A Journey Through an Extremely Narrow Space: A Simple Guide to Examine Late Endosomal–Mitochondrial Membrane Contact Sites

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ABSTRACT

The question of how small molecules such as cholesterol move between organelles through membrane contact sites is still unclear. Nara et al. recently published a paper in *Experimental Cell Research* on the successful three-dimensional observation of a space between two organelles measuring approximately 50 nm. Electron microscopy can provide new insights into the organelle-mediated communication for the production of steroid hormones, contributing to research on fetal development.

Keywords: membrane contact site, organelle, MLN64, EM, electron tomography

ABBREVIATIONS

MCSs: membrane contact sites; EM: electron microscopy; ER: endoplasmic reticulum; TEM: transmission electron microscopy; VPS: vacuolar protein sorting; MLN64: metastatic lymph node 64; HBSS: Hanks' balanced salt solution; 3D: three dimensional; Lamp1: lysosomal-associated membrane protein 1; VAP: VAMP-associated proteins; MSP: major sperm protein; FFAT: two phenylalanines (FF) in an acidic tract (AT); KDEL: lysine, aspartic acid, glutamic acid, and leucine.

COMMENTARY

In eukaryotic cells, intracellular membrane-bound compartments can communicate with each other to maintain their functional identity and cell activity. To keep its functions, each compartment contains specific components, such as proteins and lipids, so that these can be received in compartment-specific ways. While the mechanism of delivering specific proteins to compartments (a protein sorting) is well-known as vesicular trafficking, little is known about how lipids and ions are accurately sorted into appropriate compartments. Unfortunately, unlike proteins, which have a signal sequence that assigns them to appropriate organelles, lipids and ions do not have a signature or sequence to deliver them to their destination. Recently, membrane contact sites (MCSs) have been identified as one of the tools that allow communication between organelles and have been located where two organelles meet, in very close proximity (1, 2). MCSs are considered a functional spot for the rapid and efficient transport of water-insoluble molecules, such as lipids and Ca^{2+} ions. It has also been reported that MCSs can play an important role in the modification of organelle morphology (3), attracting significant attention from the scientific community.

Indeed, numerous organelles have been found to be in proximity to other organelles inside the cell. The endoplasmic reticulum (ER), which has a central role in biosynthesizing and transporting lipids and in signaling, forms MCSs in various organelles allowing them to communicate with each other (4). The ultrastructure of MCSs between the ER and the plasma membrane has been extensively analyzed (5, 6); however, the detailed morphologies of MCSs remain to be clarified. To conduct TEM analyses, ultrathin sections of the embedded sample need to be prepared, which often makes it difficult to identify the organelles present on each section. Depending on the cut, ERs and endosomes

are often observed as vesicles, and labeling with specific antibodies is crucial. Importantly, in our experiments, we used immuno-EM to identify late endosomes and the ER. Lamp1-labeled late endosomes and mitochondria were approximately 150 nm touching, which was half the length of the interface between the KDEL-labeled ER and mitochondria (7). Our study is of great value because it reveals details of the proximity of structures between organelles labeled using immunoelectron microscopy.

Previous EM studies of MCSs have suggested that in many cases the facing organelle membranes are not attached to each other but are connected by a thin rod-like structure called tether, which keeps them at a certain distance and confers them stability. Each MCS tether has been shown to have a specific length (8), and our study demonstrated that late endosome–mitochondria and ER–mitochondria tethers are < 20 nm in length while the ER–late endosome tether is approximately 10 nm long (7). However, as numerous molecules contribute to the ER–late endosome contacts, it is unknown whether these molecules have a specific length. In ER–lysosome contacts, these distances depend on their tether molecules (9). In the present study, immunoelectron 3D tomography revealed the presence of numerous tethers in the MCSs. These tethers were approximately 19 nm long. Tethers contribute to stabilize the conformation of two closely located organelle membranes. Moreover, some tethers are postulated to have multiple functions, such as using their own lipid-binding domain to transfer lipids between MCSs (10). Future research should focus on whether EM can reveal unique material exchange and transfer in the vicinity of MCSs, which could be referred to as a "cultural exchange" between organelles. To this end, it may be necessary to capture images of the accumulation of transport molecules such as cholesterol in the MCSs.

To date, there is still little information on the tether components that support various inter-organelle MCSs, although the nature of these components is of great interest. Our study supports the hypothesis that the MLN64 protein might function as a tether that connects late endosomes and mitochondria. A previous study has shown that, in MLN64 knockdown cells, tethers between the ER and mitochondria were consistently observed but not between late endosomes and mitochondria, suggesting that MLN64 is specialized in tethering functions (7). Our analysis of whether MLN64 is a tether component has not been able to confirm this. The FFAT motif of MLN64 can bind the VAP ER protein to a cytoplasmic side of the MSP domain (11). However, there are several known interactions between late endosomal proteins and ER proteins (10), and there is still no direct evidence that MLN64 is the tether between late endosomes and the ER. Partial deletion of Vps13C was shown to shorten the distance between the ER and lysosomes, decreasing the length of the tether by 5 nm compared to the full length (9). In the near future, tethering components will be identified and this may lead to a better understanding of the function of the MCSs in lipid transfer.

One of the findings of our study was that the formation of the endosome–mitochondria MCSs is altered in the presence or absence of lipids. A previous study has shown that the median distance between endosomes and mitochondria under lipid-depleted conditions was close to that during MLN64 knockdown (7). The total number and perimeter of ER–mitochondria MCSs was shown to increase under HBSS starvation conditions (12). These results suggested that MCSs adjust their structures under different conditions. Therefore, it is of particular interest to investigate how MCSs are formed and maintained. The ultrastructural analysis of MCSs by EM plays an important role in elucidating the mechanism of these structures from a morphological point of view.

Furthermore, it should be emphasized that placental cells were used in this study. This type of cells is specialized to secrete large amounts of progesterone, which helps to maintain pregnancy. MLN64 is assumed to be responsible for transporting cholesterol, which represents the basis for progesterone production, to the mitochondria, i.e., the centers of production (13). Our experiments suggested that the MCSs associated with MLN64 may act as a bridge for the transport of cholesterol to the

mitochondria. Moreover, the late endosomal–mitochondrial MCSs may represent a specialized membrane proximity structure found in placental cells, and it is not yet known whether cholesterol passes through this structure. It should be emphasized that the use of placental cells is crucial to understand the physiological significance of endosomal–mitochondrial MCSs.

CONCLUSION

EM analysis elucidated the ultrastructure of MCSs providing insights into the mechanism of small molecule transport between organelles. Future studies may reveal that MCSs also play a role in steroid hormone secretion, contributing to the maintenance of pregnancy.

CONFLICTS OF INTEREST

The author declares no competing interests.

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