic processes. Given the growing body of evidence for altered inter-organelle interactions in aging and age-dependent disease, experimentally assigning causality between specific inter-organelle interactions will unlock their potential as therapeutic targets.

Perspectives

- Aging is characterized by a progressive inability to maintain homeostasis and resilience. Several modes of inter-organelle communication are emerging as key mechanisms by which cells adapt to changing or stressful conditions and are thus likely to provide new therapeutic targets for the diseases of aging.
- Remodeling of multiple organelle contact sites is increasingly associated with age-onset diseases, but evidence that aberrant inter-organelle communication is a systemic driver of aging is just emerging.
- As the technology for quantitatively assessing both the physical and functional interactions between organelles continues expanding our insights into how cells regulate the dynamics of inter-organelle communication, we can experimentally manipulate these processes to test for causal roles in aging and age-related disease.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

The authors are supported by National Institutes of Health F31AG076290 to E.D. and the Glenn Foundation for Medical Research/American Federation for Aging Research and National Institutes of Health R01AG073354 and R21AG071975 to K.B

Author Contributions

E.D., E.R. and K.B. conceived, drafted and critically revised the manuscript; E.R. generated the figures.

Acknowledgements

We apologize to all colleagues whose work could not be cited due to length restrictions. We thank all members of the Burkewitz lab and Vivian Gama for discussions and helpful comments.

Abbreviations

Arf1, ADP-ribosylation factor 1; ATF, activating transcription factor; ATG14, autophagy-related 14; ER, endoplasmic reticulum; InsP3R, inositol triphosphate receptor; IRE, Inositol-requiring enzyme; LIPL, lipase like; Ltc, lipid transfer at contact site; LTP, lipid transfer protein; MAMs, mitochondria-associated ER membranes; MITOL, mitochondrial ubiquitin ligase; mTOR, mechanistic target of rapamycin; mTORC1, mTOR Complex 1; mTORC2, mTOR Complex 2; PERK, PKR-like endoplasmic reticulum kinase; ROS, reactive oxygen species; STX17, syntaxin-17; TCA, tricarboxylic acid; UPR, unfolded protein response; Vac, vacuolar protein; VAP, VAMP-associated protein; XBP, X-box binding protein.

References

1 Gottschling, D.E. and Nyström, T. (2017) The upsides and downsides of organelle interconnectivity. *Cell* **169**, 24–34 https://doi.org/10.1016/j.cell.2017.02.030
2 Bohnert, M. and Schuldiner, M. (2018) Stepping outside the comfort zone of membrane contact site research. *Nat. Rev. Mol. Cell Biol.* **19**, 483–484 https://doi.org/10.1038/s41580-018-0022-1
3 Frakes, A.E. and Dillin, A. (2017) The UPRER: sensor and coordinator of organismal homeostasis. *Mol. Cell* **66**, 761–771 https://doi.org/10.1016/j.molcel.2017.05.031
4 Melber, A. and Haynes, C.M. (2018) UPRmt regulation and output: a stress response mediated by mitochondrial-nuclear communication. *Cell Res.* **28**, 281–295 https://doi.org/10.1038/cr.2018.16
5 Merkwirth, C., Jovaisaite, V., Durieux, J., Matilainen, O., Jordan, S.D., Quiros, P.M. et al. (2016) Two conserved histone demethylases regulate mitochondrial stress-induced longevity. *Cell* **165**, 1209–1223 https://doi.org/10.1016/j.cell.2016.04.012
6 Petkovic, M., O'Brien, C.E. and Jan, Y.N. (2021) Interorganelle communication, aging, and neurodegeneration. *Genes Dev.* **35**, 449–469 https://doi.org/10.1101/gad.346759.120
7 Cohen, S., Valm, A.M. and Lippincott-Schwartz, J. (2018) Interacting organelles. *Curr. Opin. Cell Biol.* **53**, 84–91 https://doi.org/10.1016/j.ceb.2018.06.003
8 Bohnert, M. (2020) Tether me, tether me not-dynamic organelle contact sites in metabolic rewiring. *Dev. Cell* **54**, 212–225 https://doi.org/10.1016/j.devcel.2020.06.026
9 Jain, A. and Zoncu, R. (2022) Organelle transporters and inter-organelle communication as drivers of metabolic regulation and cellular homeostasis. *Mol. Metab.* **60**, 101481 https://doi.org/10.1016/j.molmet.2022.101481
10 Prinz, W.A., Toulmay, A. and Balla, T. (2020) The functional universe of membrane contact sites. *Nat. Rev. Mol. Cell Biol.* **21**, 7–24 https://doi.org/10.1038/s41580-019-0180-9
11 Feng, Q. and Kornmann, B. (2018) Mechanical forces on cellular organelles. *J. Cell Sci.* **131**, jcs218479 https://doi.org/10.1242/jcs.218479
12 Henne, W.M. (2016) Organelle remodeling at membrane contact sites. *J. Struct. Biol.* **196**, 15–19 https://doi.org/10.1016/j.jsb.2016.05.003
13 Hoyer, M.J., Chitwood, P.J., Ebmeier, C.C., Striepen, J.F., Qi, R.Z., Old, W.M. et al. (2018) A novel class of ER membrane proteins regulates ER-associated endosome fission. *Cell* **175**, 254–265.e14 https://doi.org/10.1016/j.cell.2018.08.030
14 Wu, H. and Voeltz, G.K. (2021) Reticulon-3 promotes endosome maturation at ER membrane contact sites. *Dev. Cell* **56**, 52–66.e7 https://doi.org/10.1016/j.devcel.2020.12.014
15 Friedman, J.R., Lackner, L.L., West, M., DiBenedetto, J.R., Nunnari, J. and Voeltz, G.K. (2011) ER tubules mark sites of mitochondrial division. *Science* **334**, 358–362 https://doi.org/10.1126/science.1207385
16 Chakrabarti, R., Ji, W.-K., Stan, R.V., de Juan Sanz, J., Ryan, T.A. and Higgs, H.N. (2018) INF2-mediated actin polymerization at the ER stimulates mitochondrial calcium uptake, inner membrane constriction, and division. *J. Cell Biol.* **217**, 251–268 https://doi.org/10.1083/jcb.201709111
17 Lee, J.E., Cathey, P.I., Wu, H., Parker, R. and Voeltz, G.K. (2020) Endoplasmic reticulum contact sites regulate the dynamics of membraneless organelles. *Science* **367**, eaay7108 https://doi.org/10.1126/science.aay7108
18 Ji, W.-K., Chakrabarti, R., Fan, X., Schoenfeld, L., Strack, S. and Higgs, H.N. (2017) Receptor-mediated Drp1 oligomerization on endoplasmic reticulum. *J. Cell Biol.* **216**, 4123–4139 https://doi.org/10.1083/jcb.201610057
19 Abrisch, R.G., Gumbin, S.C., Wisniewski, B.T., Lackner, L.L. and Voeltz, G.K. (2020) Fission and fusion machineries converge at ER contact sites to regulate mitochondrial morphology. *J. Cell Biol.* **219**, e201911122 https://doi.org/10.1083/jcb.201911122
20 Sharma, A., Smith, H.J., Yao, P. and Mair, W.B. (2019) Causal roles of mitochondrial dynamics in longevity and healthy aging. *EMBO Rep.* **20**, e48395 https://doi.org/10.15252/embr.201948395
21 Kleele, T., Rey, T., Winter, J., Zaganelli, S., Mahecic, D., Perreten Lambert, H. et al. (2021) Distinct fission signatures predict mitochondrial degradation or biogenesis. *Nature* **593**, 435–439 https://doi.org/10.1038/s41586-021-03510-6
22 Gerhold, J.M., Cansiz-Arda, Ş., Lõhmus, M., Engberg, O., Reyes, A., van Rennes, H. et al. (2015) Human mitochondrial DNA-protein complexes attach to a cholesterol-rich membrane structure. *Sci. Rep.* **5**, 15292 https://doi.org/10.1038/srep15292
23 Lewis, S.C., Uchiyama, L.F. and Nunnari, J. (2016) ER-mitochondria contacts couple mtDNA synthesis with mitochondrial division in human cells. *Science* **353**, aaf5549 https://doi.org/10.1126/science.aaf5549

24 Meeusen, S. and Nunnari, J. (2003) Evidence for a two membrane-spanning autonomous mitochondrial DNA replisome. *J. Cell Biol.* **163**, 503–510 https://doi.org/10.1083/jcb.200304040
25 Murley, A., Lackner, L.L., Osman, C., West, M., Voeltz, G.K., Walter, P. et al. (2013) ER-associated mitochondrial division links the distribution of mitochondria and mitochondrial DNA in yeast. *eLife* **2**, e00422 https://doi.org/10.7554/eLife.00422
26 Wong, Y.C., Ysselstein, D. and Krainc, D. (2018) Mitochondria-lysosome contacts regulate mitochondrial fission via RAB7 GTP hydrolysis. *Nature* **554**, 382–386 https://doi.org/10.1038/nature25486
27 Nagashima, S., Tábara, L.-C., Tilokani, L., Paupe, V., Anand, H., Pogson, J.H. et al. (2020) Golgi-derived PI**(**4**)**P-containing vesicles drive late steps of mitochondrial division. *Science* **367**, 1366–1371 https://doi.org/10.1126/science.aax6089
28 Csordás, G., Thomas, A.P. and Hajnóczky, G. (1999) Quasi-synaptic calcium signal transmission between endoplasmic reticulum and mitochondria. *EMBO J.* **18**, 96–108 https://doi.org/10.1093/emboj/18.1.96
29 Rizzuto, R., Brini, M., Murgia, M. and Pozzan, T. (1993) Microdomains with high Ca^{2+} close to IP3-sensitive channels that are sensed by neighboring mitochondria. *Science* **262**, 744–747 https://doi.org/10.1126/science.8235595
30 Rizzuto, R., Pinton, P., Carrington, W., Fay, F.S., Fogarty, K.E., Lifshitz, L.M. et al. (1998) Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca^{2+} responses. *Science* **280**, 1763–1766 https://doi.org/10.1126/science.280.5370.1763
31 Kirichok, Y., Krapivinsky, G. and Clapham, D.E. (2004) The mitochondrial calcium uniporter is a highly selective ion channel. *Nature* **427**, 360–364 https://doi.org/10.1038/nature02246
32 Perocchi, F., Gohil, V.M., Girgis, H.S., Bao, X.R., McCombs, J.E., Palmer, A.E. et al. (2010) MICU1 encodes a mitochondrial EF hand protein required for Ca^{2+} uptake. *Nature* **467**, 291–296 https://doi.org/10.1038/nature09358
33 De Stefani, D., Raffaello, A., Teardo, E., Szabò, I. and Rizzuto, R. (2011) A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter. *Nature* **476**, 336–340 https://doi.org/10.1038/nature10230
34 Baughman, J.M., Perocchi, F., Girgis, H.S., Plovanich, M., Belcher-Timme, C.A., Sancak, Y. et al. (2011) Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter. *Nature* **476**, 341–345 https://doi.org/10.1038/nature10234
35 Herrera-Cruz, M.S. and Simmen, T. (2017) Over six decades of discovery and characterization of the architecture at mitochondria-associated membranes (MAMs). *Adv. Exp. Med. Biol.* **997**, 13–31 https://doi.org/10.1007/978-981-10-4567-7_2
36 Eisenberg-Bord, M., Shai, N., Schuldiner, M. and Bohnert, M. (2016) A tether is a tether is a tether: tethering at membrane contact sites. *Dev. Cell* **39**, 395–409 https://doi.org/10.1016/j.devcel.2016.10.022
37 Rossi, A., Pizzo, P. and Filadi, R. (2019) Calcium, mitochondria and cell metabolism: a functional triangle in bioenergetics. *Biochim. Biophys. Acta Mol. Cell Res.* **1866**, 1068–1078 https://doi.org/10.1016/j.bbamcr.2018.10.016
38 Cárdenas, C., Miller, R.A., Smith, I., Bui, T., Molgó, J., Müller, M. et al. (2010) Essential regulation of cell bioenergetics by constitutive InsP3 receptor Ca^{2+} transfer to mitochondria. *Cell* **142**, 270–283 https://doi.org/10.1016/j.cell.2010.06.007
39 Bravo, R., Vicencio, J.M., Parra, V., Troncoso, R., Munoz, J.P., Bui, M. et al. (2011) Increased ER-mitochondrial coupling promotes mitochondrial respiration and bioenergetics during early phases of ER stress. *J. Cell Sci.* **124**, 2143–2152 https://doi.org/10.1242/jcs.080762
40 Arruda, A.P., Pers, B.M., Parlakgül, G., Güney, E., Inouye, K. and Hotamisligil, G.S. (2014) Chronic enrichment of hepatic endoplasmic reticulum-mitochondria contact leads to mitochondrial dysfunction in obesity. *Nat. Med.* **20**, 1427–1435 https://doi.org/10.1038/nm.3735
41 Franceschi, C., Garagnani, P., Parini, P., Giuliani, C. and Santoro, A. (2018) Inflammaging: a new immune-metabolic viewpoint for age-related diseases. *Nat. Rev. Endocrinol.* **14**, 576–590 https://doi.org/10.1038/s41574-018-0059-4
42 Cardenas, C., Lovy, A., Silva-Pavez, E., Urra, F., Mizzoni, C., Ahumada-Castro, U. et al. (2020) Cancer cells with defective oxidative phosphorylation require endoplasmic reticulum-to-mitochondria Ca^{2+} transfer for survival. *Sci. Signal.* **13**, eaay1212 https://doi.org/10.1126/scisignal.aay1212
43 Arruda, A.P. and Hotamisligil, G.S. (2015) Calcium homeostasis and organelle function in the pathogenesis of obesity and diabetes. *Cell Metab.* **22**, 381–397 https://doi.org/10.1016/j.cmet.2015.06.010
44 Zung, N. and Schuldiner, M. (2020) New horizons in mitochondrial contact site research. *Biol. Chem.* **401**, 793–809 https://doi.org/10.1515/hsz-2020-0133
45 Garrido-Maraver, J., Loh, S.H.Y. and Martins, L.M. (2020) Forcing contacts between mitochondria and the endoplasmic reticulum extends lifespan in a Drosophila model of Alzheimer's disease. *Biol. Open* **9**, bio047530 https://doi.org/10.1242/bio.047530
46 Burkewitz, K., Feng, G., Dutta, S., Kelley, C.A., Steinbaugh, M., Cram, E.J. et al. (2020) Atf-6 regulates lifespan through ER-mitochondrial calcium homeostasis. *Cell Rep.* **32**, 108125 https://doi.org/10.1016/j.celrep.2020.108125
47 Hughes, A.L. and Gottschling, D.E. (2012) An early age increase in vacuolar pH limits mitochondrial function and lifespan in yeast. *Nature* **492**, 261–265 https://doi.org/10.1038/nature11654
48 Hughes, C.E., Coody, T.K., Jeong, M.-Y., Berg, J.A., Winge, D.R. and Hughes, A.L. (2020) Cysteine toxicity drives age-related mitochondrial decline by altering iron homeostasis. *Cell* **180**, 296–310.e18 https://doi.org/10.1016/j.cell.2019.12.035
49 Higuchi-Sanabria, R., Shen, K., Kelet, N., Frankino, P.A., Durieux, J., Bar-Ziv, R. et al. (2020) Lysosomal recycling of amino acids affects ER quality control. *Sci Adv* **6**, eaaz9805 https://doi.org/10.1126/sciadv.aaz9805
50 Kim, J. and Bai, H. (2022) Peroxisomal stress response and inter-organelle communication in cellular homeostasis and aging. *Antioxidants (Basel)* **11**, 192 https://doi.org/10.3390/antiox11020192
51 Fransen, M., Lismont, C. and Walton, P. (2017) The peroxisome-mitochondria connection: how and why? *Int. J. Mol. Sci.* **18**, 1126 https://doi.org/10.3390/ijms18061126
52 Ivashchenko, O., Van Veldhoven, P.P., Brees, C., Ho, Y.-S., Terlecky, S.R. and Fransen, M. (2011) Intraperoxisomal redox balance in mammalian cells: oxidative stress and interorganellar cross-talk. *Mol. Biol. Cell* **22**, 1440–1451 https://doi.org/10.1091/mbc.E10-11-0919
53 Weir, H.J., Yao, P., Huynh, F.K., Escoubas, C.C., Goncalves, R.L., Burkewitz, K. et al. (2017) Dietary restriction and AMPK increase lifespan via mitochondrial network and peroxisome remodeling. *Cell Metab.* **26**, 884–896.e5 https://doi.org/10.1016/j.cmet.2017.09.024
54 Yoboue, E.D., Sitia, R. and Simmen, T. (2018) Redox crosstalk at endoplasmic reticulum (ER) membrane contact sites (MCS) uses toxic waste to deliver messages. *Cell Death Dis.* **9**, 331 https://doi.org/10.1038/s41419-017-0033-4
55 Wu, Y., Whiteus, C., Xu, C.S., Hayworth, K.J., Weinberg, R.J., Hess, H.F. et al. (2017) Contacts between the endoplasmic reticulum and other membranes in neurons. *Proc. Natl Acad. Sci. U.S.A.* **114**, E4859–E4867 https://doi.org/10.1073/pnas.1701078114

56 Vance, J.E. (1990) Phospholipid synthesis in a membrane fraction associated with mitochondria. *J. Biol. Chem.* **265**, 7248–7256 https://doi.org/10.1111/tra.12230
57 Reinisch, K.M. and Prinz, W.A. (2021) Mechanisms of nonvesicular lipid transport. *J. Cell Biol.* **220**, e202012058 https://doi.org/10.1083/jcb.202012058
58 Monteiro-Cardoso, V.F., Rochin, L., Arora, A., Houcine, A., Jääskeläinen, E., Kivelä, A.M. et al. (2022) ORP5/8 and MIB/MICOS link ER-mitochondria and intra-mitochondrial contacts for non-vesicular transport of phosphatidylserine. *Cell Rep.* **40**, 111364 https://doi.org/10.1016/j.celrep.2022.111364
59 Gallo, A., Danglot, L., Giordano, F., Hewlett, B., Binz, T., Vannier, C. et al. (2020) Role of the Sec22b-E-Syt complex in neurite growth and ramification. *J. Cell Sci.* **133**, jcs247148 https://doi.org/10.1242/jcs.247148
60 Weber-Boyvat, M., Trimbuch, T., Shah, S., Jäntti, J., Olkkonen, V.M. and Rosenmund, C. (2021) ORP/osh mediate cross-talk between ER-plasma membrane contact site components and plasma membrane SNAREs. *Cell. Mol. Life Sci.* **78**, 1689–1708 https://doi.org/10.1007/s00018-020-03604-w
61 Ng, A.Y.E., Ng, A.Q.E. and Zhang, D. (2018) ER-PM contacts restrict exocytic sites for polarized morphogenesis. *Curr. Biol.* **28**, 146–153.e5 https://doi.org/10.1016/j.cub.2017.11.055
62 Papsdorf, K. and Brunet, A. (2019) Linking lipid metabolism to chromatin regulation in aging. *Trends Cell Biol.* **29**, 97–116 https://doi.org/10.1016/j.tcb.2018.09.004
63 Kannan, M., Lahiri, S., Liu, L.-K., Choudhary, V. and Prinz, W.A. (2017) Phosphatidylserine synthesis at membrane contact sites promotes its transport out of the ER. *J. Lipid Res.* **58**, 553–562 https://doi.org/10.1194/jlr.M072959
64 Borkenhagen, L.F., Kennedy, E.P. and Fielding, L. (1961) Enzymatic formation and decarboxylation of phosphatidylserine. *J. Biol. Chem.* **236**, PC28–PC30 https://doi.org/10.1016/S0021-9258(19)63319-3
65 Vance, J.E. (2015) Phospholipid synthesis and transport in mammalian cells. *Traffic* **16**, 1–18 https://doi.org/10.1111/tra.12230
66 Friedman, J.R., Kannan, M., Toulmay, A., Jan, C.H., Weissman, J.S., Prinz, W.A. et al. (2018) Lipid homeostasis is maintained by dual targeting of the mitochondrial PE biosynthesis enzyme to the ER. *Dev. Cell* **44**, 261–270.e6 https://doi.org/10.1016/j.devcel.2017.11.023
67 Gok, M.O., Speer, N.O., Henne, W.M. and Friedman, J.R. (2022) ER-localized phosphatidylethanolamine synthase plays a conserved role in lipid droplet formation. *Mol. Biol. Cell* **33**, ar11 https://doi.org/10.1091/mbc.E21-11-0558-T
68 Rockenfeller, P., Koska, M., Pietrocola, F., Minois, N., Knittelfelder, O., Sica, V. et al. (2015) Phosphatidylethanolamine positively regulates autophagy and longevity. *Cell Death Differ.* **22**, 499–508 https://doi.org/10.1038/cdd.2014.219
69 Herker, E., Vieyres, G., Beller, M., Krahmer, N. and Bohnert, M. (2021) Lipid droplet contact sites in health and disease. *Trends Cell Biol.* **31**, 345–358 https://doi.org/10.1016/j.tcb.2021.01.004
70 Walther, T.C., Chung, J. and Farese, Jr, R.V. (2017) Lipid droplet biogenesis. *Annu. Rev. Cell Dev. Biol.* **33**, 491–510 https://doi.org/10.1146/annurev-cellbio-100616-060608
71 Mejhert, N., Gabriel, K.R., Frendo-Cumbo, S., Krahmer, N., Song, J., Kuruvilla, L. et al. (2022) The lipid droplet knowledge portal: a resource for systematic analyses of lipid droplet biology. *Dev. Cell* **57**, 387–397.e4 https://doi.org/10.1016/j.devcel.2022.01.003
72 Ugrankar, R., Bowerman, J., Hariri, H., Chandra, M., Chen, K., Bossanyi, M.-F. et al. (2019) Drosophila snazarus regulates a lipid droplet population at plasma membrane-droplet contacts in adipocytes. *Dev. Cell* **50**, 557–572.e5 https://doi.org/10.1016/j.devcel.2019.07.021
73 Herms, A., Bosch, M., Ariotti, N., Reddy, B.J.N., Fajardo, A., Fernández-Vidal, A. et al. (2013) Cell-to-cell heterogeneity in lipid droplets suggests a mechanism to reduce lipotoxicity. *Curr. Biol.* **23**, 1489–1496 https://doi.org/10.1016/j.cub.2013.06.032
74 Mutlu, A.S., Duffy, J. and Wang, M.C. (2021) Lipid metabolism and lipid signals in aging and longevity. *Dev. Cell* **56**, 1394–1407 https://doi.org/10.1016/j.devcel.2021.03.034
75 Hansen, M., Flatt, T. and Aguilaniu, H. (2013) Reproduction, fat metabolism, and life span: what is the connection? *Cell Metab.* **17**, 10–19 https://doi.org/10.1016/j.cmet.2012.12.003
76 Rambold, A.S., Cohen, S. and Lippincott-Schwartz, J. (2015) Fatty acid trafficking in starved cells: regulation by lipid droplet lipolysis, autophagy, and mitochondrial fusion dynamics. *Dev. Cell* **32**, 678–692 https://doi.org/10.1016/j.devcel.2015.01.029
77 Rambold, A.S., Kostelecky, B., Elia, N. and Lippincott-Schwartz, J. (2011) Tubular network formation protects mitochondria from autophagosomal degradation during nutrient starvation. *Proc. Natl Acad. Sci. U.S.A.* **108**, 10190–10195 https://doi.org/10.1073/pnas.1107402108
78 Freyre, C.A.C., Rauher, P.C., Ejsing, C.S. and Klemm, R.W. (2019) MIGA2 links mitochondria, the ER, and lipid droplets and promotes *de novo* lipogenesis in adipocytes. *Mol. Cell* **76**, 811–825.e14 https://doi.org/10.1016/j.molcel.2019.09.011
79 Benador, I.Y., Veliova, M., Mahdaviani, K., Petcherski, A., Wikstrom, J.D., Assali, E.A. et al. (2018) Mitochondria bound to lipid droplets have unique bioenergetics, composition, and dynamics that support lipid droplet expansion. *Cell Metab.* **27**, 869–885.e6 https://doi.org/10.1016/j.cmet.2018.03.003
80 Wang, H., Sreenivasan, U., Hu, H., Saladino, A., Polster, B.M., Lund, L.M. et al. (2011) Perilipin 5, a lipid droplet-associated protein, provides physical and metabolic linkage to mitochondria. *J. Lipid Res.* **52**, 2159–2168 https://doi.org/10.1194/jlr.M017939
81 Schulze, R.J., Krueger, E.W., Weller, S.G., Johnson, K.M., Casey, C.A., Schott, M.B. et al. (2020) Direct lysosome-based autophagy of lipid droplets in hepatocytes. *Proc. Natl Acad. Sci. U.S.A.* **117**, 32443–32452 https://doi.org/10.1073/pnas.2011442117
82 Ramachandran, P.V., Savini, M., Folick, A.K., Hu, K., Masand, R., Graham, B.H. et al. (2019) Lysosomal signaling promotes longevity by adjusting mitochondrial activity. *Dev. Cell* **48**, 685–696.e5 https://doi.org/10.1016/j.devcel.2018.12.022
83 Folick, A., Oakley, H.D., Yu, Y., Armstrong, E.H., Kumari, M., Sanor, L. et al. (2015) Lysosomal signaling molecules regulate longevity in *Caenorhabditis elegans*. *Science* **347**, 83–86 https://doi.org/10.1126/science.1258857
84 Choudhary, V., Ojha, N., Golden, A. and Prinz, W.A. (2015) A conserved family of proteins facilitates nascent lipid droplet budding from the ER. *J. Cell Biol.* **211**, 261–271 https://doi.org/10.1083/jcb.201505067
85 Jacquier, N., Choudhary, V., Mari, M., Toulmay, A., Reggiori, F. and Schneiter, R. (2011) Lipid droplets are functionally connected to the endoplasmic reticulum in saccharomyces cerevisiae. *J. Cell Sci.* **124**, 2424–2437 https://doi.org/10.1242/jcs.076836
86 Kassan, A., Herms, A., Fernández-Vidal, A., Bosch, M., Schieber, N.L., Reddy, B.J.N. et al. (2013) Acyl-CoA synthetase 3 promotes lipid droplet biogenesis in ER microdomains. *J. Cell Biol.* **203**, 985–1001 https://doi.org/10.1083/jcb.201305142
87 Sugiura, A., Mattie, S., Prudent, J. and McBride, H.M. (2017) Newly born peroxisomes are a hybrid of mitochondrial and ER-derived pre-peroxisomes. *Nature* **542**, 251–254 https://doi.org/10.1038/nature21375

88 Stefan, C.J., Trimble, W.S., Grinstein, S., Drin, G., Reinisch, K., De Camilli, P. et al. (2017) Membrane dynamics and organelle biogenesis-lipid pipelines and vesicular carriers. *BMC Biol.* **15**, 102 https://doi.org/10.1186/s12915-017-0432-0
89 Hamasaki, M., Furuta, N., Matsuda, A., Nezu, A., Yamamoto, A., Fujita, N. et al. (2013) Autophagosomes form at ER-mitochondria contact sites. *Nature* **495**, 389–393 https://doi.org/10.1038/nature11910
90 Morel, E. (2020) Endoplasmic reticulum membrane and contact site dynamics in autophagy regulation and stress response. *Front. Cell Dev. Biol.* **8**, 343 https://doi.org/10.3389/fcell.2020.00343
91 Böckler, S. and Westermann, B. (2014) Mitochondrial ER contacts are crucial for mitophagy in yeast. *Dev. Cell* **28**, 450–458 https://doi.org/10.1016/j.devcel.2014.01.012
92 Betz, C., Stracka, D., Prescianotto-Baschong, C., Frieden, M., Demaurex, N. and Hall, M.N. (2013) Feature Article: mTOR complex 2-Akt signaling at mitochondria-associated endoplasmic reticulum membranes (MAM) regulates mitochondrial physiology. *Proc. Natl Acad. Sci. U.S.A.* **110**, 12526–12534 https://doi.org/10.1073/pnas.1302455110
93 Bravo-Sagua, R., López-Crisosto, C., Parra, V., Rodriguez-Peña, M., Rothermel, B.A., Quest, A.F.G. et al. (2016) mTORC1 inhibitor rapamycin and ER stressor tunicamycin induce differential patterns of ER-mitochondria coupling. *Sci. Rep.* **6**, 36394 https://doi.org/10.1038/srep36394
94 Murley, A., Yamada, J., Niles, B.J., Toulmay, A., Prinz, W.A., Powers, T. et al. (2017) Sterol transporters at membrane contact sites regulate TORC1 and TORC2 signaling. *J. Cell Biol.* **216**, 2679–2689 https://doi.org/10.1083/jcb.201610032
95 Lim, C.-Y., Davis, O.B., Shin, H.R., Zhang, J., Berdan, C.A., Jiang, X. et al. (2019) ER-lysosome contacts enable cholesterol sensing by mTORC1 and drive aberrant growth signalling in Niemann-Pick type C. *Nat. Cell Biol.* **21**, 1206–1218 https://doi.org/10.1038/s41556-019-0391-5
96 Davis, O.B., Shin, H.R., Lim, C.-Y., Wu, E.Y., Kukurugya, M., Maher, C.F. et al. (2021) NPC1-mTORC1 signaling couples cholesterol sensing to organelle homeostasis and is a targetable pathway in Niemann-pick type C. *Dev. Cell* **56**, 260–276.e7 https://doi.org/10.1016/j.devcel.2020.11.016
97 López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M. and Kroemer, G. (2013) The hallmarks of aging. *Cell* **153**, 1194–1217 https://doi.org/10.1016/j.cell.2013.05.039
98 Takeda, K., Nagashima, S., Shiiba, I., Uda, A., Tokuyama, T., Ito, N. et al. (2019) MITOL prevents ER stress-induced apoptosis by IRE1α ubiquitylation at ER-mitochondria contact sites. *EMBO J.* **38**, e100999 https://doi.org/10.15252/embj.2018100999
99 Muñoz, J.P., Ivanova, S., Sánchez-Wandelmer, J., Martínez-Cristóbal, P., Noguera, E., Sancho, A. et al. (2013) Mfn2 modulates the UPR and mitochondrial function via repression of PERK. *EMBO J.* **32**, 2348–2361 https://doi.org/10.1038/emboj.2013.168
100 Lebeau, J., Saunders, J.M., Moraes, V.W.R., Madhavan, A., Madrazo, N., Anthony, M.C. et al. (2018) The PERK arm of the unfolded protein response regulates mitochondrial morphology during acute endoplasmic reticulum stress. *Cell Rep.* **22**, 2827–2836 https://doi.org/10.1016/j.celrep.2018.02.055
101 Verfaillie, T., Rubio, N., Garg, A.D., Bultynck, G., Rizzuto, R., Decuypere, J.-P. et al. (2012) PERK is required at the ER-mitochondrial contact sites to convey apoptosis after ROS-based ER stress. *Cell Death Differ.* **19**, 1880–1891 https://doi.org/10.1038/cdd.2012.74
102 Carreras-Sureda, A., Jaña, F., Urra, H., Durand, S., Mortenson, D.E., Sagredo, A. et al. (2019) Non-canonical function of IRE1α determines mitochondria-associated endoplasmic reticulum composition to control calcium transfer and bioenergetics. *Nat. Cell Biol.* **21**, 755–767 https://doi.org/10.1038/s41556-019-0329-y
103 Wiley, C.D., Velarde, M.C., Lecot, P., Liu, S., Sarnoski, E.A., Freund, A. et al. (2016) Mitochondrial dysfunction induces senescence with a distinct secretory phenotype. *Cell Metab.* **23**, 303–314 https://doi.org/10.1016/j.cmet.2015.11.011
104 Wiley, C.D. and Campisi, J. (2021) The metabolic roots of senescence: mechanisms and opportunities for intervention. *Nat. Metab.* **3**, 1290–1301 https://doi.org/10.1038/s42255-021-00483-8
105 Lin, W., Chen, S., Wang, Y., Wang, M., Lee, W.Y.-W., Jiang, X. et al. (2021) Dynamic regulation of mitochondrial-endoplasmic reticulum crosstalk during stem cell homeostasis and aging. *Cell Death Dis.* **12**, 794 https://doi.org/10.1038/s41419-021-03912-4
106 Cohen, S., Rambold, A.S. and Lippincott-Schwartz, J. (2018) Mitochondrial and lipid droplet dynamics regulate intra- and intercellular fatty acid trafficking. *Mol. Cell. Oncol.* **5**, e1043038 https://doi.org/10.1080/23723556.2015.1043038
107 Savini, M., Folick, A., Lee, Y.-T., Jin, F., Cuevas, A., Tillman, M.C. et al. (2022) Lysosome lipid signalling from the periphery to neurons regulates longevity. *Nat. Cell Biol.* **24**, 906–916 https://doi.org/10.1038/s41556-022-00926-8
108 Tirosh, A., Tuncman, G., Calay, E.S., Rathaus, M., Ron, I., Tirosh, A. et al. (2021) Intercellular transmission of hepatic ER stress in obesity disrupts systemic metabolism. *Cell Metab.* **33**, 319–333.e6 https://doi.org/10.1016/j.cmet.2020.11.009
109 Hoffman, D.P., Shtengel, G., Xu, C.S., Campbell, K.R., Freeman, M., Wang, L. et al. (2020) Correlative three-dimensional super-resolution and block-face electron microscopy of whole vitreously frozen cells. *Science* **367**, eaaz5357 https://doi.org/10.1126/science.aaz5357
110 Guo, Y., Li, D., Zhang, S., Yang, Y., Liu, J.-J., Wang, X. et al. (2018) Visualizing intracellular organelle and cytoskeletal interactions at nanoscale resolution on millisecond timescales. *Cell* **175**, 1430–1442.e17 https://doi.org/10.1016/j.cell.2018.09.057
111 Valm, A.M., Cohen, S., Legant, W.R., Melunis, J., Hershberg, U., Wait, E. et al. (2017) Applying systems-level spectral imaging and analysis to reveal the organelle interactome. *Nature* **546**, 162–167 https://doi.org/10.1038/nature22369
112 Pareek, V., Tian, H., Winograd, N. and Benkovic, S.J. (2020) Metabolomics and mass spectrometry imaging reveal channeled *de novo* purine synthesis in cells. *Science* **368**, 283–290 https://doi.org/10.1126/science.aaz6465
113 Arrojo E Drigo, R., Lev-Ram, V., Tyagi, S., Ramachandra, R., Deerinck, T., Bushong, E. et al. (2019) Age mosaicism across multiple scales in adult tissues. *Cell Metab.* **30**, 343–351.e3 https://doi.org/10.1016/j.cmet.2019.05.010