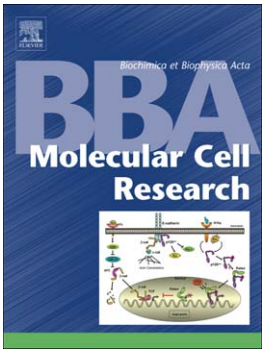

Mitochatting –if only we could be a fly on the cell wall

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Mitochatting –if only we could be a fly on the cell wall

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Abstract

Mitochondria, cellular metabolic hubs, perform many essential processes and are required for the production of metabolites such as ATP, iron-sulfur clusters, heme, amino acids and nucleotides. To fulfill their multiple roles, mitochondria must communicate with all other organelles to exchange small molecules, ions and lipids. Since mitochondria are largely excluded from vesicular trafficking routes, they heavily rely on membrane contact sites. Contact sites are areas of close proximity between organelles that allow efficient transfer of molecules, saving the need for slow and untargeted diffusion through the cytosol. More globally, multiple metabolic pathways require coordination between mitochondria and additional organelles and mitochondrial activity affects all other cellular entities and *vice versa*. Therefore, uncovering the different means of mitochondrial communication will allow us a better understanding of mitochondria and may illuminate disease processes that occur in the absence of proper cross-talk. In this review we focus on how mitochondria interact with all other organelles and emphasize how this communication is essential for mitochondrial and cellular homeostasis.

Introduction

Over a billion years ago, mitochondria, which evolved from α-proteobacteria, became endosymbionts in the eukaryotic cell [1]. Some characteristics of the mitochondrial bacterial ancestors endured, such as the double membrane, the ability to aerobically synthesize ATP and the possession of their own DNA. However, as they evolved, mitochondria became dependent on their host cells, and had to work as part of a complex cellular system.
For eukaryotic cells to work as a coherent unit, all organelles must communicate and coordinate their functions amongst themselves. Mitochondria are responsible for production of ATP and lipids, synthesis and breakdown of various amino acids, biogenesis of heme and iron-sulfur clusters and more. To perform this array of activities, mitochondria evolved ways to transfer molecules to and from other organelles. Indeed it is now becoming apparent that mitochondria communicate with their surrounding cellular entities through an extensive network of signaling pathways and contact sites, areas of close apposition between membranes. This "Mitochatting" allows mitochondria to signal their availability, stress condition or dysfunction to the rest of the cell, thus ensuring that the cell will not commit to a biological process without an assurance that mitochondria are ready to meet its demands [2].
Not surprisingly, the most extensive communication path occurs between mitochondria and the nucleus. This "*tete a tete*" consists of signaling cascades, transition of mitochondrial

products to the nucleus and dual targeted proteins and we have recently written extensively about this cross-talk [3]. In this review we summarize some of the ways by which mitochondria communicate with their neighboring-non-nuclear organelles and describe how this communication maintains mitochondrial homeostasis. Since this topic is extremely wide, we focus on studies carried out using the yeast *Saccharomyces cerevisiae* (from now on referred to simply as yeast), which serves as a widely-used model for studying mitochondrial and cellular biology, as its basic cellular functions and metabolic pathways are highly conserved throughout the eukaryotic kingdom. Apprehending how mitochondria transfer information to other organelles can promote a better understanding of their essential roles in health and disease.

Mitochondria-endoplasmic reticulum

One of the cell's largest and most intricate organelles is the endoplasmic reticulum (ER) which is essential for the folding and modifications of all endomembrane and secretory proteins, for maintenance of calcium homeostasis and for biosynthesis of the majority of cellular lipids. It is therefore of no surprise that mitochondria must communicate accurately and frequently with the ER. Communication between these two organelles can be done by several means which are described below.

Membrane contact sites

Machinery

An important way by which mitochondria communicate with the ER is through membrane contact sites, distinct areas where organelles come into close proximity [4]. One tethering complex that holds the membranes of the ER and mitochondria in yeast is the ER mitochondrial encounter structure (ERMES). ERMES is composed of four core subunits - two mitochondrial (Mdm10, Mdm34), one on the ER membrane (Mmm1), and a soluble protein (Mdm12) [5]. In addition it contains a non-essential subunit (Gem1) [6,7]. Interestingly Mdm10 has an additional non-ERMES-role in protein import as part of the mitochondrial sorting and assembly machinery (SAM) [8].

The importance of ERMES can be highlighted by the fact that the loss of this complex results in respiration defects, alterations in mitochondrial morphology and inheritance, problems with mtDNA maintenance and more [9].

Another suggested structure that facilitates the tethering of the ER and mitochondria is the ER membrane protein complex (EMC) [10]. This multi-subunit complex is, unlike the ERMES complex, conserved to vertebrates [11]. One study suggested that the EMC proteins bridge the ER-mitochondria interface through interactions with Tom5, a member of the translocase of the outer membrane (TOM) complex [10]. However, while the EMC can be found in many diverse eukaryotic taxa, Tom5 can be found only in animals and fungi, implying that an additional partner should be present in order for the EMC to function as a

tether throughout the different eukaryotic lineages [12]. Unlike other suggested functions of the EMC [11], where deletion of any single protein caused functional loss of the entire complex, in the case of the contact site, loss of five EMC proteins was required before an altered mitochondrial lipid profile was observed [10]. Loss of both ERMES and EMC is lethal, suggesting that these two complexes might compensate for each other. However more studies should be carried out in order to determine whether the EMC is a direct tethering complex or whether it exerts its function through other contact site proteins.
Recently, a novel ER-mitochondria contact resident protein, Lam6, was identified as being involved in both contact site regulation as well as lipid transfer [13–15]. Lam6 has a steroidogenic acute regulatory transfer (StART) -like domain, is part of a large family of StART containing proteins, all found in contact sites, and is conserved from yeast to humans [15]. Lam6 was shown to co-localize and interact with ERMES, and loss of Lam6 and ERMES leads to a synthetic sick/lethal phenotype. It appears to be recruited to ER-mitochondria contact-sites by the TOM subunits Tom70 and Tom71 [13,14].
Overexpression of Lam6 resulted in the expansion of the contact between mitochondria and ER [13] and could do this in an ERMES independent manner [16], suggesting that it might potentially have some tethering capacities. Lam6 was shown to be important for the cross-talk between the ERMES and the mitochondria- vacuole contact site - vCLAMP (vaCuoLe And Mitochondria Patch) [13].
Additional proteins seem to modulate the ER-mitochondria contact sites albeit by an unknown mechanism. First, Vps13, a peripheral membrane protein, has the ability to suppress phenotypes resulting from the loss of ERMES when it is harboring a single amino acid substitution [17]. Like Lam6, its deletion exhibits a synthetic lethal phenotype when combined with the deletion of the ERMES component Mmm1 [18]. Vps13 was shown to localize to several contact sites including the ERMES and vCLAMP [17,18]. These studies further hint on the redundant nature of the ER-mitochondria and mitochondria-vacuole contact sites.
In addition, over expression of the mitochondrial proteins, Mcp1 and Mcp2, restored mitochondrial morphology and reversed respiration and alterations in mitochondrial lipid homeostasis in cells lacking Mdm10, however their mechanism of action is not yet clear [19].
Another novel regulator of the ER-mitochondria contact site is Sar1, a small GTPase that is active in the first step of vesicular transport from the ER to the Golgi apparatus. Sar1 was shown to negatively regulate the size of ER-mitochondria contact sites by introducing curvature in the ER membrane [20]. Most probably additional regulators await discovery.

Function

The contact site between the ER and mitochondria has been shown to have several roles:
Lipid transfer: One of the first functions shown for ER-mitochondria contacts is lipid transfer.

While some phospholipids such as phosphatidylethanolamine (PE), cardiolipin (CL), and Phosphatidylglycerol (PG) can be synthesized, at least partially, inside mitochondria, phosphatidylserine (PS), phosphatidylinositol (PI) , phosphatidylcholine (PC) and phosphatidic acid (PA), need to be imported into mitochondria from other organelles [21]. As the synthesis of many lipids starts in the ER, including those of structural phospholipids, sterols, storage lipids and precursors for the formation of sphingolipids, the ER supplies lipids to many other organelles, including mitochondria [22]. The ER-mitochondria contact was shown to have a major role in this lipid transfer, and enzymes involved in the synthesis of lipids are enriched in this contact [21]. The most well-studied example is the transfer of PS, which is synthesized in the ER, must be moved to mitochondria, both to serve as a constituent of mitochondrial membranes and as a precursor to be converted to PE by mitochondrial Phosphatidylserine Decarboxylase 1 (*PSD1*). PE can then be used for mitochondrial membranes and may be transported back to the ER to be further converted to PC [9].

For the majority of these lipids, the mechanism governing their transfer in contact sites is yet to be discovered. ERMES was suggested to have a role in the shuttling of PS and PE between the ER and mitochondria. However whether it has a direct role in shuttling lipids or merely connects the two membranes enabling efficient function of other lipid transfer proteins is still an open question. In support of the role of ERMES as an active lipid transporter, three of the ERMES subunits harbor a synaptotagmin-like mitochondrial lipid-binding protein (SMP) domain, and two of them show strong binding to PC [23]. In addition, a role for Mdm10 in PE transport from the mitochondria to the ER was found in cells where ER stress was induced [24]. Furthermore, by an *in-vitro* assay on yeast membrane fractions to monitor phospholipid exchange between the ER and mitochondria, it was shown that ERMES facilitates the transfer of PS from the ER to mitochondria, but it is not required for the transport of PE from mitochondria back to the ER. Moreover, it was suggested that Mmm1 and Mdm34 may have a negative regulatory role in PE transport, as in their absence the transport was accelerated [25]. Recently it was shown that PS synthesis at ER-mitochondria contact sites promotes the transfer of PS from the ER to mitochondria. This transfer was increased upon induction of an artificial ER-mitochondria tether in these cells [26]. However, the fact that cells lacking ERMES only show a mild change in their total lipid composition and are still able to convert PS to PC, implies that additional paths for lipid transfer between mitochondria and the endomembrane system exist. One such way is through alternative contact sites such as vCLAMP [27,28]. Indeed, in the absence of both ERMES and vCLAMP, the levels of phospholipids in mitochondria are significantly reduced [27].

In yeast the main sterol species is ergosterol, which is synthesized in the ER and has a role in mitochondrial membranes and morphology [29]. Therefore, sterols are an additional group of lipids that might be transferred through the ER-mitochondria contact site. In support of this notion, loss of ERMES components Mdm10, Mdm12 or Mmm1 resulted in an increase of mitochondrial ergosterol levels, suggesting a connection between this contact site and ergosterol transfer to or from mitochondria [19]. Indeed, Lam6, which is localized

to ERMES contact sites, was suggested to have a role in sterol transfer between the two organelles, as it was able to extract sterols from membranes [14,15]. In addition Lam6 was shown to bind sterols through its StART-like domain [15] and was able to transport sterols between membranes *in vitro* [14].
Another clue about sterol transport comes from studies in mammalian cells, as the oxysterol-binding protein (OSBP)-related proteins ORP5 and ORP8, which are known to transfer PS at the ER-PM contact site, were recently shown to localize to the contact areas between ER and mitochondria, termed mitochondrial associated membranes (MAMs) [30]. Moreover, this localization required a functional lipid transfer domain (ORD), which is able to bind sterols [31]. As depletion of these proteins resulted in defects in mitochondrial morphology it was suggested that they might have a role in lipid transfer also in the ER-mitochondria contact site, however whether PS or sterols are transferred is unclear.
Another group of lipids whose routes of import into mitochondria are not clear is sphingolipids. The biosynthesis of ceramide and sphingolipids occurs in the ER and golgi however ceramide metabolic enzymes were found in mitochondria [22]. Some sphingolipid enzymes can be localized to both compartments: the Inositol phosphosphingolipid phospholipase C (Isc1) protein can be found in the ER during logarithmic growth phase, however upon diauxic shift, it translocates to mitochondria [32]. In mammalian cells a sphingomyelinase, that can produce ceramide by hydrolysis of sphingomyelin, was suggested to be located in the MAM [33], however more studies should be carried out in order to understand whether the ER-mitochondria contact site is involved in the import of sphingolipids into mitochondria and how this occurs.

Mitochondrial dynamics: Another important aspect of ER-mitochondria communication through contact sites is its effect on organelle fission. ER-mitochondria contact sites were shown to coincide with mitochondrial constriction at sites that precede the assembly of the fission machinery [34]. Gem1 and ERMES are involved in this process by marking the sites of ER-associated mitochondrial division and play a role in the correct segregation of mitochondrial DNA between the two newly formed tips of a mitochondrion following division [35]. Moreover, it has recently been shown in mammalian cells that the ER has a role in nucleoid (the mtDNA-protein complexes) replication, a process that occurs in the mitochondrial matrix [36].
In addition, mitofusin 2 (MFN2), a protein that mediates mitochondrial fusion, has also been shown to tether the ER to mitochondria in mammalian cells by creating homotypic or heterotypic complexes with MFN2 or MFN1 respectively [37,38], suggesting that the ER-mitochondria contact may also play a role in regulating mitochondrial fusion.

Calcium (Ca^{+2}) homeostasis: In mammalian cells, MAMs are involved in maintaining Ca^{+2} homeostasis. Upon its release from the ER, Ca^{+2} enters mitochondria through the mitochondrial calcium channel uniporter (MCU). The MAMs enable the formation of Ca^{+2} hotspots which allow accumulation of high concentrations of Ca^{+2}. These are required for the activation of the low-affinity MCU and the regulation of mitochondrial activity as well

as cell death [39,40]. Yeast Gem1 was also shown to respond to Ca^{+2} levels, a process which is mediated through its two Ca^{+2} binding motifs [41]. It is therefore tempting to speculate that through Gem1, Ca^{+2} reservoirs in the ER can affect mitochondria. However yeast ER contains much less Ca^{+2} than the mammalian one [39,42], Ca^{+2} release from yeast ER has not been described [43] and *Saccharomyces cerevisiae* lack the MCU [44].

Mitochondrial inheritance: Gem1 was also suggested to play a role in mitochondrial inheritance due to an effect on Myo2, a class V myosin that drives mitochondrial movements into the bud of budding yeast [45]. Two redundant Myo2 receptors, Mmr1 and Ypt11, recruit Myo2 to mitochondria. Mmr1is also involved in tethering mitochondria to the cortical ER of the newly-formed bud [46]. Interestingly, the Gem1 and Mmr1 double mutant results in synthetic sickness and in a mitochondrial inheritance defect. However, mitochondria accumulation in the bud, which occurs when Mmr1 or Ypt11 are overexpressed, is not dependent on Gem1 [47]. Therefore it was suggested that Gem1 has an independent role in recruiting Myo2.

Mitophagy: The ER-mitochondria contact site was shown to have a role in mitophagy, the mitochondria-specific autophagy, as ERMES subunits were shown to co-localize with the site of autophagosome formation and ERMES mutants present severe mitophagy defects. Therefore ER–mitochondria contact sites may be important to supply lipids for the mitophagy autophagosome [48].
In mammalian cells, autophagosomes were shown to form at MAMs and the ER SNARE protein STX17 recruits the autophagosomal machinery to the contact site under starvation conditions [49].
The outer mitochondrial membrane protein FUNDC1 was also reported to be localized to MAMs, where it interacts with calnexin. In the MAM it is involved in hypoxia-induced mitophagy and mitochondrial fission through interaction with the fission protein DRP1 [50].

Life and death decisions: Since many essential processes concentrate in the ER-mitochondria contact sites, including calcium transfer, mitophagy and inheritance, it is not surprising that this contact plays a critical role in the cell's life and death decisions [51]. In mammalian cells these contacts were suggested to be involved in Drp1 recruitment and subsequent induction of apoptosis [52] as well as in ER-stress response [53].

Metabolic cross-talk

Several metabolic pathways include enzymatic steps that occur at more than a single organelle but don't necessarily occur at contact sites. Such processes require tight communication between the different organelles to maintain them continuous, and inhibit the accumulation of intermediate products. In addition, local concentrations of the different intermediates of these biosynthesis pathways might serve as a way of communication by

themselves. An example for such a pathway, shared between ER and mitochondria, is the biosynthesis pathway of Heme A and ubiquinone (Coenzyme Q, CoQ). CoQ functions in the respiratory chain as an electron carrier between complex I or II to complex III [54] and Heme is an essential co-factor for respiration as well as for many different enzymes [55]. The precursors for both Heme A and CoQ originate in the mevalonate pathway, which is localized to the ER and cytosol, while the final steps of biosynthesis occur in the mitochondrial matrix [56]. Hence, it is clear that a tight communication between the organelles is necessary for these processes to occur smoothly, without accumulation of unwanted intermediate products. How this occurs and whether contact sites take part in this communication remains to be seen. If this does occur at ER-mitochondria contact sites it may explain the requirement of these contacts for optimal respiration capacity [5].

Mitochondria-vacuoles

Vacuoles (yeast lysosomes) have many essential roles such as being a hub for ion storage and serving as a final destination for both endosomal and autophagic vesicles [57]. As such, vacuoles have evolved to break-down and recycle a wide variety of macromolecules delivered for degradation. Vacuolar processes can affect mitochondrial fitness and degradation, and the vacuole serves as another connection between mitochondria and the endomembrane system in the cell. Hence different types of communication exist between these organelles.

Membrane contact sites

Machinery and Function

Recently a contact site connecting mitochondria to vacuoles was discovered and termed vCLAMP [27,28]. The vCLAMP requires the HOPS (Homotypic fusion and vacuole protein sorting complex) protein Vps39, which is recruited to contact sites via its vacuolar binding partner, the Rab GTPase Ypt7 [28]. The discovery of which mitochondrial membrane protein is bound by Vps39 is forthcoming. The functions of vCLAMP are yet to be explored but concomitant loss of vCLAMP and ERMES (see above) results in dramatic decrease in phospholipid accumulation in mitochondria, suggesting a strong role in lipid transport. In addition, transporters of small molecules were enriched in vCLAMPs, pointing to a role in coordinating the transfer of these between the vacuole and mitochondria [27]. The regulation of vCLAMP seems to be intricate and interesting. First, the fact that Vps39 has several vacuolar roles and is both part of the HOPS complex, required for endosomal fusion, and part of the vCLAMP, poses that these two events might be inversely regulated. Second, ERMES and vCLAMP are co-regulated and a tight cross-talk exists between these contacts, most probably to ensure that lipid transfer rates to mitochondria remain constant. The vCLAMP seems to be more important when cells are grown on glucose, whereas

ERMES contacts are essential during growth on non-fermentable carbon sources [28]. Moreover, deletion of ERMES subunits and disassembly of the complex resulted in the expansion of the vCLAMP contact site [27], and overexpression of Vps39 rescued the growth defects of ERMES mutants grown on glucose medium [28].
It was recently shown that overexpression of Lam6, a sterol binding protein [15] and a ERMES contact site resident [14] results in an extension of both contacts [13].
Vps13, which also localizes to both ERMES and vCLAMP might also play a role in the coordination between the two [17]. These studies support the notion that the ER-mitochondria and mitochondria-vacuole contacts can work in a redundant fashion.
vCLAMP was also shown to be regulated by phosphorylation of Vps39, which might serve as a metabolic switch translating the metabolic state of the cell to changes in contact site size [28]. Interestingly, physical contacts between mitochondria and melanosomes, pigment-synthesizing lysosome-like organelles, were discovered in mouse, and were correlated with melanosome biogenesis and maturation [58]. In addition, mitochondria-lysosome communication has been shown to be essential for iron transfer in reticulocytes [59]. Therefore, contacts between mitochondria and late endomembrane organelles are conserved from yeast to higher eukaryotes and their functions remain to be explored.

Metabolic cross-talk

Iron: In yeast, mitochondria are major iron hubs and are responsible for the production of the essential iron sulfur clusters (ISC) [60]. However, the regulation of cellular iron levels and the balance between cytosolic iron and sequestered iron is the outcome of a tight communication between the mitochondria and vacuoles.
Iron is imported into mitochondria by the two paralogous inner membrane transporters Mrs3 and Mrs4 [61,62]. At high iron concentrations, iron is also transported into vacuoles through the Ccc1 importer [63,64], making the vacuole the second iron storage pool in the cell. Yap5, a bZIP transcription factor, coordinates these two pools. Yap5 has two ISC binding domains, which allow it to sense intracellular iron levels. As iron levels go up, the association of the ISC with Yap5 induces a conformational change, which in turn activates it to transcribe Ccc1 [65], as well as other genes [66]. Impairment in ISC formation, which is a result of mitochondrial malfunction, results in activation of the Aft1/2 signaling cascade and expression of the "iron regulon". Among the genes regulated by this signaling are Fet5 and Fth1, which form a complex on the vacuolar membrane to export iron to the cytosol [67], as well as Smf3 that also exports iron from the vacuole [68]. Interestingly, Aft1/2 can also control the expression of Cth2, a negative regulator of Ccc1, which can bind and destabilize Ccc1 mRNA, thus inhibiting vacuolar import under iron deficiency conditions [69,70].
In the above examples, the cross-talk of mitochondria and vacuoles occurs indirectly through activation of nuclear transcription. However, a more direct mitochondrial-vacuolar signaling pathway also exists [71]. Deletion of *MRS3* and *MRS4*, which encode the mitochondrial iron importers, led to an increase in the activity of the vacuolar iron importer Ccc1, and resulted in low cytosolic iron levels. This was mediated by oxidant damage [72].

Deletion of Ccc1 in these cells restored iron levels in the cytosol and mitochondria. This indicates that mitochondrial transporters are able to affect vacuolar iron homeostasis [71]. Indeed, overexpression of Mmt1 and Mmt2, which are suggested to function as the mitochondrial iron exporters, results in a similar phenotype to that of deletion of Mrs3 and Mrs4, which include increased iron sensitivity and oxidant generation and can be also suppressed by deletion of Ccc1[73].

Cardiolipin: Cardiolipin (CL) is an important anionic phospholipid which is predominantly localized to the inner mitochondrial membrane. It is required for optimal function and structural integrity of mitochondrial proteins and complexes. Mutants altered in CL synthesis exhibit reduced mitochondrial membrane potential, instability of electron transport chain complexes and impaired protein import [74]. Besides its role in mitochondrial integrity, CL has a role in the cross-talk between mitochondria and vacuoles [74]. Mutants of Crd1, the mitochondrial cardiolipin synthase enzyme, have severe vacuolar defects, including enlarged vacuoles, low Vacuolar H+-ATPase (V-ATPase) activity and concomitant loss of vacuolar acidification. It is unclear whether this is a direct or indirect effect.
Supporting an indirect effect is the fact that deletion of the vacuolar Na+/H+ and K+/H+ exchanger, Nhx1, suppressed the growth defects and vacuolar dysfunction, suggesting that the Crd1 deficient strain had an impaired ion homeostasis. Mitochondria are important regulators of cellular ion levels; therefore CL reduction might lead to an increase in the ion influx. This leads to induction of Nhx1-mediated sequestering of ions in the vacuole and to its swelling [74].
Regardless, the decreased V-ATPase activity could be rescued by deletion of the retrograde protein Rtg2, which also suppressed the growth defects. The retrograde response is a pathway that allows mitochondria to communicate reduced activity to the nucleus, resulting in activation of a transcriptional response aiming to restore mitochondrial homeostasis [3]. As deletion of other retrograde proteins did not result in the suppression of vacuolar defects, the involvement of the retrograde response is unclear. Therefore Rtg2 might be involved in this cross-talk due to its role as a component in the histone acetyltransferase (HAT) complex [75] and might be regulating vacuolar-associated genes.

Vacuole acidity affects mitochondrial function: Ageing in yeast is accompanied by mitochondrial dysfunction, mitochondrial fragmentation, increased levels of reactive oxygen species (ROS) and loss of mtDNA [76]. Surprisingly, it was found that overexpression of Vma1 and Vph2, members of the V-ATPase, suppresses mitochondrial dysfunction in aging cells by acidifying the vacuole [76]. Interestingly, young cells treated with a V-ATPase inhibitor or lacking V-ATPase subunits, display impaired mitochondrial morphology, reminiscent of old cells. This suggests that the decrease in vacuolar acidity in aged cells is one of the causes for mitochondrial dysfunction, and indicates a strong link between vacuoles and mitochondria during aging. The connection seems to be the proton-

dependent neutral amino acid transporter Avt1, as its overexpression prevents mitochondrial aging and its deletion prevents the suppression of mitochondrial dysfunction caused by overexpression of Vma1 and Vph2. This suggests that disruption in import of neutral amino acids into the vacuole, as a result of decreased vacuolar acidity, leads to mitochondrial dysfunction. However, since the deletion or overexpression of Avt1 alone resulted in a milder mitochondrial phenotype relative to the one observed in cells with increased or decreased vacuolar pH, there are probably additional V-ATPase-dependent transporters that can lead to age-induced mitochondrial dysfunction when vacuolar pH changes.
A beneficial role for an increase in vacuolar pH was shown in cells lacking mtDNA. Deletion of Vma2 or Vma13, proteins involved in the V-ATPase assembly and function, resulted in the suppression of the lethality of cells lacking Mgr1, a subunit of the i-AAA protease complex. Moreover, this rise in vacuolar pH resulted in increased membrane potential and improved mitochondrial import; however the mechanism underlying these improvements is still unclear [77].
Overall, as both increase and decrease in vacuolar pH levels can result in a beneficial effect on mitochondrial functions it is probably the metabolic state of the cell, as well as the environmental conditions, that determine the outcomes of these changes in vacuole acidity.

Apoptosis: Although debated for many years it is now becoming clear that yeast also undergo apoptosis. Upon prolonged growth in acetic acid, alterations in mitochondrial structure and function can occur which include reduction in cristae number, mitochondrial swelling, ROS accumulation, depolarization of the inner membrane and decreased respiration. Release of lethal factors from mitochondria, such as cytochrome C, contributes to an apoptotic death process [78]. Moreover, following the induction of apoptosis, mitochondria are degraded, either through mitophagy [79] or by proteolysis through the Pep4 peptidase that is released from vacuoles at this stage [78]. Pep4 mediated proteolysis requires the presence of the mitochondrial ADP/ATP carrier (AAC) proteins, potentially to enable mitochondrial permeabilization. The mechanism governing the release of proteases from the vacuole and its involvement in mitochondrial degradation is not worked out yet.

Autophagy: One mechanism to deal with non-functional, damaged mitochondria is through mitophagy, the selective removal of mitochondria by the autophagic machinery [80]. In this process, excess and damaged mitochondria are delivered to the vacuole for degradation [81]. Mitophagy is initiated upon mitochondrial dysfunction, such as after long growth on a non-fermentable carbon source, which forces the cells to carry out respiration [82]. Mitophagy can also occur following loss of the mitochondrial F1F0 ATP synthase [83] or deletion of Mdm38, which is part of the mitochondrial K+/H+ exchange system [84]. However, in both cases it is not yet clear what exactly is being sensed. Although both mutations lead to loss of membrane potential, treatment with an uncoupling agent, which also leads to membrane potential loss, did not result in a significant induction of mitophagy in yeast [80]. Hence simple loss of membrane potential is not sufficient to induce mitophagy and what drives the process is still unknown. Increase in mitochondrial ROS levels and

cellular redox state were suggested to be inducers of the process [85]. In mammalian cells, the Pink1/Parkin pathway was extensively studied as a way to recognize mitochondria marked for degradation [86]. However, neither Pink1 nor Parkin are present in yeasts.
Like macroautophagy, mitophagy also requires involvement of the core AuTophaGy related (ATG) genes. Atg32 and Atg33 were identified as mitophagy specific proteins, as they are located in the mitochondrial outer membrane [87–89]. Atg33 was suggested to be involved in detection of aged mitochondria for degradation at stationary phase [90]. Atg32 appears to function as a mitochondrial receptor that binds Atg11, which links cargo to the vesicle-forming machinery [91].
Interestingly, the C terminus of Atg32 is positioned in the intermembrane space of mitochondria, and is proteolytically processed by the i-AAA protease Yme1. Therefore Yme1 is an additional regulator of mitophagy [92].
Mitochondrial dynamics were linked to mitophagy but the exact mechanism is still unclear. In mammalian cells, inhibition of mitochondrial fission resulted in impaired mitophagy and accumulation of dysfunctional mitochondria [93]. Several studies in yeast showed that the fission factor Dnm1is required for efficient mitophagy [84,89,94,95]. Dnm1 was further shown to directly interact with Atg11 [95]. However, the role of fission in mitophagy is still controversial in yeast, and it is still unclear whether fission is directly involved in mitophagy, and whether Dnm1 has a non-fission contribution to the process [80,87,96,97].
The mitochondrial phosphatase, Aup1, which is conserved to humans, was shown to be required for survival of cells during prolonged respiration and to mediate mitophagy. Aup1 might therefore be part of a signal transduction mechanism that marks mitochondria for degradation [82]. It may work through the retrograde protein Rtg3 as Δ*aup1* strains affect the phosphorylation pattern of Rtg3 under these conditions, and deletion of Rtg3 abrogates stationary phase mitophagy. Moreover conditions that lead to the induction of mitophagy also lead to induction of retrograde target genes in an Aup1-dependent fashion [98]. However, which signals activate or repress Aup1 are yet to be uncovered.

Mitochondria-peroxisomes

In recent years it is becoming clear that peroxisomes, which are key metabolic organelles that participate in many central catabolic pathways such as β-oxidation of fatty acids, amino acid catabolism and detoxification of ROS, have tight communication with mitochondria [99]. Peroxisomes and mitochondria share key elements of their division and biogenesis, and they function together to regulate many central metabolic processes. The extent of interaction is so high that it was suggested that peroxisomes have a mitochondrial origin and that they emerged as functionally specialized mitochondria [100]. Indeed, new evidence now demonstrates that pre-peroxisomal vesicles bud from mitochondria [101].

Membrane contact sites

Machinery and Function

In yeast, mitochondria and peroxisomes form a contact site at the junction where mitochondria are also in contact with the ER [102]. Cells lacking subunits of the ERMES complex, the tethering complex between the ER and mitochondria, show aberrant peroxisome structure and size. In this geographically unique contact site a three-way junction can be formed that would allow bi-directional transfer of molecules between the three organelles.

Interestingly, this contact site is localized next to a specific mitochondrial niche, where the pyruvate dehydrogenase (PDH) complex is found in the mitochondrial matrix. This proximity might promote

the concentration of acetyl-CoA, which can be transferred from peroxisomes to a specific mitochondrial area in order to allow rapid entrance into the Tricarboxylic acid (TCA) cycle [102].

It has been suggested that certain enzymes in the mevalonate pathways reside in peroxisomes in mammals [103]. Although this is still controversial, this may be an example for a pathway that would require such a three-way junction to occur efficiently. There may be additional metabolic pathways that require this three-way proximity.

Recently it was suggested that Pex11, a peroxisomal protein which is involved in peroxisome proliferation [104], has a physical interaction with Mdm34, the subunit of the ERMES complex [105]. However, as this was shown using the split-venus technique more studies should be done in order to find the tethering complex between these organelles, as physical interaction between the two venus halves, attached to proteins of opposing membranes, is not restricted only to proteins that are actually tethering the membranes, but also to proteins that are found in the vicinity of the contact site [16].

Metabolic cross-talk

β-oxidation of fatty acids: In mammals, β-oxidation of very long chain fatty acids requires cross-talk between peroxisomes, where the very long chains are broken down to long chains, and mitochondria, where the final breakdown of the long chain fatty acids occurs [106]. However, in the yeast *Saccharomyces cerevisiae* it is thought that only peroxisomes perform β-oxidation of fatty acids. Communication between the organelles is still essential since the acetyl-CoA, which is the final product of this process, must be transported to the mitochondrial matrix, where it would be metabolized to CO_2 and water during respiration [107]. Aceyl-CoA also has a role in acetylation of mitochondrial proteins and acetyl-CoA levels were correlated with acetylation changes in mitochondria [108]. As it is an amphiphilic and bulky molecule, acetyl-CoA cannot freely cross biological membranes [109]. Two pathways therefore exist for acetyl CoA transport [107]: (i) the enzyme carnitine acetyltransferase (Cat2) converts acetyl-CoA to acetylcarnitine, which is able to cross through mitochondrial membranes; (ii) acetyl-CoA can enter the cytosolic glyoxylate cycle

and be converted to succinate, which in turn is transported to mitochondria. These two transport pathways are somewhat redundant, as single deletion of either Cat2 or Cit2 (a key enzyme in the glyoxylate cycle) does not result in growth defects when cells are grown on oleic acid as the sole carbon source. However double deletion of both genes abolishes growth, emphasizing the importance of the transport between mitochondria and peroxisomes [107].

The glyoxylate cycle: This cycle allows the conversion of two acetyl-CoA molecules into succinate, a C4-unit, which can then be utilized in the TCA cycle or function as a precursor for amino-acid biosynthesis. This cycle allows yeast to grow on C2-carbons, such as ethanol or acetate, as a sole carbon source. The conventional view was that the glyoxylate cycle is contained solely within peroxisomes, however it is now clear that the glyoxylate cycle enzymes are located both inside and outside peroxisomes [110]. The net product of the glyoxylate cycle is succinate, which needs to be imported into mitochondria. Succinate can be imported via the dicarboxylate carrier (Dic1), where it can be used to replenish the TCA cycle or to act as precursor for amino acid biosynthesis. Alternately, it can be transformed to fumarate by the mitochondrial succinate dehydrogenase and exported as fumarate through the succinate-fumarate carrier (Sfc1) [110]. Cytosolic fumarate can then be converted into malate, oxaloacetate and finally phosphoenolpyruvate, which serve as a precursor for gluconeogenesis. The malate formed may then be transported back to peroxisomes [110]. The fact that both Dic1 and Sfc1 are required for growth on ethanol or acetate as carbon sources, emphasizes that the coordinated transfer of metabolites from peroxisomes to mitochondria is essential under certain conditions [111,112]. It would therefore make a lot of sense if the glyoxylate cycle would be performed mainly in peroxisome-mitochondria contact sites yet this remains to be shown.

Reactive oxygen species (ROS): Both mitochondria and peroxisomes are involved in maintaining the cellular redox balance and homeostasis [113]. A reducing environment is maintained in the cytosol, mitochondrial matrix and peroxisomes, in order to facilitate protein folding and activity [114]. Excess ROS, predominantly in the form of superoxide radicals or hydrogen peroxide (H_2O_2), can be removed by different ways, including by the catalase enzyme in peroxisomes, mitochondria and the cytosol and the super oxide dismutase (SOD) and peroxiredoxin pathways in mitochondria and peroxisomes [106]. Hence, under conditions of excess ROS in one organelle, the cell may shuttle the "overflow" ROS for breakdown in the other. Indeed, in mammalian cells, deficiencies in peroxisomal ROS metabolism impact the mitochondrial redox balance and lead to mitochondrial fragmentation and cell death [113]. In addition, excess oxygen radical production inside peroxisomes causes cellular lipid peroxidation, and triggers a complex network of signaling events eventually resulting in increased mitochondrial H_2O_2 production [115].

In yeast the load of ROS also seems to be shared between the two organelles. For example, loss of the peroxisomal protein Opt2, which is a close homolog of the plasma membrane

glutathione transporter Opt1, causes an inability to rectify oxidative stress in both peroxisomes and mitochondria [116]. Indeed, a synthetic lethality screen revealed that among the genes that are synthetic lethal with Δ*opt2*, mitochondrial proteins were highly enriched [116].

The retrograde response: The retrograde pathway has evolved to maintain glutamate supplies in respiratory-deficient yeast. In those cells, the TCA-cycle fails to fully operate and limits the production of α-ketoglutarate, which is the direct precursor of glutamate [3]. To compensate for this, respiratory deficient cells use the retrograde response to up-regulate the expression of genes involved in anaplerotic pathways, aimed to supply the mitochondria with TCA-cycle intermediates such as acetyl-CoA and oxaloacetate. This leads to peroxisome proliferation, resulting in a dramatic elevation in peroxisome number, and an increase in fatty-acid oxidation, resulting in more acetyl-CoA production, which is then transferred from peroxisomes to mitochondria. Moreover, the glyoxylate cycle activity increases and key elements of this cycle, such as the citrate synthase Cit2, are upregulated. Finally, genes encoding for permeases that allow the transport of metabolites from peroxisomes to mitochondria, such as acetyl-CoA, are upregulated [117].

Mitochondria Derived Vesicles (MDVs): In COS7 mammalian cells, it was shown that mitochondria-anchored protein ligase (Mapl) is incorporated within MDVs which fuse with a subset of peroxisomes [118]. Vesicles were also shown to deliver cargo from mitochondria to lysosomes, and *in vitro* studies revealed an enrichment of oxidized proteins within these vesicles [119,120]. The content and purpose of these peroxisome targeted vesicles is still unknown, however one possibility is that mitochondria deliver oxidized proteins to peroxisomes via these vesicles, and this might serve as a base for organelle communication. Whether these vesicles exist also in yeast is yet to be discovered.

Dual targeted proteins

Peroxisomes and mitochondria share several proteins as well as some isoenzymes that catalyze a variety of reactions. The fact that two organelles share the same protein can create a way of communication between them. Assuming that the expression levels of a protein stays constant but that the relative distribution between the two organelles can change depending on conditions in each organelle, then the levels of the protein on the membrane serve to assess the status of the partner organelle. For example, if a protein is targeted evenly between mitochondria and peroxisomes, when both organelles are fully functional the concentration of this protein in peroxisomes will be 50% of its total. However, when mitochondrial import is impaired, more of the dual-targeted protein will be targeted to peroxisomes and this can be sensed and used to transmit a metabolic/regulatory signal.

Catalase A (Cta1), an essential enzyme in the decomposition of intracellular H_2O_2 [121], is primarily localized to peroxisomes under conditions that induce peroxisome formation but can also be imported into the mitochondrial matrix under respiratory growth conditions

[122]. While Cta1 possesses two peroxisomal targeting signals (PTS), the enzyme has no classical mitochondrial targeting sequence (MTS) and hence how it is imported to mitochondria is still unclear.

A second example for a dual targeted protein is the carnitine acetyltransferase Cat2 [123]. Cat2 can be found both in mitochondria and in peroxisomes in yeast grown on oleic acid. Cat2 contains a MTS at its N-terminus and a type 1 PTS (PTS-1) at the C-terminus. It appears that under normal growth the full length protein is synthesized and the majority of proteins target to mitochondria. During growth in oleic acid, a shorter transcript lacking the MTS is produced, and is now targeted to peroxisomes. In vertebrates, the two CAT isoforms are formed not by alternative start sites but rather by alternative splicing. One human splice variant contains both an MTS and PTS1 and is localized to mitochondria whereas the other lacks the MTS and is therefore localized to peroxisomes [123].

Mitochondria and peroxisomes were shown to share components of their fission machinery [124] as well as the nuclear transcription factors that control their biogenesis [100]. The yeast dynamin-like protein, Dnm1, is recruited to mitochondria by Fis1 and its adaptors Mdv1 and Caf4, and is required for mitochondrial fission and morphology [124]. Dnm1 was shown to be involved in peroxisome division [125]. Moreover, Fis1, Caf4 and Mdv1 were also shown to have a role in Dnm1-dependent peroxisome fission, and Caf4 and Mdv1 are recruited to peroxisomes in a Fis1 dependent manner [126]. Vps1, an additional dynamin-related protein that is found on both peroxisomes and vacuoles, is partially redundant to Dnm1 in the regulation of peroxisome abundance. *Δvps1* cells display a peroxisomal fission defect that can be reversed by manipulating Fis1 to be exclusively localized to peroxisomes [126]. The peroxisomal located Fis1 was unable to restore mitochondrial fission in a background of *Δfis1*. These observations indicated that peroxisomes and mitochondria compete for Dnm1, and that Fis1 plays a pivotal role in recruiting Dnm1 to peroxisomes and mitochondria. Indeed, mitochondrial dysfunction was shown to result in an increase in peroxisome number [127] in a Dnm1 dependent manner [126], suggesting that the relative distribution of Dnm1 can function as coordinator between the division of peroxisomes to the functional state of mitochondria. However, how Fis1 distributes between mitochondria and peroxisomes is unkown.

Another dual localized protein is the conserved membrane-anchored AAA-ATPase Msp1[128,129]. In mitochondria, Msp1 can sense and degrade tail-anchored (TA) proteins that are mistargeted to the outer mitochondrial membrane (OMM). The dual localization of Msp1 to both the OMM and peroxisomes might imply that it has a similar role in degrading mistargeted membrane proteins in peroxisomes.

Mitochondria-Lipid droplets

Lipid droplets (LDs) are important lipid reservoirs of the cell, storing high amounts of neutral lipids such as triacylglycerols and sterol-esters [130], and are required both for structural and metabolic purposes in mitochondria. Close interactions between LDs and mitochondria were seen in many different cell types, and dually targeted proteins have been

suggested. Not much is known about the different ways of communication between these two metabolic organelles and more aspects of their interaction await discovery.

Membrane contact sites

Machinery and Function

In mammalian cells, LDs were shown to localize near mitochondria [131]. In skeletal muscle cells, LDs were shown to be associated with mitochondria and this association became more abundant upon physical exercise, when energy demands increased [132,133]. Perlipin 5 was suggested to be a mediator of LD-mitochondria contact sites in oxidative cells and tissues and to recruit LDs to mitochondrial surfaces [134].

In yeast, physical interactions between LDs and mitochondria have been observed [131,135]. Using a split-venus approach it was suggested that the LD protein Erg6 interacts with the mitochondrial protein Mcr1, most probably in areas of contact sites between these organelles. Both Erg6 and Mcr1 are involved in the formation of ergosterol and therefore it is possible that LDs and mitochondria exchange metabolites between them, via physical interactions, to complete ergosterol biosynthesis [131]. The tethering machinery of this contact site remains to be elucidated.

Interestingly, a role for LDs in mediating lipid balance and mobilization was described in mammalian cells. In starved cells LDs are the primary source of fatty acids for β-oxidation in mitochondria, and supplementation happens in areas of close proximity, most probably contact sites between the organelles [136].

Recently, the LD-mitochondria contact was suggested to be involved in the regulation of stress responses and apoptosis through a mechanism conserved from yeast to mammals. A special domain termed V-domain was shown to enable the shuttling of pro and anti-apoptotic proteins from the outer mitochondrial membrane to LDs, thus allowing modulation of the apoptotic process [137].

Overall it is clear that the mitochondria-LD contact site should be more intensively explored in order to fully grasp the extent of the functions and regulation processes that are occurring within it.

Dual targeted proteins

The majority of proteins that were found to be dual-targeted to LDs and mitochondria were found using proteomic assays, which makes it difficult to differentiate between *bona fide* events of dual targeting or contaminations in the preparations of pure mitochondria/LD fractions for mass spectrometry. One example of a suggested dual localized protein is the phosphatidylglycerol phospholipase C (Pgc1) that is involved in the regulation of PG content in membranes [138]. Pgc1 was shown to be found in the subcellular fractions containing both mitochondria and LDs. However, only purified mitochondrial fractions showed a significant *in-vitro* phospholipase C type activity against PG, implying that active Pgc1 is localized to mitochondria [138]. Therefore, it remains to be studied if the lipid

droplet localization of Pgc1 serves to sequester Pgc1 from mitochondria or whether it does not represent a real dually targeted protein.
PG, which is normally a low abundance phospholipid, can be utilized for cardiolipin biosynthesis by cardiolipin synthase. Interestingly, Taz1, another enzyme in the cardiolipin biosynthesis pathway, was fractionated with LD proteins [139], and was found to be dual localized in independent observations (data not published).
More studies should be carried out in order to establish whether dual localized proteins between these two organelles actually exist, to define such proteins, and to better understand the factors determining the relative distribution of the proteins between the two organelles.

Mitochondria-Plasma membrane

The extent of communication between mitochondria and the plasma-membrane (PM) became clear recently, with the discovery that mitochondria have contact sites with the PM that are important in many cellular aspects, including mitochondrial inheritance and dynamics [140]. Moreover, the communication between mitochondria and the PM promotes cellular iron homeostasis and mitochondria might have a role in the targeting of PM proteins. The full scope of communication between the two organelles is still unclear.

Membrane contact sites

Machinery and Function

Mitochondria have been shown to be in close proximity to the PM in several mammalian cell types [141,142]. However, the proteins mediating this tethering have not yet been identified. In yeast, the PM protein Num1 mediates mitochondria-PM contact sites [143,144]. This association is important for mitochondrial motility, distribution and morphology. PM-mitochondria contacts also promote the movement of mitochondria into the daughter cell when budding occurs [140]. Num1 is thought to mediate the interaction with mitochondria by binding Mdm36, a soluble protein which is peripherally attached to mitochondria [143,145], and through its possession of two lipid binding domains each binding one of the membranes [146].
As ER proteins were also found to co-immunoprecipitate with Num1, and cortical ER was seen in close vicinity of mitochondria-PM contacts facilitated by Num1, it is suggested that the ER is a component in this contact, which was therefore termed Mitochondria–ER–Cortex-Anchor or MECA [143].
Num1 becomes essential under conditions of impaired mitochondrial dynamics, and its loss can lead to mother cells that are devoid of all mitochondria, resulting in death of the mother and loss of cell proliferation [140]. These mitochondrial inheritance defects and lethality were rescued by expression of a chimeric mitochondria–PM tether, consisting of the C-terminal PH domain of Num1[144], demonstrating that the distribution of mitochondria during cell growth is mediated by Num1 tethering activity.
An additional possible role for the mitochondria-PM contact sites, beside their role in proper inheritance, is in mitochondrial dynamics [144]. It was suggested that the dynamin-related protein Dnm1 requires membrane tension in order to perform efficient fission of

mitochondria. This tension can be generated by mitochondrial movement on actin, which is encountered by the tethering of Num1 to the cortex [145,147].
Mitochondria-PM contacts which are not mediated by ER were also seen [144] and interestingly, the contacts were located within areas of invaginations of the PM resembling eisosomes, specialized sites of plasma membrane organization. This suggests that tethering of mitochondria to the PM may not exist only due to physical reasons, but may also promote sensing of membrane status to influence mitochondrial inheritance and dynamics. However, eisosomes don't seem to be involved in the formation of these contacts and the nature of these invaginations is yet to be determined [140].

Metabolic cross-talk

Iron: In yeast strains impaired in their ISC biogenesis and export, a constitutive activation of the Aft1/2 transcription factors leads to increased iron uptake at the PM [148]. Among the PM genes that are induced by Aft1 are members of the FRE family which are PM localized metalloreductases (Fre1-4), all involved in the reduction of ferric iron to the ferrous form [149]. The reduced iron is then imported to the cell by the high-affinity ferrous transport complex which is composed of the multicopper oxidase, Fet3 [150] and the iron permease, Ftr1 [151]. Yeast can also increase their iron uptake by the production (this does not occur in *S.cerevisiae*) or capture of siderophores, organic molecules that have a high affinity for iron. Iron-bound siderophores that were secreted into the environment can be uptaken by siderophore transporters on the PM [152]. Genes involved in siderophore binding and transport are also upregulated by Aft1/2.
Altogether, this increase in iron import by the PM, as a result of inefficient or impaired iron metabolism in mitochondria, points to the cross-talk between the two organelles, which aims to maintain cellular iron homeostasis and mitochondrial function.

Protein targeting: One unique case of mitochondria affecting PM protein localization was observed upon disruption of genes from the class C VPS complex, which is involved in SNARE-mediated membrane fusion at the vacuole [153]. Deletion of these genes resulted in mitochondrial defects as cells were not able to grow on a non-fermentable carbon source, and accumulation of Ras proteins on mitochondrial membranes [154]. In these conditions, the small lipidated GTPases, Ras1 and Ras2, relocalized from PM to mitochondria. It was suggested that these proteins are targeted to the PM by mitochondria derived vesicles, such as those recently identified in mammalian cells [155]. However, since Ras proteins tether to membranes by a prenyl residue, alterations in membrane composition may also account for such localization alterations.

Summary

For many years, mitochondria were seen as autonomous regions in the cell, having evolved from bacteria, containing their own DNA and ribosomes and excluded from the endomembrane vesicular routes. However, it is now clear that mitochondria rely on tight

communication with all other organelles for their many functions, biogenesis, division, inheritance and metabolism.

Following the recent characterization of mitochondria derived vesicles [156], it was postulated that the mitochondrial proteobacterial ancestor may have, in fact, given rise to the entire eukaryotic endomembrane system [157]. According to this model, the endomembrane apparatus evolved from outer membrane vesicles (OMV), released from the mitochondrial ancestor, which fused into an endomembrane system within the cytosol of its archaeal host. If this theory is true, it might help explain why the eukaryotic endomembrane system has a bacterial lipid composition and not an archaeal one, and why mitochondrial functions and pathways are so intertwined with those of the secretory pathway. One example for this is the phospholipid biosynthesis pathway which requires transfer of precursors between the ER and mitochondria [157]. Support to this model comes from recent proof that mammalian peroxisomes have mitochondrial precursors [101].

During the last decade many mitochondrial contact sites with other organelles have been demonstrated. Although cellular communication through contact sites is important, it also poses a geographical problem. As contact sites expand, they may consume a large part of the outer mitochondrial membrane perimeter (Figure 1) leaving very little for other, essential, mitochondrial functions such as import of proteins and nutrients from the cytosol. More than 99% of the ~1000 mitochondrial proteins in yeast are translated in the cytosol and should be imported to their designated mitochondrial subdomains [3]. Therefore enough space should be dedicated to the efficient translocation of mitochondrial proteins. It is therefore clear that a regulated balance must be maintained between areas that would be dedicated to contact site formation and areas that are solely allowing translocation of proteins from the cytosol. Interestingly, several mitochondrial tethers bind proteins that have a role in translocation and this might serve as a way to regulate these functions. One of the most studied examples is the shuttling of Mdm10 between the ERMES complex, where it functions in tethering, and the SAM complex, where it promotes the biogenesis of α-helical and β-barrel proteins [8]. Lam6 also interacts with Tom70/71 and EMCs interact with Tom5 [10,13,14]. Such "dual function" proteins might have evolved to ensure that under conditions where contact sites extend - translocation complexes will be reduced, and will enable dedication of the membrane space to the contact site, and *vice versa*.

More generally, most contact site proteins have additional functions. For example the vCLAMP protein Vps39 is part of the HOPS complex [27], Erg6 has a role in the biosynthesis of ergosterol [158] and Pex11 is required for peroxisome proliferation [159,160]. Having contact residents carry out additional functions may serve one of two functions. On the one hand, it may simply localize a function at contacts, as occurs with lipid transfer proteins that also act as tethers, and therefore ensure that these functions occur more efficiently at the contact sites. conversely, and similarly to the case of translocation, such "dual function" proteins may serve to compete the two functions ensuring that they do not occur at the same time. For example in the case of Vps39, vacuoles that devote large membrane areas to contacts with mitochondria will have less endosome docking.

In the future, studies that will uncover the functionality of contact site proteins and the exact localization of enzymes in shared pathways should reveal how mitochondria create and utilize communication routes with all other cellular entities, and will help us to see them as if we were a fly on the cell wall.

Figure legend:

Figure 1: Mitochondrial contact sites occupy a large portion of the mitochondrial outer membrane. Depicted are proteins that were suggested to reside in the contact sites between mitochondria and ER, vacuole (V), lipid droplets (LD), peroxisomes (P) and the plasma-membrane (PM). Bulges represent a predicted trans-membrane domain. Mitochondrial proteins are in blue. PDH = pyruvate dehydrogenase complex.

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References:

[1] S.D. Dyall, Ancient Invasions: From Endosymbionts to Organelles, Science, 304 (2004) 253–257.

[2] N.S. Chandel, Mitochondria as signaling organelles, BMC Biol., 12 (2014) 34.

[3] M. Eisenberg-Bord, M. Schuldiner, Ground control to major TOM: mitochondria-nucleus communication, FEBS J., 284 (2017) 196–210.

[4] Y. Elbaz, M. Schuldiner, Staying in touch: the molecular era of organelle contact sites, Trends Biochem. Sci., 36 (2011) 616–23.

[5] B. Kornmann, E. Currie, S.R. Collins, M. Schuldiner, J. Nunnari, J.S. Weissman, P. Walter, An ER-mitochondria tethering complex revealed by a synthetic biology screen, Science, 325 (2009) 477–81.

[6] B. Kornmann, C. Osman, P. Walter, The conserved GTPase Gem1 regulates endoplasmic reticulum-mitochondria connections, Proc. Natl. Acad. Sci. U. S. A., 108 (2011) 14151–6.

[7] D.A. Stroud, S. Oeljeklaus, S. Wiese, M. Bohnert, U. Lewandrowski, A. Sickmann, B. Guiard, M. van der Laan, B. Warscheid, N. Wiedemann, Composition and topology of the endoplasmic reticulum-mitochondria encounter structure, J. Mol. Biol., 413 (2011) 743–50.

[8] L. Ellenrieder, Ł. Opaliński, L. Becker, V. Krüger, O. Mirus, S.P. Straub, K. Ebell, N. Flinner, S.B. Stiller, B. Guiard, C. Meisinger, N. Wiedemann, E. Schleiff, R. Wagner, N. Pfanner, T. Becker, Separating mitochondrial protein assembly and endoplasmic reticulum tethering by selective coupling of Mdm10, Nat. Commun., 7 (2016) 13021.

[9] A. Lang, A.T. John Peter, B. Kornmann, ER-mitochondria contact sites in yeast: beyond the myths of ERMES, Curr. Opin. Cell Biol., 35 (2015) 7–12.

[10] S. Lahiri, J.T. Chao, S. Tavassoli, A.K.O. Wong, V. Choudhary, B.P. Young, C.J.R. Loewen, W. a Prinz, A Conserved Endoplasmic Reticulum Membrane Protein Complex (EMC) Facilitates Phospholipid Transfer from the ER to Mitochondria, PLoS Biol., 12 (2014) e1001969.

[11] M.C. Jonikas, S.R. Collins, V. Denic, E. Oh, E.M. Quan, V. Schmid, J. Weibezahn, B. Schwappach, P. Walter, J.S. Weissman, M. Schuldiner, Comprehensive characterization of genes required for protein folding in the endoplasmic reticulum, Science, 323 (2009) 1693–7.

[12] J.G. Wideman, Wideman, J. G., The ubiquitous and ancient ER membrane protein complex (EMC): tether or not?, F1000Research, 4 (2015) 624.

[13] Y. Elbaz-Alon, M. Eisenberg-Bord, V. Shinder, S.B. Stiller, E. Shimoni, N. Wiedemann, T. Geiger, M. Schuldiner, Lam6 Regulates the Extent of Contacts between Organelles, Cell Rep., 12 (2015) 7–14.

[14] A. Murley, R.D. Sarsam, A. Toulmay, J. Yamada, W.A. Prinz, J. Nunnari, Ltc1 is an ER-localized sterol transporter and a component of ER-mitochondria and ER-vacuole contacts, J. Cell Biol., 209 (2015) 539–548.

[15] A.T. Gatta, L.H. Wong, Y.Y. Sere, D.M. Calderón-Noreña, S. Cockcroft, A.K. Menon, T.P. Levine, A new family of StART domain proteins at membrane contact sites has a role in ER-PM sterol transport, Elife, 4 (2015) e07253.

[16] M. Eisenberg-Bord, N. Shai, M. Schuldiner, M. Bohnert, A Tether Is a Tether Is a Tether: Tethering at Membrane Contact Sites, Dev. Cell, 39 (2016) 395–409.

[17] A.B. Lang, A.T.J. Peter, P. Walter, B. Kornmann, ER-mitochondrial junctions can be bypassed by dominant mutations in the endosomal protein Vps13, J. Cell Biol., 210 (2015) 883–890.

[18] J.S. Park, M.K. Thorsness, R. Policastro, L.L. McGoldrick, N.M. Hollingsworth, P.E. Thorsness, A.M. Neiman, Yeast Vps13 promotes mitochondrial function and is localized at membrane contact sites, Mol. Biol. Cell, 27 (2016) 2435–49.

[19] T. Tan, C. Ozbalci, B. Brügger, D. Rapaport, K.S. Dimmer, Mcp1 and Mcp2, two novel proteins involved in mitochondrial lipid homeostasis, J. Cell Sci., 126 (2013) 3563–74.

[20] K.B. Ackema, C. Prescianotto-Baschong, J. Hench, S.C. Wang, Z.H. Chia, H. Mergentaler, F. Bard, S. Frank, A. Spang, Sar1, a novel regulator of ER-mitochondrial contact sites, PLoS One, 11 (2016) 1–16.

[21] K.S. Dimmer, D. Rapaport, Mitochondrial contact sites as platforms for phospholipid

exchange, Biochim. Biophys. Acta - Mol. Cell Biol. Lipids, 1862 (2017) 69–80.

[22] V. V. Flis, G. Daum, Lipid transport between the endoplasmic reticulum and mitochondria, Cold Spring Harb. Perspect. Biol., 5 (2013) 1–22.

[23] A.P. AhYoung, J. Jiang, J. Zhang, X. Khoi Dang, J.A. Loo, Z.H. Zhou, P.F. Egea, Conserved SMP domains of the ERMES complex bind phospholipids and mediate tether assembly, Proc. Natl. Acad. Sci. U. S. A., 112 (2015) E3179-88.

[24] M. Kannan, C. Sivaprakasam, W.A. Prinz, V. Nachiappan, Endoplasmic reticulum stress affects the transport of phosphatidylethanolamine from mitochondria to the endoplasmic reticulum in S cerevisiae, Biochim. Biophys. Acta - Mol. Cell Biol. Lipids, 1861 (2016) 1959–1967.

[25] R. Kojima, T. Endo, Y. Tamura, A phospholipid transfer function of ER-mitochondria encounter structure revealed in vitro, Sci. Rep., 6 (2016) 30777.

[26] M. Kannan, S. Lahiri, L.K. Liu, V. Choudhary, W.A. Prinz, Phosphatidylserine synthesis at membrane contact sites promotes its transport out of the ER, J. Lipid Res., 58 (2017) 553–562.

[27] Y. Elbaz-Alon, E. Rosenfeld-Gur, V. Shinder, A.H. Futerman, T. Geiger, M. Schuldiner, A Dynamic Interface between Vacuoles and Mitochondria in Yeast, Dev. Cell, 30 (2014) 95–102.

[28] C. Hönscher, M. Mari, K. Auffarth, M. Bohnert, J. Griffith, W. Geerts, M. van der Laan, M. Cabrera, F. Reggiori, C. Ungermann, Cellular Metabolism Regulates Contact Sites between Vacuoles and Mitochondria, Dev. Cell, 30 (2014) 86–94.

[29] K. Altmann, B. Westermann, Role of essential genes in mitochondrial morphogenesis in Saccharomyces cerevisiae, Mol. Biol. Cell, 16 (2005) 5410–7.

[30] R. Galmes, A. Houcine, A.R. van Vliet, P. Agostinis, C.L. Jackson, F. Giordano, ORP5/ORP8 localize to endoplasmic reticulum-mitochondria contacts and are involved in mitochondrial function, EMBO Rep., 17 (2016) 1–11.

[31] S. Raychaudhuri, W.A. Prinz, The diverse functions of oxysterol-binding proteins, Annu. Rev. Cell Dev. Biol., 26 (2010) 157–77.

[32] S. Vaena de Avalos, Y. Okamoto, Y.A. Hannun, Activation and localization of inositol phosphosphingolipid phospholipase C, Isc1p, to the mitochondria during growth of Saccharomyces cerevisiae, J. Biol. Chem., 279 (2004) 11537–45.

[33] S.E. Horvath, G. Daum, Lipids of mitochondria, Prog. Lipid Res., 52 (2013) 590–614.

[34] J.R. Friedman, L.L. Lackner, M. West, J.R. DiBenedetto, J. Nunnari, G.K. Voeltz, ER tubules mark sites of mitochondrial division, Science, 334 (2011) 358–62.

[35] A. Murley, L.L. Lackner, C. Osman, M. West, G.K. Voeltz, P. Walter, J. Nunnari, ER-associated mitochondrial division links the distribution of mitochondria and mitochondrial DNA in yeast, Elife, 2 (2013) e00422.

[36] S.C. Lewis, L.F. Uchiyama, J. Nunnari, ER-mitochondria contacts couple mtDNA synthesis with mitochondrial division in human cells, Science, 353 (2016).

[37] O.M. de Brito, L. Scorrano, Mitofusin 2 tethers endoplasmic reticulum to mitochondria, Nature, 456 (2008) 605–610.

[38] D. Naon, M. Zaninello, M. Giacomello, T. Varanita, F. Grespi, S. Lakshminaranayan, A. Serafini, M. Semenzato, S. Herkenne, M.I. Hernández-Alvarez, A. Zorzano, D. De Stefani, G.W. Dorn, L. Scorrano, Critical reappraisal confirms that Mitofusin 2 is an endoplasmic reticulum-mitochondria tether, Proc. Natl. Acad. Sci. U. S. A., 113 (2016) 11249–11254.

[39] R. Rizzuto, M. Brini, M. Murgia, T. Pozzan, Microdomains with high Ca2+ close to IP3-sensitive channels that are sensed by neighboring mitochondria, Science, 262 (1993) 744–7.

[40] C. Giorgi, S. Missiroli, S. Patergnani, J. Duszynski, M.R. Wieckowski, P. Pinton, Mitochondria-Associated Membranes: Composition, Molecular Mechanisms, and Physiopathological Implications, Antioxid. Redox Signal., 22 (2015) 995–1019.

[41] R.L. Frederick, J.M. McCaffery, K.W. Cunningham, K. Okamoto, J.M. Shaw, Yeast Miro GTPase, Gem1p, regulates mitochondrial morphology via a novel pathway, J. Cell Biol., 167 (2004) 87–98.

[42] J. Strayle, T. Pozzan, H.K. Rudolph, Steady-state free Ca(2+) in the yeast endoplasmic reticulum reaches only 10 microM and is mainly controlled by the secretory pathway pump pmr1, EMBO J., 18 (1999) 4733–43.

[43] B. Kornmann, P. Walter, ERMES-mediated ER-mitochondria contacts: molecular hubs for the regulation of mitochondrial biology, J. Cell Sci., 123 (2010) 1389–93.

[44] E. Kovács-Bogdán, Y. Sancak, K.J. Kamer, M. Plovanich, A. Jambhekar, R.J. Huber, M.A. Myre, M.D. Blower, V.K. Mootha, Reconstitution of the mitochondrial calcium uniporter in yeast, Proc. Natl. Acad. Sci. U. S. A., 111 (2014) 8985–90.

[45] B. Westermann, Mitochondrial inheritance in yeast, Biochim. Biophys. Acta - Bioenerg., 1837 (2014) 1039–1046.

[46] T.C. Swayne, C. Zhou, I.R. Boldogh, J.K. Charalel, J.R. McFaline-Figueroa, S. Thoms, C. Yang, G. Leung, J. McInnes, R. Erdmann, L.A. Pon, A. Quintana, C. Schwindling, A.S. Wenning, U. Becherer, J. Rettig, E.C. Schwarz, M. Hoth, S.L. Palay, I.R. Boldogh, et al., Role for cER and Mmr1p in anchorage of mitochondria at sites of polarized surface growth in budding yeast, Curr. Biol., 21 (2011) 1994–9.

[47] R.L. Frederick, K. Okamoto, J.M. Shaw, Multiple Pathways Influence Mitochondrial Inheritance in Budding Yeast, Genetics, 178 (2008) 825–837.

[48] S. Böckler, B. Westermann, O.M. de Brito, L. Scorrano, K.S. Dimmer, S. Jakobs, F. Vogel, K. Altmann, B. Westermann, G. Giaever, A.M. Chu, L. Ni, C. Connelly, L. Riles, S. Véronneau, S. Dow, A. Lucau-Danila, K. Anderson, B. André, et al., et al., Mitochondrial ER contacts are crucial for mitophagy in yeast, Dev. Cell, 28 (2014) 450–8.

[49] M. Hamasaki, N. Furuta, A. Matsuda, A. Nezu, A. Yamamoto, N. Fujita, H. Oomori, T. Noda, T. Haraguchi, Y. Hiraoka, A. Amano, T. Yoshimori, Autophagosomes form at ER-mitochondria contact sites, Nature, 495 (2013) 389–93.

[50] W. Wu, C. Lin, K. Wu, L. Jiang, X. Wang, W. Li, H. Zhuang, X. Zhang, H. Chen, S. Li, Y. Yang, Y. Lu, J. Wang, R. Zhu, L. Zhang, S. Sui, N. Tan, B. Zhao, J. Zhang, L. Li, et al., FUNDC1 regulates mitochondrial dynamics at the ER-mitochondrial contact site under hypoxic conditions, EMBO J., (2016) 1–17.

[51] D.G.J. Smethurst, K.F. Cooper, ER fatalities-The role of ER-mitochondrial contact sites in yeast life and death decisions, Mech. Ageing Dev., 161 (2016) 225–233.

[52] S. Montessuit, S.P. Somasekharan, O. Terrones, S. Lucken-Ardjomande, S. Herzig, R. Schwarzenbacher, D.J. Manstein, E. Bossy-Wetzel, G. Basañez, P. Meda, J.-C.

Martinou, Membrane Remodeling Induced by the Dynamin-Related Protein Drp1 Stimulates Bax Oligomerization, Cell, 142 (2010) 889–901.

[53] T. Namba, F. Tian, K. Chu, S.Y. Hwang, K.W. Yoon, S. Byun, M. Hiraki, A. Mandinova, S.W. Lee, CDIP1-BAP31 Complex Transduces Apoptotic Signals from Endoplasmic Reticulum to Mitochondria under Endoplasmic Reticulum Stress, Cell Rep., 5 (2013) 331–339.

[54] I. González-Mariscal, E. García-Testón, S. Padilla, A. Martín-Montalvo, T. Pomares Viciana, L. Vazquez-Fonseca, P. Gandolfo Domínguez, C. Santos-Ocaña, The regulation of coenzyme Q biosynthesis in eukaryotic cells: All that yeast can tell Us, Mol. Syndromol., 5 (2014) 107–118.

[55] S.M. Mense, L. Zhang, Heme: a versatile signaling molecule controlling the activities of diverse regulators ranging from transcription factors to MAP kinases, Cell Res., 16 (2006) 681–692.

[56] M. Turunen, J. Olsson, G. Dallner, Metabolism and function of coenzyme Q, Biochim. Biophys. Acta, 1660 (2004) 171–199.

[57] P.C. Malia, C. Ungermann, Vacuole membrane contact sites and domains: emerging hubs to coordinate organelle function with cellular metabolism, Biochem. Soc. Trans., 44 (2016) 528–533.

[58] T. Daniele, I. Hurbain, R. Vago, G. Casari, G. Raposo, C. Tacchetti, M.V. Schiaffino, B. Westermann, A.F. MacAskill, T.A. Atkin, J.T. Kittler, A. Quintana, C. Schwindling, A.S. Wenning, U. Becherer, J. Rettig, E.C. Schwarz, M. Hoth, E. Abarca-Rojano, S. Muñiz-Hernández, et al., Mitochondria and melanosomes establish physical contacts modulated by Mfn2 and involved in organelle biogenesis, Curr. Biol., 24 (2014) 393–403.

[59] A.D. Sheftel, A.S. Zhang, C. Brown, O.S. Shirihai, P. Ponka, Direct interorganellar transfer of iron from endosome to mitochondrion, Blood, 110 (2007) 125–132.

[60] J.D. Wofford, P.A. Lindahl, Mitochondrial Iron-Sulfur-Cluster Activity and Cytosolic Iron Regulate Iron Traffic in Saccharomyces cerevisiae, J. Biol. Chem., 290 (2015) 26968–77.

[61] U. Mühlenhoff, J.A. Stadler, N. Richhardt, A. Seubert, T. Eickhorst, R.J. Schweyen,

R. Lill, G. Wiesenberger, A specific role of the yeast mitochondrial carriers MRS3/4p in mitochondrial iron acquisition under iron-limiting conditions, J. Biol. Chem., 278 (2003) 40612–20.

[62] F. Foury, T. Roganti, Deletion of the mitochondrial carrier genes MRS3 and MRS4 suppresses mitochondrial iron accumulation in a yeast frataxin-deficient strain, J. Biol. Chem., 277 (2002) 24475–83.

[63] L. Li, O.S. Chen, D. McVey Ward, J. Kaplan, CCC1 is a transporter that mediates vacuolar iron storage in yeast, J. Biol. Chem., 276 (2001) 29515–9.

[64] O.S. Chen, J. Kaplan, CCC1 suppresses mitochondrial damage in the yeast model of Friedreich's ataxia by limiting mitochondrial iron accumulation, J. Biol. Chem., 275 (2000) 7626–32.

[65] N. Rietzschel, A.J. Pierik, E. Bill, R. Lill, U. Mühlenhoff, The Basic Leucine Zipper Stress Response Regulator Yap5 Senses High-Iron Conditions by Coordination of [2Fe-2S] Clusters, Mol. Cell. Biol., 35 (2015) 370–378.

[66] L. Li, X. Jia, D.M. Ward, J. Kaplan, Yap5 protein-regulated transcription of the TYW1 gene protects yeast from high iron toxicity, J. Biol. Chem., 286 (2011) 38488–97.

[67] J.L. Urbanowski, R.C. Piper, The iron transporter Fth1p forms a complex with the Fet5 iron oxidase and resides on the vacuolar membrane, J. Biol. Chem., 274 (1999) 38061–70.

[68] J.C. Rutherford, S. Jaron, E. Ray, P.O. Brown, D.R. Winge, A second iron-regulatory system in yeast independent of Aft1p, Proc. Natl. Acad. Sci. U. S. A., 98 (2001) 14322–7.

[69] E. Pedro-Segura, S. V Vergara, S. Rodríguez-Navarro, R. Parker, D.J. Thiele, S. Puig, The Cth2 ARE-binding protein recruits the Dhh1 helicase to promote the decay of succinate dehydrogenase SDH4 mRNA in response to iron deficiency, J. Biol. Chem., 283 (2008) 28527–35.

[70] L. Li, D. Bagley, D.M. Ward, J. Kaplan, Yap5 is an iron-responsive transcriptional activator that regulates vacuolar iron storage in yeast, Mol. Cell. Biol., 28 (2008) 1326–37.

[71] L. Li, J. Kaplan, A mitochondrial-vacuolar signaling pathway in yeast that affects iron and copper metabolism, J. Biol. Chem., 279 (2004) 33653–61.

[72] L. Li, G. Murdock, D. Bagley, X. Jia, D.M. Ward, J. Kaplan, Genetic dissection of a mitochondria-vacuole signaling pathway in yeast reveals a link between chronic oxidative stress and vacuolar iron transport, J. Biol. Chem., 285 (2010) 10232–10242.

[73] L. Li, R. Miao, X. Jia, D.M. Ward, J. Kaplan, Expression of the Yeast Cation Diffusion Facilitators Mmt1 and Mmt2 Affects Mitochondrial and Cellular Iron Homeostasis: Evidence for Mitochondrial Iron Export, J. Biol. Chem., 289 (2014) 17132–17141.

[74] S. Chen, M. Tarsio, P.M. Kane, M.L. Greenberg, Cardiolipin mediates cross-talk between mitochondria and the vacuole, Mol. Biol. Cell, 19 (2008) 5047–58.

[75] M.G. Pray-Grant, D. Schieltz, S.J. McMahon, J.M. Wood, E.L. Kennedy, R.G. Cook, J.L. Workman, J.R. Yates, P.A. Grant, The novel SLIK histone acetyltransferase complex functions in the yeast retrograde response pathway, Mol. Cell. Biol., 22 (2002) 8774–86.

[76] A.L. Hughes, D.E. Gottschling, An early age increase in vacuolar pH limits mitochondrial function and lifespan in yeast, Nature, 492 (2012) 261–5.

[77] C.D. Dunn, M.S. Lee, F.A. Spencer, R.E. Jensen, A genomewide screen for petite-negative yeast strains yields a new subunit of the i-AAA protease complex, Mol. Biol. Cell, 17 (2006) 213–26.

[78] C. Pereira, S. Chaves, S. Alves, B. Salin, N. Camougrand, S. Manon, M.J. Sousa, M. Côrte-Real, Mitochondrial degradation in acetic acid-induced yeast apoptosis: The role of Pep4 and the ADP/ATP carrier, Mol. Microbiol., 76 (2010) 1398–1410.

[79] J.J. Lemasters, Selective Mitochondrial Autophagy, or Mitophagy, as a Targeted Defense Against Oxidative Stress, Mitochondrial Dysfunction, and Aging, Rejuvenation Res., 8 (2005) 3–5.

[80] M. Müller, K. Lu, A.S. Reichert, Mitophagy and mitochondrial dynamics in Saccharomyces cerevisiae, Biochim. Biophys. Acta - Mol. Cell Res., 1853 (2015) 2766–2774.

[81] G. Ashrafi, T.L. Schwarz, The pathways of mitophagy for quality control and

clearance of mitochondria, Cell Death Differ., 20 (2012) 31–42.

[82] R. Tal, G. Winter, N. Ecker, D.J. Klionsky, H. Abeliovich, Aup1p, a yeast mitochondrial protein phosphatase homolog, is required for efficient stationary phase mitophagy and cell survival, J. Biol. Chem., 282 (2007) 5617–24.

[83] M. Priault, B. Salin, J. Schaeffer, F.M. Vallette, J.P. di Rago, J.C. Martinou, Impairing the bioenergetic status and the biogenesis of mitochondria triggers mitophagy in yeast, Cell Death Differ., 12 (2005) 1613–21.

[84] K. Nowikovsky, S. Reipert, R.J. Devenish, R.J. Schweyen, Mdm38 protein depletion causes loss of mitochondrial K+/H+ exchange activity, osmotic swelling and mitophagy, Cell Death Differ., 14 (2007) 1647–1656.

[85] M. Deffieu, I. Bhatia-Kissová, B. Salin, A. Galinier, S. Manon, N. Camougrand, Glutathione participates in the regulation of mitophagy in yeast, J. Biol. Chem., 284 (2009) 14828–37.

[86] A.J. Whitworth, L.J. Pallanck, PINK1/Parkin mitophagy and neurodegeneration—what do we really know in vivo?, Curr. Opin. Genet. Dev., 44 (2017) 47–53.

[87] K. Okamoto, N. Kondo-Okamoto, Y. Ohsumi, M. Baba, K. Takeshige, N. Baba, Y. Ohsumi, S. Bernales, K.L. McDonald, P. Walter, G. Bjorkoy, T. Lamark, A. Brech, H. Outzen, M. Perander, A. Overvatn, H. Stenmark, T. Johansen, C.L. Campbell, P.E. Thorsness, et al., Mitochondria-anchored receptor Atg32 mediates degradation of mitochondria via selective autophagy, Dev. Cell, 17 (2009) 87–97.

[88] T. Kanki, K. Wang, Y. Cao, M. Baba, D.J. Klionsky, M. Baba, D.F. Bogenhagen, Y. Cao, D.J. Klionsky, H. Cheong, U. Nair, J. Geng, D.J. Klionsky, S.L. Clark, W.A. Dunn, J.M. Cregg, J.A.K.W. Kiel, I.J. van der Klei, M. Oku, Y. Sakai, et al., Atg32 is a mitochondrial protein that confers selectivity during mitophagy, Dev. Cell, 17 (2009) 98–109.

[89] T. Kanki, K. Wang, M. Baba, C.R. Bartholomew, M.A. Lynch-Day, Z. Du, J. Geng, K. Mao, Z. Yang, W.-L. Yen, D.J. Klionsky, A genomic screen for yeast mutants defective in selective mitochondria autophagy, Mol. Biol. Cell, 20 (2009) 4730–8.

[90] T. Kanki, D.J. Klionsky, The molecular mechanism of mitochondria autophagy in yeast, Mol. Microbiol., 75 (2010) 795–800.

[91] N. Kondo-Okamoto, N.N. Noda, S.W. Suzuki, H. Nakatogawa, I. Takahashi, M. Matsunami, A. Hashimoto, F. Inagaki, Y. Ohsumi, K. Okamoto, Autophagy-related protein 32 acts as autophagic degron and directly initiates mitophagy, J. Biol. Chem., 287 (2012) 10631–8.

[92] K. Wang, M. Jin, X. Liu, D.J. Klionsky, Proteolytic processing of Atg32 by the mitochondrial i-AAA protease Yme1 regulates mitophagy, Autophagy, 9 (2013) 1828–1836.

[93] G. Twig, A. Elorza, A.J.A. Molina, H. Mohamed, J.D. Wikstrom, G. Walzer, L. Stiles, S.E. Haigh, S. Katz, G. Las, J. Alroy, M. Wu, B.F. Py, J. Yuan, J.T. Deeney, B.E. Corkey, O.S. Shirihai, Fission and selective fusion govern mitochondrial segregation and elimination by autophagy, EMBO J., 27 (2008) 433–46.

[94] H. Abeliovich, M. Zarei, K.T.G. Rigbolt, R.J. Youle, J. Dengjel, Involvement of mitochondrial dynamics in the segregation of mitochondrial matrix proteins during stationary phase mitophagy, Nat. Commun., 4 (2013) 2789.

[95] K. Mao, K. Wang, X. Liu, D.J.D.J. Klionsky, Y. Aoki, T. Kanki, Y. Hirota, Y. Kurihara, T. Saigusa, T. Uchiumi, D. Kang, M. Baba, M. Osumi, S.V. Scott, D.J.D.J. Klionsky, Y. Ohsumi, W. Bleazard, J.M. McCaffery, E.J. King, S. Bale, et al., The scaffold protein Atg11 recruits fission machinery to drive selective mitochondria degradation by autophagy, Dev. Cell, 26 (2013) 9–18.

[96] N. Mendl, A. Occhipinti, M. Müller, P. Wild, I. Dikic, A.S. Reichert, Mitophagy in yeast is independent of mitochondrial fission and requires the stress response gene WHI2, J. Cell Sci., 124 (2011) 1339–1350.

[97] M. Müller, A.S. Reichert, Mitophagy, mitochondrial dynamics and the general stress response in yeast, Biochem. Soc. Trans., 39 (2011) 1514–1519.

[98] D. Journo, A. Mor, H. Abeliovich, Aup1-mediated Regulation of Rtg3 during Mitophagy, J. Biol. Chem., 284 (2009) 35885–35895.

[99] N. Shai, M. Schuldiner, E. Zalckvar, No peroxisome is an island — Peroxisome contact sites, Biochim. Biophys. Acta - Mol. Cell Res., 1863 (2016) 1061–1069.

[100] A. Mohanty, H.M. McBride, Emerging roles of mitochondria in the evolution, biogenesis, and function of peroxisomes, Front. Physiol., 4 (2013) 268.

[101] A. Sugiura, S. Mattie, J. Prudent, H.M. McBride, Newly born peroxisomes are a hybrid of mitochondrial and ER-derived pre-peroxisomes, Nature, 542 (2017) 251–254.

[102] Y. Cohen, Y.A. Klug, L. Dimitrov, Z. Erez, S.G. Chuartzman, D. Elinger, I. Yofe, K. Soliman, J. Gärtner, S. Thoms, R. Schekman, Y. Elbaz-Alon, E. Zalckvar, M. Schuldiner, Peroxisomes are juxtaposed to strategic sites on mitochondria, Mol. Biosyst., 10 (2014) 1742–8.

[103] N. Aboushadi, W.H. Engfelt, V.G. Paton, S.K. Krisans, Role of peroxisomes in isoprenoid biosynthesis, J. Histochem. Cytochem., 47 (1999) 1127–32.

[104] Ł. Opaliński, J.A.K.W. Kiel, C. Williams, M. Veenhuis, I.J. van der Klei, Membrane curvature during peroxisome fission requires Pex11, EMBO J., 30 (2011) 5–16.

[105] M. Mattiazzi Ušaj, M. Brložnik, P. Kaferle, M. Žitnik, H. Wolinski, F. Leitner, S.D.D. Kohlwein, B. Zupan, U. Petrovič, Genome-wide localization study of yeast Pex11 identifies peroxisome-mitochondria interactions through the ERMES complex, J. Mol. Biol., 427 (2015) 2072–2087.

[106] M. Schrader, J. Costello, L.F. Godinho, M. Islinger, Peroxisome-mitochondria interplay and disease, J. Inherit. Metab. Dis., 38 (2015) 681.

[107] C.W.T. Van Roermund, E.H. Hettema, M. Van Den Berg, H.F. Tabak, R.J.A. Wanders, Molecular characterization of carnitine-dependent transport of acetyl-CoA from peroxisomes to mitochondria in Saccharomyces cerevisiae and identification of a plasma membrane carnitine transporter, Agp2p, EMBO J., 18 (1999) 5843–5852.

[108] B.T. Weinert, V. Iesmantavicius, T. Moustafa, C. Schölz, S.A. Wagner, C. Magnes, R. Zechner, C. Choudhary, Acetylation dynamics and stoichiometry in Saccharomyces cerevisiae, Mol. Syst. Biol., 10 (2014) 1–13.

[109] K. Strijbis, B. Distel, Intracellular acetyl unit transport in fungal carbon metabolism, Eukaryot. Cell, 9 (2010) 1809–1815.

[110] M. Kunze, I. Pracharoenwattana, S.M. Smith, A. Hartig, A central role for the peroxisomal membrane in glyoxylate cycle function, Biochim. Biophys. Acta - Mol. Cell Res., 1763 (2006) 1441–1452.

[111] L. Palmieri, F.M. Lasorsa, A. De Palma, F. Palmieri, M.J. Runswick, J.E. Walker,

Identification of the yeast ACR1 gene product as a succinate-fumarate transporter essential for growth on ethanol or acetate, FEBS Lett., 417 (1997) 114–118.

[112] L. Palmieri, A. Vozza, A. Honlinger, K. Dietmeier, A. Palmisano, V. Zara, F. Palmieri, The mitochondrial dicarboxylate carrier is essential for the growth of Saccharomyces cerevisiae on ethanol or acetate as the sole carbon source, Mol. Microbiol., 31 (1999) 569–577.

[113] C. Lismont, M. Nordgren, P.P. Van Veldhoven, M. Fransen, Redox interplay between mitochondria and peroxisomes, Front. Cell Dev. Biol., 3 (2015) 35.

[114] A. Ayer, J. Sanwald, B.A. Pillay, A.J. Meyer, G.G. Perrone, I.W. Dawes, Distinct Redox Regulation in Sub-Cellular Compartments in Response to Various Stress Conditions in Saccharomyces cerevisiae, PLoS One, 8 (2013) e65240.

[115] B. Wang, P.P. Van Veldhoven, C. Brees, N. Rubio, M. Nordgren, O. Apanasets, M. Kunze, M. Baes, P. Agostinis, M. Fransen, Mitochondria are targets for peroxisome-derived oxidative stress in cultured mammalian cells, Free Radic. Biol. Med., 65 (2013) 882–94.

[116] Y. Elbaz-Alon, B. Morgan, A. Clancy, T.N.E. Amoako, E. Zalckvar, T.P. Dick, B. Schwappach, M. Schuldiner, The yeast oligopeptide transporter Opt2 is localized to peroxisomes and affects glutathione redox homeostasis, FEMS Yeast Res., 14 (2014) 1055–1067.

[117] Z. Liu, R. a Butow, Mitochondrial retrograde signaling, Annu. Rev. Genet., 40 (2006) 159–185.

[118] M. Neuspiel, A.C. Schauss, E. Braschi, R. Zunino, P. Rippstein, R.A. Rachubinski, M.A. Andrade-Navarro, H.M. McBride, Cargo-Selected Transport from the Mitochondria to Peroxisomes Is Mediated by Vesicular Carriers, Curr. Biol., 18 (2008) 102–108.

[119] V. Soubannier, G.L. McLelland, R. Zunino, E. Braschi, P. Rippstein, E.A. Fon, H.M. McBride, A Vesicular Transport Pathway Shuttles Cargo from Mitochondria to Lysosomes, Curr. Biol., 22 (2012) 135–141.

[120] V. Soubannier, P. Rippstein, B.A. Kaufman, E.A. Shoubridge, H.M. McBride, Reconstitution of mitochondria derived vesicle formation demonstrates selective

enrichment of oxidized cargo, PLoS One, 7 (2012) e52830.

[121] V.Y. Petrova, T.V. Rasheva, A. V. Kujumdzieva, Catalase enzyme in mitochondria of Saccharomyces cerevisiae, Electron. J. Biotechnol., 5 (2002).

[122] V.Y. Petrova, D. Drescher, A. V Kujumdzieva, M.J. Schmitt, Dual targeting of yeast catalase A to peroxisomes and mitochondria, Biochem. J., 380 (2004) 393–400.

[123] Y. Elgersma, C.W. van Roermund, R.J. Wanders, H.F. Tabak, Peroxisomal and mitochondrial carnitine acetyltransferases of *Saccharomyces cerevisiae* are encoded by a single gene, EMBO J., 14 (1995) 3472–3479.

[124] M. Schrader, H.D. Fahimi, Growth and Division of Peroxisomes, Int. Rev. Cytol., 255 (2006) 237–290.

[125] K. Kuravi, Dynamin-related proteins Vps1p and Dnm1p control peroxisome abundance in Saccharomyces cerevisiae, J. Cell Sci., 119 (2006) 3994–4001.

[126] A.M. Motley, G.P. Ward, E.H. Hettema, Dnm1p-dependent peroxisome fission requires Caf4p, Mdv1p and Fis1p, J. Cell Sci., 121 (2008) 1633–40.

[127] R.A. Butow, N.G. Avadhani, Mitochondrial Signaling: The Retrograde Response, Mol. Cell, 14 (2004) 1–15.

[128] Y.C. Chen, G.K.E. Umanah, N. Dephoure, S.A. Andrabi, S.P. Gygi, T.M. Dawson, V.L. Dawson, J. Rutter, Msp1/ATAD1 maintains mitochondrial function by facilitating the degradation of mislocalized tail-anchored proteins, EMBO J., 33 (2014) 1–17.

[129] V. Okreglak, P. Walter, The conserved AAA-ATPase Msp1 confers organelle specificity to tail-anchored proteins, Proc. Natl. Acad. Sci., 111 (2014) 8019–8024.

[130] T.C. Walther, R. V Farese, Lipid droplets and cellular lipid metabolism, Annu. Rev. Biochem., 81 (2012) 687–714.

[131] J. Pu, C.W. Ha, S. Zhang, J.P. Jung, W.-K. Huh, P. Liu, Interactomic study on interaction between lipid droplets and mitochondria, Protein Cell, 2 (2011) 487–496.

[132] C.S. Shaw, D.A. Jones, A.J.M. Wagenmakers, Network distribution of mitochondria and lipid droplets in human muscle fibres, Histochem. Cell Biol., 129 (2008) 65–72.

[133] M.A. Tarnopolsky, C.D. Rennie, H.A. Robertshaw, S.N. Fedak-Tarnopolsky, M.C.

Devries, M.J. Hamadeh, Influence of endurance exercise training and sex on intramyocellular lipid and mitochondrial ultrastructure, substrate use, and mitochondrial enzyme activity, Am. J. Physiol. Regul. Integr. Comp. Physiol., 292 (2007) R1271–R1278.

[134] H. Wang, U. Sreenivasan, U. Sreenevasan, H. Hu, A. Saladino, B.M. Polster, L.M. Lund, D. Gong, W.C. Stanley, C. Sztalryd, Perilipin 5, a lipid droplet-associated protein, provides physical and metabolic linkage to mitochondria, J. Lipid Res., 52 (2011) 2159–68.

[135] A. Perktold, B. Zechmann, G. Daum, G. Zellnig, Organelle association visualized by three-dimensional ultrastructural imaging of the yeast cell, FEMS Yeast Res., 7 (2007) 629–38.

[136] A.S. Rambold, S. Cohen, J. Lippincott-Schwartz, Fatty Acid Trafficking in Starved Cells: Regulation by Lipid Droplet Lipolysis, Autophagy, and Mitochondrial Fusion Dynamics, Dev. Cell, 32 (2015) 678–692.

[137] J. Bischof, M. Salzmann, M.K. Streubel, J. Hasek, F. Geltinger, J. Duschl, N. Bresgen, P. Briza, D. Haskova, R. Lejskova, M. Sopjani, K. Richter, M. Rinnerthaler, Clearing the outer mitochondrial membrane from harmful proteins via lipid droplets, Cell Death Discov., 3 (2017) 17016.

[138] M. Simocková, R. Holic, D. Tahotná, J. Patton-Vogt, P. Griac, Yeast Pgc1p (YPL206c) controls the amount of phosphatidylglycerol via a phospholipase C-type degradation mechanism, J. Biol. Chem., 283 (2008) 17107–15.

[139] E. Currie, X. Guo, R. Christiano, C. Chitraju, N. Kory, K. Harrison, J. Haas, T.C. Walther, R. V Farese, High confidence proteomic analysis of yeast LDs identifies additional droplet proteins and reveals connections to dolichol synthesis and sterol acetylation, J. Lipid Res., 55 (2014) 1465–1477.

[140] B. Westermann, The mitochondria–plasma membrane contact site, Curr. Opin. Cell Biol., 35 (2015) 1–6.

[141] T. Klecker, S. Böckler, B. Westermann, Making connections: interorganelle contacts orchestrate mitochondrial behavior, Trends Cell Biol., 24 (2014) 537–545.

[142] M. Frieden, S. Arnaudeau, C. Castelbou, N. Demaurex, Subplasmalemmal

mitochondria modulate the activity of plasma membrane Ca2+-ATPases, J. Biol. Chem., 280 (2005) 43198–208.

[143] L.L. Lackner, H. Ping, M. Graef, A. Murley, J. Nunnari, Endoplasmic reticulum-associated mitochondria-cortex tether functions in the distribution and inheritance of mitochondria, Proc. Natl. Acad. Sci. U. S. A., 110 (2013) E458-67.

[144] T. Klecker, D. Scholz, J. Förtsch, B. Westermann, The yeast cell cortical protein Num1 integrates mitochondrial dynamics into cellular architecture, J. Cell Sci., 126 (2013) 2924–2930.

[145] M. Hammermeister, K. Schödel, B. Westermann, Mdm36 is a mitochondrial fission-promoting protein in Saccharomyces cerevisiae, Mol. Biol. Cell, 21 (2010) 2443–52.

[146] H.A. Ping, L.M. Kraft, W.T. Chen, A.E. Nilles, L.L. Lackner, Num1 anchors mitochondria to the plasma membrane via two domains with different lipid binding specificities, J. Cell Biol., 213 (2016) 513–524.

[147] A.C. Schauss, H.M. McBride, K.L. Cerveny, S.L. Studer, R.E. Jensen, H. Sesaki, M. Farkasovsky, H. Kuntzel, J.W. Yu, J.M. Mendrola, A. Audhya, S. Singh, D. Keleti, D.B. DeWald, D. Murray, S.D. Emr, M.A. Lemmon, R.A. Heil-Chapdelaine, J.R. Oberle, J.A. Cooper, et al., Mitochondrial fission: a non-nuclear role for Num1p, Curr. Biol., 17 (2007) R467-70.

[148] S. V Sambasivarao, Iron sensing and regulation in Saccharomyces cerevisiae: Ironing out the mechanistic details, 18 (2013) 1199–1216.

[149] M. Shakoury-Elizeh, J. Tiedeman, J. Rashford, T. Ferea, J. Demeter, E. Garcia, R. Rolfes, P.O. Brown, D. Botstein, C.C. Philpott, Transcriptional remodeling in response to iron deprivation in Saccharomyces cerevisiae, Mol. Biol. Cell, 15 (2004) 1233–43.

[150] C. Askwith, D. Eide, A. Van Ho, P.S. Bernard, L. Li, S. Davis-Kaplan, D.M. Sipe, J. Kaplan, G.S. Adrian, M.T. McCammon, D.L. Montgomery, M.G. Douglas, S.F. Altschul, W. Gish, W. Miller, E.W. Myres, D.G. Lipman, W. Bao, D.M. O'Malley, R. Whetten, et al., The FET3 gene of S cerevisiae encodes a multicopper oxidase required for ferrous iron uptake, Cell, 76 (1994) 403–10.

[151] R. Stearman, D.S. Yuan, Y. Yamaguchi-Iwai, R.D. Klausner, A. Dancis, A Permease-

Oxidase Complex Involved in High-Affinity Iron Uptake in Yeast, Science, 271 (1996) 1552–1557.

[152] C.D. Kaplan, J. Kaplan, Iron Acquisition and Transcriptional Regulation, Chem. Rev., 109 (2009) 4536–4552.

[153] M.R. Peterson, S.D. Emr, The Class C Vps Complex Functions at Multiple Stages of the Vacuolar Transport Pathway, Traffic, 2 (2001) 476–486.

[154] G. Wang, R.J. Deschenes, Plasma Membrane Localization of Ras Requires Class C Vps Proteins and Functional Mitochondria in Saccharomyces cerevisiae, Mol. Cell. Biol., 26 (2006) 3243–3255.

[155] A. Sugiura, G.-L. McLelland, E. a Fon, H.M. McBride, A new pathway for mitochondrial quality control: mitochondrial-derived vesicles, EMBO J., 33 (2014) 1–15.

[156] M.A. Andrade-Navarro, L. Sanchez-Pulido, H.M. McBride, Mitochondrial vesicles: an ancient process providing new links to peroxisomes, Curr. Opin. Cell Biol., 21 (2009) 560–567.

[157] S.B. Gould, S.G. Garg, W.F. Martin, Bacterial Vesicle Secretion and the Evolutionary Origin of the Eukaryotic Endomembrane System, Trends Microbiol., 24 (2016) 525–534.

[158] N.D. Lees, B. Skaggs, D.R. Kirsch, M. Bard, Cloning of the late genes in the ergosterol biosynthetic pathway of Saccharomyces cerevisiae—A review, Lipids, 30 (1995) 221–226.

[159] R. Erdmann, G. Blobel, Giant peroxisomes in oleic acid-induced Saccharomyces cerevisiae lacking the peroxisomal membrane protein Pmp27p, J. Cell Biol., 128 (1995) 509–523.

[160] P.A. Marshall, Y.I. Krimkevich, R.H. Lark, J.M. Dyer, M. Veenhuis, J.M. Goodman, Pmp27 promotes peroxisomal proliferation, J. Cell Biol., 129 (1995) 345–355.

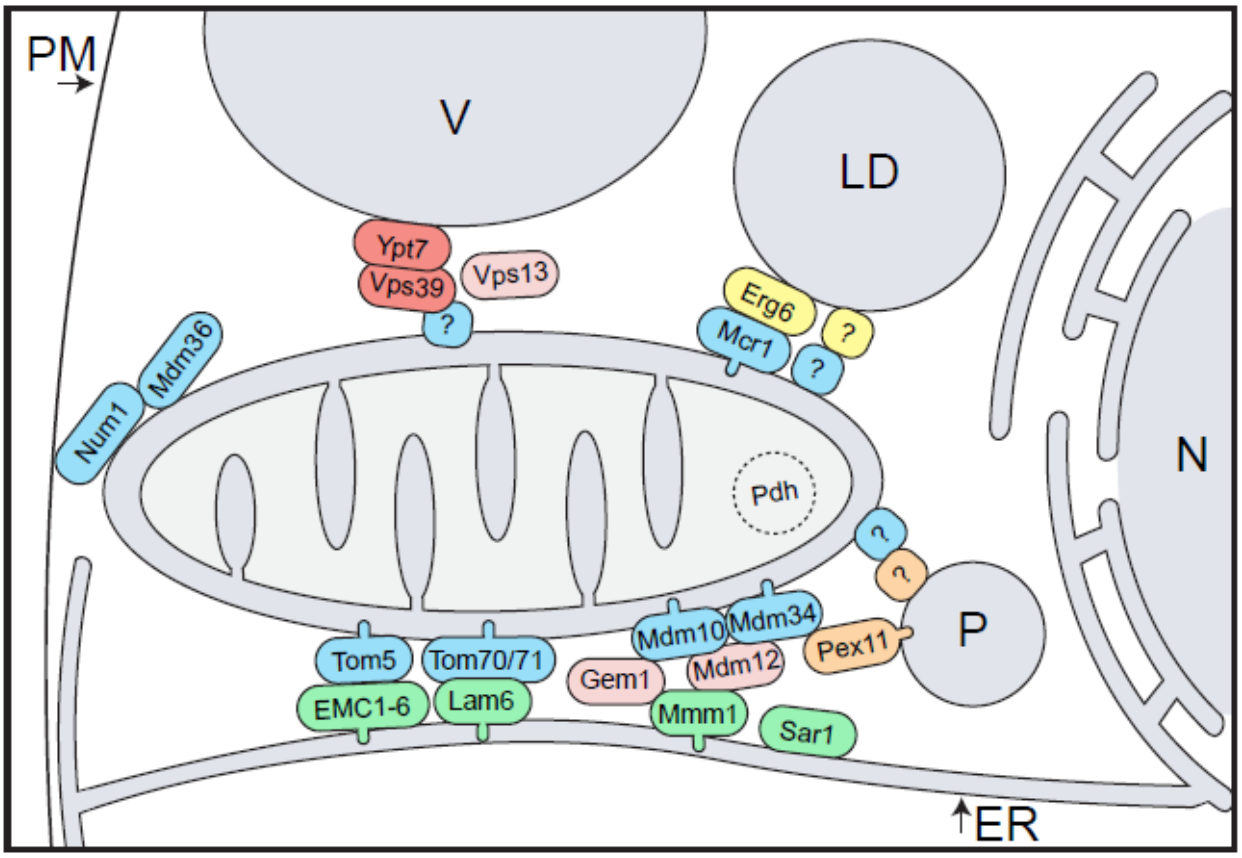

Figure 1

Highlights

- Mitochondria must communicate with all other organelles to perform their array of activities.
- Mitochondrial communication includes an extensive network of signaling cascades, physical contacts between membranes and shared metabolic pathways and proteins.
- This review summarizes different aspects of mitochondrial communication emphasizing its role in cellular and mitochondrial homeostasis.