

Review

The Expanding and Unexpected Functions of Mitochondria Contact Sites

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Mitochondria make functionally relevant contacts with most, if not all, other organelles in the cell. These contacts impact on mitochondrial behavior and function as well as on a wide variety of cellular functions. Many recent advances have been made in the rapidly growing field of mitochondria contact site biology, and these advances have expanded the known functions of mitochondria contact sites in exciting and unexpected ways.

Organelle Contact within the Cell

Organelles perform distinct, essential functions in the cell. Although once thought to operate independently, it is clear that organelles contact other organelles and that these contacts are crucial for organelle function and overall cellular homeostasis [1–3]. Contact is achieved through proteins that function to directly tether organelles. Although some proteins solely serve to physically bridge organelles, others actively participate in interorganelle communication and function. Importantly, the proteins that localize to contact sites establish, maintain, and functionally alter contacts in response to different physiological contexts and impact on a wide variety of fundamental cellular functions.

The vast extent to which organelles contact one another has become increasingly evident. Although organelle contacts were first visualized by electron microscopy six decades ago [4,5], advances in the ability to visualize organelles and identify the proteins that localize to sites of contact as well as to manipulate these contacts have greatly advanced the study of contact site formation and function. Exciting, cutting-edge imaging techniques now allow the visualization of multiple organelles simultaneously over time with increased spatial and temporal resolution. Thus, the sheer number, extent, and spatiotemporal dynamics of interorganelle contacts within cells can be more fully appreciated. Recent work demonstrates that most organelles make functionally relevant contact, with most, if not all, other organelles in the cell, with functionally relevant contact referring to a tether-mediated contact that impacts on the behavior or function of one or both organelles. The focus here will be on contacts made between mitochondria and other organelles – specifically, on recent advances that highlight the expanding and unexpected functions of mitochondria–organelle contacts.

The Many Organelles That Mitochondria Contact

Contact between mitochondria and the endoplasmic reticulum (ER) has been visually appreciated since the 1950s and functionally appreciated since the 1990s [4–7]. Excitingly, over the past decade, contacts between mitochondria and many additional organelles have been identified (Figure 1). In addition to the ER, mitochondria contact vacuoles/lysosomes, peroxisomes, lipid droplets, endosomes, the Golgi, the plasma membrane (PM), and melanosomes. The number of contacts mitochondria make with a specific organelle can vary dramatically from only a few contacts to hundreds per cell [8]. These contacts are often mediated by multiple, distinct tethering complexes. Although these tethering complexes often share some functional overlap, it is clear, as discussed below, that distinct complexes have unique, nonoverlapping functions and are dif-

Highlights

Mitochondria make functionally relevant contacts with most, if not all, other organelles in the cell.

Mitochondria–organelle contacts impact on many aspects of mitochondrial behavior and function.

The functions of mitochondria–organelle contact extend beyond the mitochondrion itself and impact on an interorganelle communication network and, consequently, on a wide variety of cellular functions.

Many mitochondrial contact site proteins localize to multiple contact sites.

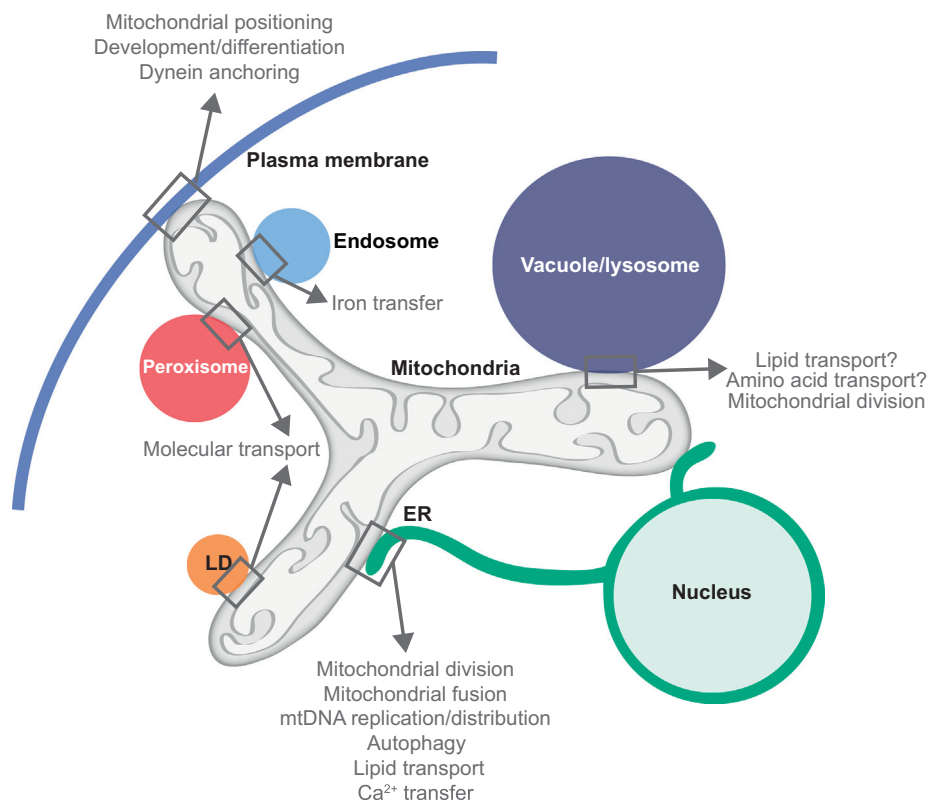
Many mitochondrial contact site proteins function in other crucial cellular processes, such as protein import, vesicular trafficking, and membrane remodeling pathways. It is unclear how these canonical functions are integrated with contact site function.

Mitochondria–organelle contacts are part of a complex and dynamic organelle interaction network that integrates organelle communication and function with cellular metabolism and homeostasis.

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Figure 1. Mitochondria–Organelle Contacts and Functions. Mitochondria make functionally relevant contact with many organelles in the cell. A subset of mitochondria–organelle contacts and the functions ascribed to these contacts are shown (text for details). Abbreviations: ER, endoplasmic reticulum; LD, lipid droplet; mtDNA, mitochondrial DNA.

ferentially regulated in specific biological contexts. In addition, the molecular mechanisms mediating contact and the dynamics and duration of contact can vary dramatically. For example, contacts between mitochondria and lipid droplets can range from tens to hundreds of seconds in Cos7 cells, and a mitochondria–PM contact in budding yeast can persist for greater than 45 minutes [8,9]. Thus, not all mitochondria–organelle contacts are created equal; mitochondrial contacts exhibit functional, architectural, and dynamic differences, and differentially impact on many aspects of mitochondrial behavior and function.

Mitochondria–Organelle Contacts and Mitochondrial Dynamics

Mitochondria form highly elaborate, dynamic, reticular networks in many cell types. The structure and dynamics of the mitochondrial network are, in part, maintained by division and fusion of the organelle [10,11]. Early investigations into the activities of mitochondrial division and fusion were, not surprisingly, approached from a highly mitochondria-centric perspective. However, the finding that sites of mitochondrial division are spatially linked to ER–mitochondria contact sites rapidly changed that view. In both yeast and mammalian cells, the ER is present at the vast majority of mitochondrial division events [12]. The ER–mitochondria encounter structure (ERMES) mediates contact between mitochondria and the ER at sites of mitochondrial division in yeast [13,14], although the identity of the functionally equivalent tether in higher eukaryotes remains to be determined. ER contact at nascent division sites is associated with the initial constriction of mitochondria, and precedes the recruitment of the dynamin-related proteins that drive further

constriction and scission of the mitochondrial membranes, Dnm1/Drp1 and Dyn2 [11,12,15]. In mammals, the ER-localized formin INF2 has been proposed to stimulate actin polymerization at nascent division sites [16]. Activation of INF2 at sites of ER–mitochondria contact is thought to be mediated by an interaction with the mitochondria-anchored actin-nucleating protein, Spire1C [17]. Actin polymerization has been proposed to facilitate mitochondrial division in multiple ways: by providing a force-generating system to drive the initial constriction of mitochondria, by functioning in the direct recruitment of Drp1 to the site of contact, and by enhancing ER–mitochondria contact and consequently ER-to-mitochondria calcium transfer leading to constriction of the mitochondrial inner membrane [16,18,19]. Thus, ER–mitochondria contact impacts on mechanisms that influence the structure of both the outer and inner mitochondrial membranes during division.

Interestingly, ER–mitochondria contacts at sites of division are spatially linked to nucleoids, complexes of mitochondrial DNA (mtDNA) and associated proteins. Studies in yeast first demonstrated a link between ER-associated mitochondrial division and the maintenance and distribution of nucleoids [14], and this has more recently been described in mammalian cells [20]. Specifically, the small subset of ER–mitochondria contact sites that go on to divide are spatially and temporally linked to actively replicating nucleoids. The coupling of ER-associated mitochondrial division with mtDNA replication is proposed to serve as a mechanism to distribute newly replicated mtDNA to daughter mitochondria and facilitate the accurate distribution of nucleoids in cells [14,20]. The identity of factors that physically and functionally coordinate mtDNA replication, which occurs in the mitochondrial matrix, with ER–mitochondria contacts and the division machine on the mitochondrial surface are at this point unknown.

A recent study has added yet another unexpected player to the process of mitochondrial division, the lysosome, because sites of mitochondria–lysosome contact have also been spatially linked to sites of mitochondrial division [21]. Lysosomal GTP-bound RAB7 has been shown to be involved in the formation and stabilization of mitochondria–lysosome contacts, which are subsequently destabilized by TBC1D15, a RAB7 GAP (GTPase-activating protein) that is recruited to mitochondria by the outer mitochondrial membrane protein Fis1. The ER and Drp1 are present at sites of lysosome-marked mitochondrial division, raising the question of how contact between mitochondria and multiple organelles at sites of division is regulated and integrated.

The connection between mitochondria–ER contacts and mitochondrial dynamics might extend beyond division. In a recent study using grazing incidence structured illumination microscopy (GI-SIM) to examine the ER and mitochondrial dynamics with increased spatial and temporal resolution over standard SIM techniques, the ER was found to be present at over half of the mitochondrial fusion events observed [22]. In comparison with fusion events not associated with the ER, the duration of fusion events from initial contact to completion of fusion were shorter for fusion events associated with the ER. Although the molecular basis and functional contributions of ER–mitochondria contact at sites of fusion remain to be determined, it is clear that ER–mitochondria contacts are intimately connected to the division and fusion dynamics of the mitochondrial network.

Mitochondria–Organelle Contacts and Organelle Distribution

In addition to regulating the cellular distribution of mitochondria by impacting on mitochondrial dynamics, mitochondria contact sites can also more directly affect the position of mitochondria in cells. In yeast, the mitochondria–ER–cortex anchor (MECA) tethers mitochondria to the PM and cortical ER, bringing three cellular membranes into close proximity [23]. The core protein component of MECA, Num1, interacts directly with the mitochondrial membrane and PM; the molecular basis for the interaction between Num1 and the ER is poorly understood [24–27]. Num1 forms

stable contacts between mitochondria and the cell cortex that persist for extended periods of time [9], and the tethering activity of Num1 is required for proper mitochondrial distribution and inheritance during budding yeast mitosis [23,28,29]. Interestingly, the mitochondria–PM contacts sites formed by Num1 also serve as cortical attachment sites for dynein [9], where, once anchored, dynein captures and walks along astral microtubules to help to orient the mitotic spindle [30,31]. When mitochondrial inheritance is inhibited, Num1-mediated mitochondria–PM contacts are not formed in buds, and defects in dynein-mediated spindle positioning are observed [9,32]. Thus, Num1-mediated mitochondria–PM tethering impacts not only on the spatial distribution of mitochondria within cells but also on when and where dynein anchoring occurs and, consequently, on the function of dynein in spindle orientation. This surprising connection between a mitochondria–PM contact site and dynein anchoring was recently shown to be conserved in the evolutionarily distant fission yeast *Schizosaccharomyces pombe* [33]. The functional and physiological significance of why mitochondria–PM contacts serve as cortical attachment sites for dynein remains to be determined.

Although MECA-mediated tethering is stable in mitotic cells, a recent study indicates that the protein components of MECA, Num1 and Mdm36, are degraded during meiosis, specifically in anaphase II, which results in the release of mitochondria from the cell cortex [34]. This programmed release of mitochondria is proposed to be important for subsequent incorporation of mitochondria into spores. Interestingly, after being released from the cell cortex, mitochondria become closely opposed to the nuclear envelope, raising the possibility that a yet to be determined ER–mitochondria tethering complex is upregulated in meiosis to mediate this extensive contact. Thus, the regulation of at least two mitochondria contact sites facilitates mitochondrial repositioning during the meiotic developmental program.

A role for mitochondria–PM tethering in development and differentiation, more specifically in stem cell fate determination, has been recently reported for mammary stem cells [35]. During the asymmetric division of a mammary stem cell, highly fused mitochondria are tethered to the PM by a complex between Mfn1, an outer mitochondrial membrane fusion protein, and PCKζ, a cortically anchored kinase. The tethered mitochondria, which have enhanced reactive oxygen species scavenging capacity, are asymmetrically inherited by the daughter cell that retains stem cell identity. Even in simple asymmetrically dividing budding yeast cells, Mmr1-mediated ER–mitochondria tethering has been implicated in the asymmetric inheritance and retention of higher-functioning mitochondria by daughter cells [36,37]. Although we are still in the early stages of understanding the full mechanistic and functional contributions of mitochondria–organelle contacts to the function-dependent distribution of mitochondria in asymmetrically dividing cells, it is clear that mitochondria contact sites will play pivotal roles in development and differentiation.

Mitochondria–Organelle Contacts and Molecular Transport

In addition to influencing the dynamics and distribution of mitochondria, contacts between mitochondria and other organelles have been implicated in the interorganelle exchange of Ca^{2+} , lipid, and various metabolites. The role of ER–mitochondria contacts in Ca^{2+} transfer has been appreciated and well-studied for some time [6]. Ca^{2+} transfer between the two organelles impacts on mitochondrial bioenergetics, metabolism, and dynamics, as well as on cell death and autophagy, and perturbations in Ca^{2+} transfer are associated with many disease states. A discussion of the many players involved in as well as of the functions of Ca^{2+} transfer between the ER and mitochondria warrants its own reviews [38–42].

The transfer of another metal, iron, has also been associated with mitochondria contacts. Kiss-and-run contacts between mitochondria and endosomes have been implicated in iron transfer between the two organelles [43–45]. Although the molecular basis for mitochondria–endosome

contacts is unclear, the duration of contact between the two organelles is regulated by intraendosomal iron, suggesting that the contact is influenced by endosomal cargo.

A role for mitochondria contacts in interorganelle lipid transport has also been appreciated for decades [6,7], and several exciting advances have recently been made in our understanding of the molecular bases and mechanisms of transport. Multiple proteins that mediate mitochondria–organelle contact contain lipid-binding and -transport domains. For example, three of the four proteins in the ER–mitochondria tether ERMES (Mmm1, Mdm12, and Mdm34) contain a synaptotagmin-like mitochondrial lipid-binding protein (SMP) domain [13,46,47]. SMP domains are conserved lipid-binding domains found in proteins that reside at membrane contact sites [48,49]. Mmm1 and Mdm12 have been shown to bind phospholipids *in vitro*, and the structure of the Mmm1 and Mdm12 complex indicates that the proteins come together to form an extended, continuous hydrophobic channel that likely facilitates phospholipid transport [50–52]. Cells that lack ERMES exhibit defects in phospholipid transport between the ER and mitochondria [13]. Thus, similarly to many proteins containing SMP domains, ERMES components serve to tether as well as to facilitate transfer of phospholipids between two organelle membranes. Although a functional ortholog of the entire ERMES complex remains to be identified, the SMP domain protein PDZD8 has been recently suggested to be a functional ortholog, or possible paralog, of the ERMES component Mmm1 in metazoans [53,54]. PDZD8 mediates ER–mitochondria contact, and PDZD8-mediated contact is proposed to facilitate ER-to-mitochondria Ca^{2+} transfer. A role for PDZD8 in ER-associated mitochondrial division or lipid transfer between the two organelles remains to be examined.

Vps13 has been suggested to fulfill some of the functions of the ERMES complex in yeast and metazoans [55–58]. In yeast, Vps13 localizes to multiple interorganelle contact sites, including sites of mitochondria–vacuole contact [56–58], and its closest homolog in metazoans, VPS13A, localizes to ER–mitochondria contacts [55]. Vps13 has phospholipid-binding and -transfer activities *in vitro* and, based on the crystal structure of the N-terminal region of Vps13 from *Chaetomium thermophilum*, the protein has a hydrophobic cavity large enough to accommodate several lipid molecules simultaneously [55]. Consistent with the possibility that Vps13 may serve some ERMES-like functions, Vps13 is required in the absence of ERMES, and dominant mutations in Vps13 have been identified as suppressors of ERMES deficiency in yeast [56,58].

The ER–membrane protein complex (EMC) is yet another protein complex in yeast that has been localized to ER–mitochondria contacts and implicated in phospholipid transfer between the two organelles [59]. Although the EMC, a complex of six proteins (Emc1–6) that has been implicated in ER protein folding [60], is found throughout the ER, a fraction of the complex interacts with Tom5, a component of the translocase of the mitochondrial outer membrane. Cells lacking the EMC exhibit a reduction in ER–mitochondria tethering as well as in the transfer of phospholipids from the ER to mitochondria [59]. The proportion of the EMC that interacts with Tom5 colocalizes with ERMES; however, data suggest that the EMC and ERMES function independently in their tethering and lipid-transport roles. Dissecting the contact site-specific functions of the EMC are complicated by the fact that the EMC has recently been shown to be involved in the biogenesis of multipass transmembrane proteins, and thus could indirectly affect contact site formation and function [61–63].

In addition to contacts mediating transfer between the ER and mitochondria, mitochondria–lipid droplet and mitochondria–peroxisome contacts have also been implicated in interorganelle transport. In metazoans, mitochondria–lipid droplet contacts have been shown to participate in both the breakdown and synthesis of fatty acids. During starvation, fatty acids are transferred via direct contact from lipid droplets to mitochondria, where the fatty acids are subsequently metabolized

to drive mitochondrial ATP production [64]. Conversely, in brown adipocytes, mitochondria recruited to lipid droplets by perilipin 5 provide ATP to fuel fatty acid synthesis and lipid droplet expansion [65,66]. For mitochondria–peroxisome contacts, Pex34, a peroxisomal protein [67], and Fzo1, a dynamin-related GTPase that drives fusion of the mitochondrial outer membrane [68], have recently been identified as mitochondria–peroxisome tethering proteins in yeast [69]. Pex34 is anchored in the peroxisomal membrane, and the molecular basis for the interaction with mitochondria is unknown. Fzo1 is known to be anchored in the mitochondrial outer membrane, and recent evidence suggests that the protein may also localize to the peroxisomal membrane [69]. Thus, Fzo1–Fzo1 interactions may mediate mitochondria–peroxisome tethering similarly to the mitochondria–mitochondria tethering role for Fzo1 in outer mitochondrial membrane fusion, but without the subsequent membrane fusion event [70]. Interestingly, Pex34-mediated, but not Fzo1-mediated, mitochondria–peroxisome contacts are functionally linked to the transfer of β -oxidation products from peroxisomes to mitochondria [69], highlighting that not all contact sites are created equal.

Mitochondria–Organelle Contacts and Cellular Metabolism and Homeostasis

The formation and function of many mitochondria–organelle contacts are integrated with cellular metabolism and homeostasis. vCLAMP (vacuole–mitochondria patch) tethers mitochondria to the vacuole in yeast, and vCLAMP formation is integrated with cellular metabolism and is decreased in respiratory conditions [71,72]. A core component of vCLAMP is the HOPS (homotypic fusion and vacuole-sorting complex) tethering complex subunit Vps39. As a component of HOPS, Vps39 functions in the endolysosomal fusion pathway [73]. Interestingly, Vps39 is the only component of the HOPS complex that is required for vCLAMP, and it is unclear whether Vps39 localizes to vCLAMP alone or together with the HOPS complex [71,72].

Functional studies on vCLAMP were initially plagued by a problem common to any contact site composed of a protein that also functions outside the context of the contact site – it is difficult to discern whether the phenotypes observed in the absence of the protein are due to loss of contact site activity or reflect the canonical function of the protein. For vCLAMP, this difficulty was overcome by the identification of Vps39 separation-of-function mutants, which are defective for vCLAMP formation but not for HOPS complex function [74]. Previous work had demonstrated that Vps39 function is crucial in the absence of ERMES [71,72]. Although the loss of Vps39 function in vCLAMP was thought to underlie the synthetic interaction observed, the vCLAMP-impaired Vps39 mutants were elegantly used to demonstrate that the role of Vps39 in HOPS-dependent trafficking to the vacuole is what becomes crucial in the absence of ERMES. This unexpected result, in combination with additional studies using vesicular trafficking mutants, suggests that HOPS function and, more generally, trafficking to the vacuole are crucial in ERMES-deficient cells [74]. Although not essential in the absence of ERMES, the vCLAMP-specific functions of Vps39 impact on specific cellular stress response pathways and survival during starvation; however, the underlying functional basis for these phenotypes remains to be determined.

Unlike vCLAMP-mediated mitochondria–vacuole contact, mitochondria–vacuole contact mediated by Vps13 is crucial in the absence of ERMES [56–58], highlighting that the mitochondria–vacuole contacts mediated by vCLAMP and Vps13 are functionally distinct. Interestingly, however, Vps39 and other components of the vesicular trafficking pathway are required for the ability of Vps13 dominant mutants to suppress defects associated with ERMES deficiency [74]. Thus, transport to the vacuole is required for Vps13-mediated ERMES bypass. These findings suggest that the vacuole likely acquires a factor via vesicular transport that is required for ERMES bypass, and transport of this factor, potentially a phospholipid, from the vacuole to mitochondria may be facilitated by Vps13.

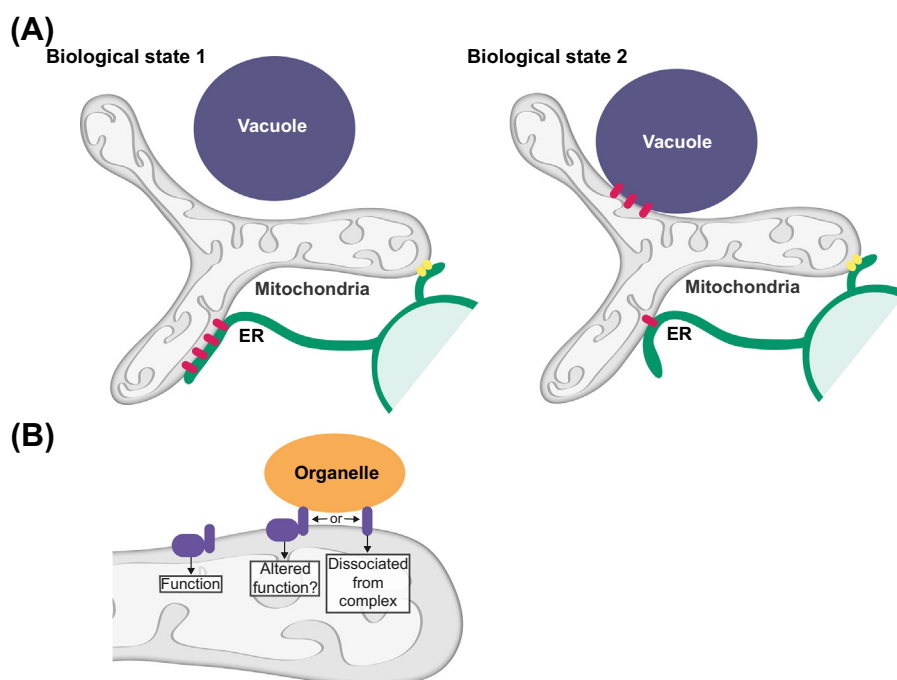
Lam6, also known as Ltc1, localizes to vCLAMP as well as to ER-mitochondria and ER-vacuole contacts [75,76]. Lam6 belongs to a StART/VASt domain family of sterol-transporting proteins [77]. Indeed, Lam6 has been shown to transport sterols *in vitro* [76,78]. Lam6 is required for cell viability in the absence of ERMES and is suggested to function in the regulation of local membrane lipid composition perhaps via the regulation of sterols on mitochondria [75,76]. Although the function of Lam6 at ER-mitochondria contacts remains to be fully defined, Lam6 functions at ER-vacuole contacts to create sterol-enriched vacuole membrane domains that regulate TORC1 signaling by spatially segregating TORC1 regulators [79]. Given that Lam6 localizes to multiple contact sites in the cell, it has been proposed that coordinate regulation of the relative levels of Lam6 at each contact may regulate an organelle interaction network that integrates organelle communication and function with cellular metabolism and homeostasis [75,79]. Consistent with this idea, the organelle-specific receptors for Lam6 are involved in many aspects of organelle biogenesis and function. Lam6 is recruited to mitochondria by Tom70/71, mitochondrial preprotein import receptors, and to the vacuole by Vac8, which has functions in vacuole transport, autophagy, and nucleus-vacuole junction formation [76,80–82]. If and how Lam6 affects the canonical functions of its organelle receptors and integrates these crucial functions with contact site formation and function are outstanding questions.

ER-mitochondria contacts are also intimately tied to cellular homeostasis via their impact on autophagy. Mitophagosomes in yeast and autophagosome in metazoans have been shown to form at ER-mitochondria contacts, and have been suggested to be a source of phospholipid for the forming autophagosome. Disrupting ER-mitochondria contacts, using mutants of ERMES in yeast and by depleting Mfn2 or PACS2 in metazoans, results in decreased formation of autophagic vesicles [83–85]. However, once again highlighting the fact that not all contacts are created equal, decreasing ER-mitochondria contact mediated by the VAPB-PTPIP51 tethering complex in metazoans induces basal autophagy, whereas increasing VAPB-PTPIP51-mediated contact impairs basal autophagy [86,87]. The effects of VAPB-PTPIP51 on autophagy are likely due to the role the tethering complex plays in the regulation of Ca^{2+} transfer between the two organelles. Further adding to the complexity, the effects of ER-mitochondria contacts on autophagy vary depending on the autophagic stimulus [87]. Thus, an important but difficult challenge will be to tease apart the direct and indirect contributions of distinct mitochondria contacts to the mechanisms and regulation of various autophagic pathways that differ in terms of the stimulus received and the intended cargo to be cleared.

Shared Components of Mitochondria Contact Sites

As more proteins that mediate or localize to sites of interorganelle contact are identified, it has become increasingly clear that contact site proteins can localize to more than one site of contact. Thus, a challenge in going forward is to understand how the localization of a protein to a specific contact site is determined in specific biological circumstances (Figure 2A). Changes in nutrient status have been shown to affect the localization of contact site proteins. In glucose conditions, Vps13 localizes to mitochondria-vacuole and mitochondria-endosome contacts, and in respiratory conditions Vps13 relocates to the nucleus-vacuole junction [56,57]. Organelle-specific adaptors recruit Vps13 to specific membranes: Vps13 is recruited to mitochondria by Mcp1, and to endosomes and vacuoles by Ypt35 [58,88]. These organelle-specific adaptors share a related motif, termed the Vps13 adaptor-binding (VAB) domain, that mediates the interaction with Vps13 [88]. It has been suggested the adaptors compete for Vps13 binding, and changes in the level of a specific adaptor and/or the accessibility or modification state of its VAB domain are used to control Vps13 localization in specific cellular circumstances. Perhaps similar mechanisms are used to regulate the cellular localization of Lam6 and other proteins that localize to multiple sites of contact.

Adding to the complexity of mitochondria contact site biology is the increasing number of proteins found at mitochondria contact sites that have well-studied functions in other crucial cellular processes (Figure 2B). For numerous proteins that localize to mitochondria–organelle contacts, the mitochondrial receptor is a component of the outer-membrane import machinery: Lam6 interacts with Tom70/71, EMC with Tom5, and Vps39 with Tom40 [59,74,76]. In addition, the ERMES component Mdm10 functions in the biogenesis of mitochondrial outer-membrane β -barrel proteins [80]. Other mitochondria contact site proteins have canonical functions in membrane-trafficking and -remodeling pathways. The vCLAMP component Vps39 functions in endolysosomal trafficking as a member of the HOPS complex [73], and proteins that drive outer mitochondrial membrane fusion, Fzo1/MFN1 and MFN2 (yeast/mammals) [70], also mediate mitochondria–organelle contacts. Fzo1 functions in mitochondria–peroxisome tethering [69], whereas its mammalian homologs have been implicated in contacts between mitochondria and the ER, melanosomes, and lipid droplets in the case of Mfn2, and between mitochondria and the PM in the case of Mfn1 [35,89–92]. It is unclear in most, if not all, of these circumstances whether and how the canonical functions of the proteins impact on or are affected by contact site formation and function (Figure 2B). In addition, for proteins that canonically function within a larger complex, it is not clear



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Figure 2. Shared Components of Mitochondrial Contact Sites. (A) The biological state of the cell can impact on the extent of mitochondria–organelle contacts as well as on the localization of contact site proteins. Many mitochondria contact site proteins localize to multiple, distinct contact sites, and the localization of a protein to a specific contact site may differ in specific biological circumstances. In the example shown in (A), a mitochondria contact site protein (shown in red) primarily localizes to and mediates endoplasmic reticulum (ER)–mitochondria contact in biological state 1, and primarily localizes to and mediates mitochondria–vacuole contact in biological state 2. The localization of a distinct mitochondria contact site protein (shown in yellow) is not altered between biological states 1 and 2. (B) Many mitochondrial contact site proteins have well-studied functions in other crucial cellular processes. The localization of such proteins to a contact site may impact on the canonical functions of that protein. For proteins that canonically function within a larger complex, it is not clear if the protein, when present at mitochondria contacts, remains with the complex or dissociates.

whether the protein, when present at mitochondria contacts, remains with the complex or dissociates. Furthermore, proteins that function in *cis* within their resident membrane may also act in *trans* when present at a contact, similarly to the resident ER protein Sac1 that can modify PM lipids in *trans* at contact sites [93]. Going forward, it will be exciting to determine how localization to a contact site impacts on the canonical activities of proteins and integrates these activities with contact site function to coordinately regulate organelle biogenesis and behavior.

Concluding Remarks

The contacts mitochondria make with other organelles play crucial roles in many aspects of mitochondrial biology and behavior. Furthermore, the functions of mitochondria–organelle contact extend beyond the mitochondrion itself and impact on a larger interorganelle communication network and, consequently, on a wide variety of cellular functions. Although great progress in our understanding of the formation and function of many mitochondria contacts sites has been made, we still have a long way to go before we are able to paint a complete picture of the molecular basis, function, and regulation of all mitochondria–organelle contacts (see Outstanding Questions). Merely understanding each contact in isolation, however, will not be enough. It will be crucial to understand how distinct mitochondria contacts communicate with one another and with other interorganelle contacts. As our knowledge of interorganelle contact site biology rapidly advances, it is increasingly clear that a complex, dynamic, and interdependent organelle interaction network is present in cells and that this network responds to cellular needs. Therefore, the exciting and unexpected knowledge gained about the biology of mitochondria–organelle contacts must be placed into the larger context of the complete organelle–interaction network of the cell.

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Outstanding Questions

How many distinct types of mitochondria–organelle contacts are there?

What functions of mitochondria–organelle contacts remain to be discovered?

How do distinct mitochondrial contacts communicate with one another and with other interorganelle contacts?

For mitochondrial contact site proteins that have well-studied functions in other crucial cellular processes, how does the localization of the protein to a contact site impact on the canonical activities of that protein and integrate those activities with contact site function?

How can we place the knowledge gained about the biology of mitochondria–organelle contacts into the larger context of the complete organelle–interaction network of the cell?

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