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Interacting Organelles

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Abstract

Eukaryotic cells are organized into membrane-bound organelles. These organelles communicate with one another through vesicular trafficking pathways and membrane contact sites (MCSs). MCSs are sites of close apposition between two or more organelles that play diverse roles in the exchange of metabolites, lipids and proteins. Organelle interactions at MCSs also are important for organelle division and biogenesis. For example, the division of several organelles, including mitochondria and endosomes, seem to be regulated by contacts with the endoplasmic reticulum (ER). Moreover, the biogenesis of autophagosomes and peroxisomes involves contributions from the ER and multiple other cellular compartments. Thus, organelle-organelle interactions allow cells to alter the shape and activities of their membrane-bound compartments, allowing them to cope with different developmental and environmental conditions.

Keywords

organelles; membrane contact sites; endoplasmic reticulum; Golgi; endosome; lysosome; peroxisome; lipid droplet; mitochondria; plasma membrane; light microscopy; electron microscopy

Introduction

A hallmark of eukaryotic cells is their organization into membrane-bound compartments (i.e., endoplasmic reticulum, Golgi, endosome, lysosome, peroxisome, lipid droplet and mitochondria). This allows for the spatial and temporal separation of incompatible biochemical processes. Nevertheless, cellular organelles must co-ordinate their activities for the cell to function properly as a biological system. An emerging theme in cell biology is that organelle-organelle membrane contact sites (MCSs) are hubs of metabolite exchange and organelle remodeling [1]. Ions, lipids, and proteins can all be transferred from one organelle to another at MCSs, where different organelles are at close apposition (Figure 1). In addition to the exchange of materials, MCSs play roles in organelle division. For

Disclosures

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example, division of both mitochondria and endosomes occurs at sites of close contact with endoplasmic reticulum (ER). Organelle interactions also play important roles during organelle biogenesis and growth. Indeed, organelles such as autophagosomes and peroxisomes derive membrane from multiple other organelles, including the ER and mitochondria. Here, we review several functions of organelle interactions at MCSs, and discuss emerging tools and techniques for studying these processes.

Organelle interactions via membrane contact sites

Different organelles make close contacts with each other via MCSs. These contact sites are generally thought to form via membrane proteins that act as tethers on the interacting organelles [2]. Often these tethers are part of large multiprotein complexes [2]. ER-organelle contact sites are among the best characterized MCSs [3,4]. The ER interacts with the plasma membrane, endosomes, lysosomes, mitochondria, peroxisomes, and lipid droplets (LDs; Figure 1) [3]. Many of these contacts involve the VAP family of proteins on the ER [2]. Organelle-organelle contacts occur between many other organelle pairs as well (Figure 1) [5]. For some of these MCSs, the protein machinery involved remains mysterious. Functionally, MCSs are involved in the exchange of Ca^{2+} , lipids, and proteins.

Ca^{2+} exchange occurs at many types of MCSs, including ER-plasma membrane, ER-mitochondria, and ER-endosome contacts [3]. Ca^{2+} exchange is best understood at ER-plasma membrane contact sites. The ER must maintain a high Ca^{2+} concentration for its signaling and biosynthetic activities, so its Ca^{2+} levels are approximately 10,000 times higher than that of the cytoplasm [6]. When ER Ca^{2+} stores are depleted such as during a cell signaling response, the ER membrane protein STIM1 senses this calcium depletion via its luminal domain, undergoes an allosteric transition, and is recruited to ER-plasma membrane contact sites by binding to PIP_2 [6]. STIM1 then recruits Orai1 to ER-plasma membrane junctions, triggering the opening of plasma membrane Ca^{2+} channels, and allowing Ca^{2+} to directly flow from the extracellular environment into the ER without raising cytoplasmic Ca^{2+} levels [6].

Advances have been made in understanding the mechanisms of ion transfer at ER-mitochondria contact sites as well. In yeast, a synthetic biology screen was used to identify the ER-mitochondria encounter structure (ERMES), a protein complex at ER-mitochondria MCSs implicated in Ca^{2+} signaling [7]. The ERMES complex also plays a role in maintaining iron homeostasis [8]. In mammalian cells, the voltage-dependent anion channel on the outer mitochondrial membrane is physically linked to the IP_3 receptor on the ER via the molecular chaperone glucose-regulated protein 75 [9]. In addition, contacts between VAPB on the ER and PTPIP51 on mitochondria have been implicated in Ca^{2+} homeostasis, with amyotrophic lateral sclerosis-associated mutations in VAPB causing increased Ca^{2+} uptake by mitochondria [10]. The mitofusin Mfn2 was also identified as an ER-mitochondria tether, and Mfn2 knockout cells showed decreased mitochondrial Ca^{2+} uptake in response to IP_3 -generated cytosolic Ca^{2+} transients [11]. However, the precise role of Mfn2 at ER-mitochondria MCSs has been somewhat controversial [12–15]. Finally, a recent paper has identified the protein called PDZD8 as a novel ER protein present at ER-mitochondria MCSs [16]. PDZD8 has an SMP domain that is functionally orthologous to

that of yeast Mmm1, which is part of the ERMES complex [16]. PDZD8 is necessary for the formation of ER-mitochondria MCSs in mammalian cells [16]. Moreover, when Ca^{2+} was released from the ER in cortical neurons in response to synaptic activity, PDZD8 knockdown cells showed impaired Ca^{2+} import into mitochondria [16]. In NIH3T3 cells, mitochondrial Ca^{2+} dynamics in PDZD8 knockout cells could be rescued by a synthetic ER-mitochondria tether, suggesting that the tethering function of PDZD8 is critical for ER-mitochondria Ca^{2+} transfer [16]. The relationship between these different tethering complexes at ER-mitochondria MCSs is currently not known.

Lipid transfer is another important function of MCSs. Lipid transfer has been proposed to occur at nearly every described MCS, although in some cases the type of lipid that is transferred and/or the direction of transfer remain unclear (Figure 1). For example, transfer of phospholipids including cardiolipin, phosphatidylserine (PS), and phosphatidylethanolamine (PE) occurs at ER-mitochondria MCSs [3]. In yeast, both the ERMES complex and the ER membrane protein complex (EMC) have been implicated in phospholipid transfer between ER and mitochondria [7,17]. Phospholipids also transfer from LDs to ER at MCSs in yeast under conditions where the ER undergoes expansion [18]. Fatty acid transfer occurs at both ER-LD and LD-mitochondria contacts [19]. Neutral lipid synthesis by FATP1 on the ER and DGAT2 on the LD contributes to LD biogenesis, effectively transferring fatty acids from the ER to LDs, where they are incorporated into triglycerides [20]. In contrast, when cells need to metabolize fatty acids by β -oxidation, fatty acids transfer from LDs to mitochondria at sites of close apposition [21]. Fatty acid transfer in the reverse direction, from mitochondria to LDs, is thought to protect mitochondria from lipotoxicity under certain conditions [22]. Thus, lipid exchange is likely bidirectional at both ER-LD and LD-mitochondria MCSs. It is currently unclear whether phospholipid transfer occurs at the same type of MCS as neutral lipid synthesis, or whether there are multiple types of ER-LD MCSs. Finally, lysosome-peroxisome contacts play a key role in cholesterol metabolism. When peroxisome genes are disrupted, cholesterol accumulates in lysosomes, suggesting that cholesterol internalized from lipoprotein particles must traffic to peroxisomes via MCSs before being routed to other cellular compartments [23].

Proteins can transfer at MCSs as well. So far, this has only been documented at ER-LD MCSs. LDs originate from the ER, which is a major site of lipid synthesis [24,25]. In mammalian cells, but not in yeast, most LDs seem to detach from the ER after biogenesis [25]. However, these detached LDs can form “membrane bridges” with the ER, which allow proteins such as GPAT4 to transfer from the ER to LDs [26]. The formation of these membrane bridges requires the Arf1/COPI machinery, which may remodel the LD surface to allow for the formation of membrane bridges [27]. Protein transfer from ER to LDs also involves the ER protein seipin. In seipin-deficient cells, protein and lipid transfer from the ER to LDs was unaffected during early stages of LD biogenesis, when LDs are attached to the ER, but was impaired at later stages of LD biogenesis, after LD budding from the ER [28]. Protein transfer may be a feature that is unique to LD-organelle interactions. This is because LDs, unlike other organelles, are surrounded by a phospholipid monolayer rather than a phospholipid bilayer. This may allow LDs to make “lipidic bridges” with other organelles in a way that is not possible between two phospholipid bilayers [29].

Organelle interactions in organelle division

In addition to providing an interface for the exchange of materials, MCSs are involved in organelle remodeling. The best-studied example of this is at ER-mitochondria contact sites, although the ER plays a role in endosome division as well [3,30]. Mitochondria are dynamic organelles that constantly undergo fusion and fission. Mitochondrial fusion is mediated by the mitofusins Mfn1 and Mfn2 on the outer mitochondrial membrane and Opa1 on the inner mitochondrial membrane, while mitochondrial fission is mediated by Drp1 and other members of the dynamin family of large GTPases [31,32]. Mitochondrial fission occurs at sites where the ER wraps around mitochondrial constrictions [33] (Figure 2). Inverted formin 2 on the ER and Spire1C on mitochondria interact at ER-mitochondria contact sites to nucleate actin assembly, which in turn plays a role in the recruitment of Drp1 to the outer mitochondrial membrane [34,35]. Actin polymerization at ER-mitochondria contact sites also stimulates a rise in mitochondrial matrix calcium, activating inner mitochondrial membrane constriction and linking the dynamics of the inner and outer mitochondrial membranes [36].

Recently an additional organelle was implicated in the division of mitochondria: the lysosome. Wong and colleagues showed that RAB7 on lysosomes promoted lysosome-mitochondria contacts, and that these contacts marked sites of mitochondrial division [37]. Recruitment of FIS1 to mitochondria during mitochondrial fusion, in turn, recruited the GTPase activating protein TBC1D15 to the MCS, resulting in RAB7 GTP hydrolysis and the release of the lysosome-mitochondria contact [37]. These mitochondrial fission events were also positive for ER tubules, suggesting that mitochondrial fission occurs at three-way organelle junctions between the mitochondria, ER, and lysosomes [37]. Interestingly, a near-simultaneous report has suggested that mechanical force can induce mitochondrial division via recruitment of the mitochondrial fission machinery [38]. The authors looked at force on mitochondria applied by encounter with a motile intracellular pathogen, *Shigella flexneri*. The result raises the intriguing possibility that the force of collision between a lysosome and mitochondrion may also play a role in mitochondrial division [38]. Whether lysosomes can influence the division of any other organelle besides ER and mitochondria remains to be seen.

Organelle interactions during organelle biogenesis

Organelle crosstalk also plays interesting roles during organelle biogenesis. Autophagosome and peroxisomes are examples of metabolic organelles having complex biogeneses. Autophagosomes are double membrane structures that engulf cytoplasm and organelles, delivering material to the lysosome to be catabolized and recycled. Autophagy is initiated in response to a variety of stresses, including starvation, ER stress, and infection [39]. The source of the membrane for autophagosome biogenesis is variable and depends on the type of stress experienced by the cell. Indeed, autophagosomes have been reported to originate from the mitochondria [40], ER [41], ER-mitochondria contact sites [42], plasma membrane [43], ER-Golgi intermediate compartment [44] or trans-Golgi network [45] in different cell types under different stressors. Presumably, these different sources for autophagosome biogenesis allow cells to effectively respond to the different autophagic triggering

conditions. During nutrient starvation, for example, the source of the initiating isolation membranes for autophagosomes has been proposed to be mitochondria-associated ER membranes [46]. Consistent with this idea, tightening ER-mitochondria MCSs by overexpressing VAPB or PTPIP51 impairs rapamycin and torin 1-induced, but not starvation-induced, autophagy [47]. This suggests that ER-mitochondria MCSs promote autophagy specifically in response to starvation. Under such nutrient stress conditions, PE synthesis in mitochondria via decarboxylation of PS becomes important due to reduced PE synthesis by other pathways. The PE available on mitochondria under these conditions has been proposed to help in the formation of autophagosomes (which require PE for lipidation of LC3); moreover, fusion of these autophagosomes with lysosomes would deliver the PE into the endo-lysosome system for circulation to other membranes [40].

Peroxisomes play roles in bile acid synthesis, as well as the metabolism of glyoxylate, dicarboxylate, amino acids and lipids [48]. Peroxisomes can multiply via division of existing peroxisomes, or can form *de novo* from the ER [49,50]. It has recently been shown that mitochondria-derived vesicles play a role in the biogenesis of peroxisomes [51]. Sugiura and colleagues showed that the peroxisomal protein PEX3 can traffic from mitochondria onto vesicles that are capable of importing some peroxisomal membrane proteins; these mitochondria-derived pre-peroxisomal vesicles must then fuse with ER-derived vesicles in order to form mature peroxisomes [51]. It has been proposed that this strategy could prevent the inappropriate mixing of peroxins in the organelles of peroxisome origin, thus averting the import of peroxisomal matrix proteins into either the mitochondria or ER [49].

The fact that both autophagosomes and peroxisomes derive membrane and proteins from multiple sources suggests that this may be a common theme in organelle biogenesis. Mitochondria-derived vesicles can fuse with lysosomes, although whether this contributes to lysosome biogenesis is unknown [52]. LDs are another candidate organelle that could derive components from multiple sources. Currently, LDs are thought to originate primarily from the ER. However, the lipid synthesis enzyme DGAT2, which localizes to both the ER and mitochondria, can catalyze triacylglycerol synthesis and promote LD formation independent of its localization to the ER [53]. Thus, it is possible that the lipids comprising LDs could originate from multiple organelles, including mitochondria, as well.

Techniques for studying organelle interactions

Our understanding of MCSs has been shaped by the tools used to study them. Many biochemical techniques have been employed to study organelle contacts in fine detail and to identify molecular components involved in these contacts including membrane co-fractionation [54,55], co-immunoprecipitation [56–58] and more recently the use of proximity biotinylation with subsequent mass spectrometry [59]. In this technology section, we review imaging approaches to study organelle contacts with emphasis on recently developed techniques.

Intracellular membrane contacts were first observed using electron microscopy (EM) as regions of close contact between ER and plasma membrane [60] and between ER and mitochondria [61]. Recently, cryo-EM, which affords molecular-scale resolution of cellular

structures, has been combined with electron tomography (cryo-ET) to visualize the 3-dimensional ultrastructure of ER-plasma membrane contacts [62]. Focused Ion Beam-Scanning EM (FIB-SEM) uses a focused ion beam to collect an image while simultaneously milling the specimen surface [63] and has recently been used to systematically visualize ER contacts with multiple organelles including mitochondria, peroxisomes and multivesicular bodies, as well as plasma membrane in neurons [64] (Figure 3). The major strength of cryo-EM and FIB-SEM is that these techniques allow imaging of subcellular compartments with exquisite spatial resolution, i.e., on the order of nanometers or angstroms, while their greatest weakness is that the imaging conditions necessitate the cells being fixed.

The use of genetically-encoded fluorescent fusion proteins allows dynamic imaging of live cells and organelle contacts with molecular specificity. However, the use of more than a few fluorescent proteins in any single specimen is hampered by the inability to distinguish multiple fluorophores with overlapping emission spectra using filters. A recent study employed multispectral imaging and computational analysis to quantify the systems level dynamics of organelle contacts simultaneously among six different organelles: lysosomes, ER, mitochondria, Golgi, peroxisomes and LDs [5] (Figure 4). With this approach, it was found that individual organelles are highly dynamic and heterogeneous in their inter-organelle contacts. Summing all pair-wise organelle-organelle contacts, however, revealed that the number of inter-organelle contacts was remarkably stable over time in the absence of perturbation, leading to its description as the ‘organelle interactome’. The major strength of fluorescence imaging is that it allows observations of live cells and their dynamics. One weakness of imaging in general, whether it be light microscopy or EM, is that these approaches are best considered tools for hypothesis generation regarding organelle contacts because the observation of colocalization or close apposition of two organelles alone is not evidence of functional contact.

Förster Resonance Energy Transfer (FRET) is a molecular imaging technique in which a donor fluorophore fused to one protein transfers energy to an acceptor fluorophore, which then fluoresces if and only if the donor and acceptor fluorophores are within 10 nm or less of each other. Recently both FRET and Structured Illumination Microscopy (SIM) have been used to measure and visualize mitochondria-lysosome contacts in live cells with increased spatial resolution [37]. Bi-molecular fluorescence complementation (BiFc) is a fluorescent protein biosensor technique where two halves of a fluorescent protein (e.g. YFP) are fused to two different proteins [65,66]. Upon excitation the BiFc construct only fluoresces when the two halves of the fluorophore are fused. BiFc has recently been used to detect contacts between ER and mitochondria in yeast [67] and mammalian cells [68]. To rule out the possibility that BiFc may cause constitutive localization of inter-organelle contacts, Jing and colleagues developed light-inducible membrane-tethered peripheral ER (LiMETER), a fusion protein biosensor that contains the signal peptide and a transmembrane domain from STIM1 fused to GFP and a cytoplasmic flexible linker with the Light Oxygen Voltage-sensing (LOV2) domain derived from *Avena sativa* photorophin 1 [69]. The authors demonstrated that LiMETER underwent photo-inducible accumulation at ER-plasma membrane junctions and allowed specific labeling of these contacts. The recent further development of multiple optogenetic systems for detecting protein interactions provides a

molecular toolbox for systematic observations of dynamic organelle contacts in their native states with minimal perturbation [70].

Conclusions and future perspectives

Recent studies suggest that different organelles interact through MCSs in myriad ways, profoundly impacting cell function. One key cell function impacted by MCSs is organelle biogenesis and division. Organelle-organelle contacts not only drive division of specific organelles (i.e., ER-mitochondria contacts for mitochondria division and ER-endosome contacts for endosomal division), but could ensure unidirectional protein targeting to a newly-forming organelle. This may be important during organelle biogenesis to prevent mistargeting of proteins to the membranes where the proteins or lipids first originate [49]. Given that MCSs are interfaces for the exchange of ions, lipids, and proteins, other possible functions of MCSs could be to serve as platforms for cytoplasmic reactions (such as actin polymerization) or as assembly sites for protein complexes involved in biosynthesis and cell signaling. In the latter case, adjustments in the number, size, or duration of membrane contacts could provide another way to regulate protein complex components on separate organelles, potentially promoting or inhibiting their formation and function. As the tools for studying MCSs improve, we can look forward to many additional discoveries regarding the mechanisms underlying and functions of organelle-organelle contacts.

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Figure 1: Functions of organelle contacts.

Shown are contacts between organelles where at least one protein involved in mediating the contact has been identified. ER-lysosome and ER-endosome contacts play roles in lipid exchange, Ca^{2+} dynamics, and organelle fission [3]. Lysosomes also play a role in mitochondrial fission, at lysosome-mitochondria-ER contact sites [37]. ER-plasma membrane (PM) contact sites are involved in lipid and Ca^{2+} exchange [3,4]. All the other shown organelle contacts have proposed lipid exchange functions [3,23,29,71]. Finally, ER-LD contacts also allow proteins to transfer between these organelles [26,28].

Figure 2: Mitochondrial constriction at ER-mitochondria contact sites.

An example of organelle interactions regulating organelle division. Spire1C:actin complexes on mitochondria associate with inverted formin 2 (INF2) on the ER. Actin filaments nucleated by Spire1C are elongated by the actin polymerization activity of INF2. The actin filament elongation activity exerts pressure on the mitochondrial outer membrane, thereby driving constriction of the latter. Tethering complexes may play a role in maintaining association between ER and mitochondrial membranes. Modified with permission from [35].

ER PM Mitochondria Endosomes/Transport Vesicles Synaptic Vesicles ER Membrane Contact Sites

Figure 3: FIB-SEM showing ER-organelle contacts in a neuron.

3-dimensional model of subcellular organelles from a FIB-SEM image stack of a dendritic segment within a neuron of the nucleus accumbens brain region. Membrane areas in contact with the ER are shown in dark red. Scale bar, 1.6 μm . Figure courtesy of Pietro de Camilli.

Figure 4: Organelle organization and contacts revealed by multispectral lattice light sheet microscopy.

(a) Maximum intensity projection of a COS-7 cell expressing fluorescent proteins targeted to peroxisomes, the mitochondria, the ER, and the Golgi, and labelled with dyes to mark lysosomes and LDs. (b) Examples of complex interorganelle contacts and organization in segmented lattice light sheet images. The ER is excluded for clarity. (c) Dynamics of mitochondria-organelle interactions in segmented lattice light sheet images. Mitochondria are shown in grey, while contacts between mitochondria and other organelles are colored according to the legend. Numbers represent time in seconds. (b) is modified with permission from [5].