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A Third Musketeer on the ER: MOSPD2 is a Novel VAP-related Receptor for FFAT Motifs

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Abstract

Interorganelle membrane contact sites are subcellular structures that favor exchange and communication inside the cell. Such microdomains are built by molecular bridges that create a physical connection between two distinct organelles. The field of contact sites is now flourishing with discoveries of new tethering molecules. In that context, we identified by an unbiased proteomic approach a novel scaffold protein named MOtile SPERM Domain-containing protein 2 (MOSPD2). MOSPD2 is an endoplasmic reticulum (ER)-resident protein that is able to interact with several organelle-bound proteins that possess a small motif, named FFAT (two phenylalanines in an acidic tract). Consequently, we showed that MOSPD2 and its protein partners build contacts between the ER and endosomes, mitochondria, or Golgi. These findings highlight a new way for docking organelles on the ER.

Keywords

endoplasmic reticulum (ER), ER-organelle contact, membrane contact site, VAP proteins, FFAT motif

Eukaryotic cells are compartmentalized in distinct organelles associated with specialized functions. Interestingly, organelles do not float independently in the cytoplasm but rather interact with each other to form an interdependent network. Interorganelle membrane contact sites (MCSs) are now recognized as platforms involved in the exchange of material, organelle fission and positioning, and communication. MCSs are specific subcellular regions where heterologous membranes from two distinct organelles are in close apposition (Levine, 2004). In this network, the endoplasmic reticulum (ER)—composed of tubes and perinuclear sheets—distinguishes itself by spreading throughout the entire cytoplasm and making multiple contacts with most organelles. The ER plays a central role in interorganelle cooperation (Gatta & Levine, 2017; Phillips & Voeltz, 2015).

At the molecular level, the formation of MCSs requires protein–protein or protein–membrane interactions at the interface between the organelles involved (Eisenberg-Bord, Shai, Schuldiner, & Bohnert, 2016; Helle et al., 2013). Identifying complexes contributing

to organelle tethering is essential to understand the function of these microdomains. Of note, vesicle-associated membrane protein-associated proteins (VAP-A and B in humans) were identified as major tethering components in molecular bridges between the ER and other organelles (Loewen and Levine, 2003; Murphy & Levine, 2016). VAPs are ER-anchored proteins containing a major sperm protein (MSP) domain exposed to the cytoplasm. This domain acts as a molecular hook by interacting with small linear motifs named FFAT (two phenylalanines in an acidic tract) that are present in a wide variety of proteins either cytoplasmic or associated

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with organelles such as mitochondria, endosomes, Golgi, peroxisomes, and the plasma membrane (Loewen and Levine, 2003; Mikitova & Levine, 2012). Diverse biological functions have recently been associated with VAP-containing MCSs, such as calcium homeostasis, lipid transport, endosome positioning, and autophagy (De Vos et al., 2012; Dong et al., 2016; Gomez-Suaga et al., 2017; Hamasaki et al., 2013; Kawano, Kumagai, Nishijima, & Hanada, 2006; Nascimbeni et al., 2017; Rocha et al., 2009; Vihervaara et al., 2011; Wijdeven et al., 2016; Wilhelm et al., 2017).

Noteworthy, despite the central position of VAP proteins in ER–organelle contacts, their knockdown does not affect cell viability, and contacts are still observed between the ER and other organelles, meaning that VAPs are not the exclusive players for ER contact sites (Dong et al., 2016; Eden et al., 2016; Stoica et al., 2014). These observations support the notion that other contacts exist independently from VAP proteins. Several other tethering complexes, such as STIM1-ORAI1 and VDAC-GRP75-IP₃R, which mediate ER–plasma membrane and ER–mitochondria contacts, respectively, support this idea (Liou et al., 2005; Szabadkai et al., 2006). Alternatively, there may be a functional redundancy concerning VAPs, implicating the presence of compensatory mechanisms that are able to connect the ER with organelles harboring FFAT-proteins on their surface. As no surrogate for VAPs had yet been described, we explored this idea. We reasoned that proteins other than VAPs might bind FFAT motif-containing partners. Therefore, we set up an FFAT-pull down assay using a consensus FFAT motif (from oxysterol-binding protein-related protein 1 [ORP1L]) as bait to selectively purify FFAT-bound proteins. The FFAT peptide and a control peptide were immobilized on a solid support and further mixed with HeLa cell protein extracts. Specific FFAT-bound proteins were identified by tandem mass spectrometry. Besides VAP-A and VAP-B, the MOtile SPerm Domain-containing protein 2 (MOSPD2) protein was identified as a new FFAT motif-interacting protein (Di Mattia et al., 2018).

By its structure, MOSPD2 belongs to the VAP protein family, as it contains an MSP domain located in the middle of the protein. In human, six distinct genes encode MSP-containing proteins, namely, VAP-A, VAP-B, MOSPD1, MOSPD2, MOSPD3, and CFAP65 (Cilia- and flagella-associated protein 65 also known as coiled-coil domain-containing protein 108; CCDC108). Interestingly, the primary sequence analysis of the MSP domain led Loewen and Levine (2005) to predict in 2005 that MOSPD2 would likely bind the FFAT motif, similar to VAP-A and VAP-B, while the other members of this family would not. Moreover, three-dimensional-structure analysis confirmed that, despite sharing limited sequence homology, critical residues for FFAT binding

are conserved in the MSP domains of MOSPD2 and VAPs, supporting the notion that they share the same mode of FFAT motif recognition. This idea was supported by in vitro structure–function experiments. Eventually, we showed that the MSP domain of MOSPD2 is a bona fide FFAT-interacting domain sharing similar in vitro binding properties as VAP protein MSP domains (Di Mattia et al., 2018).

Next, we addressed whether VAPs and MOSPD2 shared the same partners in vivo. To this aim, we performed coimmunoprecipitation studies between MOSPD2 and five well-characterized organelle-bound FFAT-containing proteins from endosomes (STARD3 [StAR-related lipid transfer domain (START) protein 3], STARD3NL [STARD3 N-terminal-like protein], and ORP1L), mitochondria (PTPIP51 [protein tyrosine phosphatase-interacting protein-51]), and the Golgi apparatus (STARD11 [StAR-related lipid transfer domain protein 11]) which were coexpressed in HeLa cells. We showed that MOSPD2 interacts with the FFAT motifs present in these four proteins thanks to its MSP domain.

Like VAPs, MOSPD2 is anchored at the ER surface by a carboxyl-terminal transmembrane domain. We observed that MOSPD2 was enriched in ER domains involved in MCSs generated by FFAT motif-containing proteins. More precisely, the forced expression of any partner tested, including the endosomal (STARD3, STARD3NL, and ORP1L), mitochondrial (PTPIP51), and Golgi (STARD11) proteins, caused a predominant recruitment of MOSPD2 around endosomes, mitochondria, and Golgi, respectively.

This study showed that MOSPD2 is a novel VAP homologue anchored in the ER. MOSPD2 displays structural and functional features shared with VAP proteins, notably: an MSP domain that is able to recognize FFAT motifs and bind FFAT-containing proteins, an anchor on the ER by a C-terminal transmembrane domain, and the ability to build MCSs (Figure 1). In addition, at the resolution scale of light microscopy (~200 nm), we observed that both VAPs and MOSPD2 are localized in same subdomains of the ER engaged in a contact with a distinct organelle. We can speculate that VAP-A, VAP-B, and MOSPD2 act as ER receptors for FFAT-containing proteins and generate a binding platform for organelle docking. However, the mode of formation and the regulation of this kind of platform remain unknown.

Finally, the physiological function of MOSPD2 on interorganelle contact formation was investigated at the ultrastructural level using a loss-of-function approach. Interestingly, we observed a significant reduction in ER–endosome contacts, in MOSPD2, and in MOSPD2- and VAP-A- and VAP-B-deficient cells but not in cells deficient for VAP-A and VAP-B only.

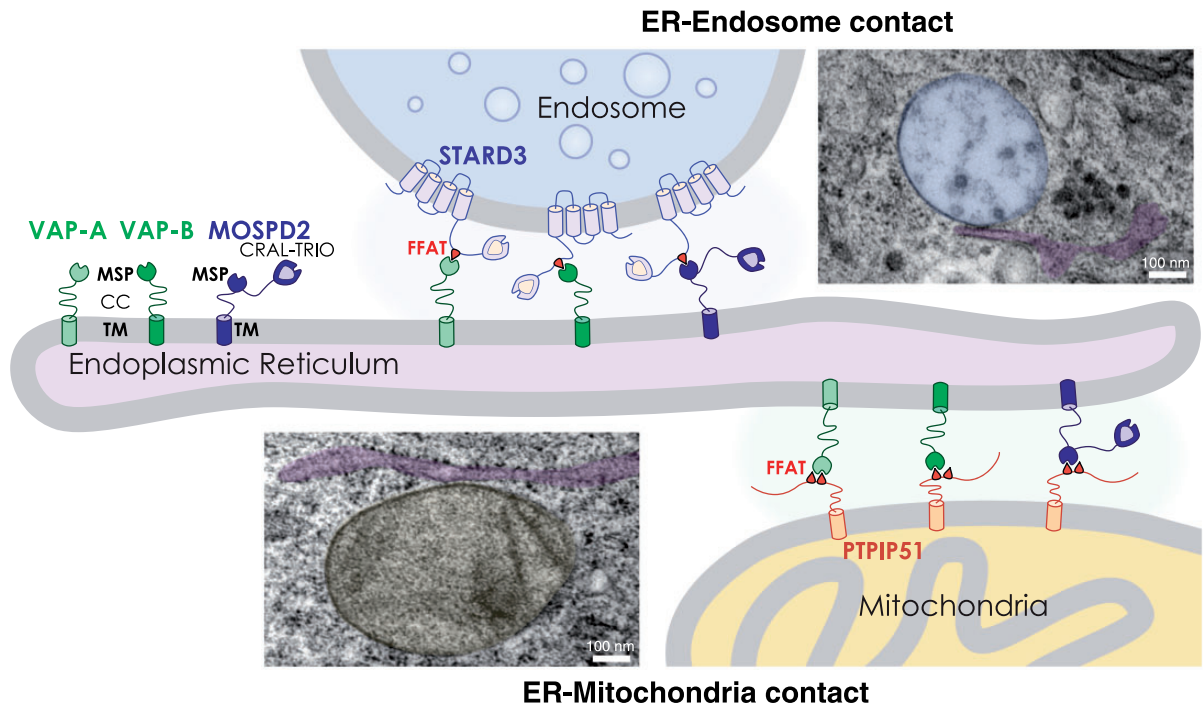


Figure 1. MOSPD2 builds ER-organelle contact sites, a shared function with VAP proteins. MOSPD2 is an ER-resident protein able to bind organelle-bound FFAT-containing proteins by its MSP domain. As illustrated here, similarly to VAP-A and VAP-B proteins, MOSPD2 builds ER–endosome contacts by interacting with STARD3; besides, MOSPD2 can build ER–mitochondria contacts, as it can interact with mitochondria PTPIP51. Electron micrographs illustrate contacts existing between the ER (purple) and endosomes (top; blue), or mitochondria (bottom; yellow). TM = transmembrane domain; CC = coiled-coil domain; MSP = major sperm protein domain; CRAL-TRIO = cellular retinaldehyde-binding protein and triple functional domain protein domain; ER = endoplasmic reticulum; VAP = vehicle-associated membrane protein-associated proteins; MOSPD2 = MOtile SPERM Domain-containing protein 2; STARD3 = StAR-related lipid-transfer domain protein 3; FFAT = two phenylalanines in an acidic tract; PTPIP51 = protein tyrosine phosphatase-interacting protein-51.

Concomitantly, we observed an increase in endosome–endosome contacts upon MOSPD2 depletion. This phenotype might be linked to the previously established role of ER–endosome contacts on the dynamics and maturation of the endosomal system (Rowland, Chitwood, Phillips, & Voeltz, 2014). These results support the notion that, despite having similar tethering functions, the physiological roles of VAPs and MOSPD2 on inter-organelle contacts are either distinct or partially redundant. Further work is necessary to clarify this issue. However, at the structural level, MOSPD2 distinguishes itself from VAP proteins by the presence of an additional protein domain located at the amino-terminal end, named CRAL-TRIO (cellular retinaldehyde-binding protein and triple functional domain protein). Moreover, in terms of evolution, MOSPD2 appeared later than the VAP proteins; VAPs are found in animals, fungi, and plants, while MOSPD2 is only present in animals. The CRAL-TRIO domain is present in many proteins implicated in lipid transport and in several kingdoms including fungi. The most described CRAL-TRIO-containing protein is the *Saccharomyces cerevisiae* Sec14p protein. This protein is able to counterexchange phosphatidylinositol (PI) and phosphatidylcholine

between membranes (Sha, Phillips, Bankaitis, & Luo, 1998). Thus, the presence of this domain potentially involved in lipid transport distinguishes MOSPD2 from VAPs as a unique tether at the ER surface. Moreover, MOSPD2 can be in complex with an FFAT-motif-containing protein partner having its own lipid-transfer domain, such as some members of the oxysterol-binding protein (ORP) and START families. It implies that a single-protein complex could integrate two distinct lipid-transfer activities. We foresee that by combining a variety of partners, MOSPD2-containing complexes might counterexchange two, three, or even more lipid species at ER-organelle contacts. In the case of ORPs, the ability of the CRAL-TRIO domain of MOSPD2 to transfer PI would be particularly beneficial. Indeed, ORPs were shown to counterexchange phosphatidylinositol-4-phosphate (PI4P) and their specific ligand (sterol for the Oxysterol binding protein [OSBP], for instance; Moser von Filseck, Vanni, Mesmin, Antony, & Drin, 2015). PI4P is synthesized from PI in one membrane and degraded into PI in the other membrane; this cycle fuels energy to allow the transport of the ORP-specific ligand against its concentration gradient. Interestingly, MOSPD2 could recycle

PI and thus close the cycle of PI or PI4P transport involved in the functioning of ORPs. Our further studies will clarify the implication of the CRAL-TRIO domain in contact sites formation and the physiological role of MOSPD2.

The discovery of MOSPD2 as a new ER-resident protein able to recognize FFAT motifs gives another dimension to VAP-related investigations. As MOSPD2 binds established VAP partners, it changes the molecular landscape of previously studied VAP-associated contact sites. However, how the balance between MCS formation involving VAP or MOSPD2 is regulated remains unclear. The fact that the MSP domain structure and the affinity for the FFAT motif are similar in VAP and MOSPD2 argues against specificities for a given partner. In contrast, their differential level of expression might provide some specificity. Indeed, the quantification of VAP proteins and MOSPD2 in HeLa cells showed that VAP-A and VAP-B are more abundant than MOSPD2 by 200 and 7 folds, respectively. It is likely that the ratio between these related proteins is cell- and context-specific. Their respective abundance is probably key to govern their specific function in a given cell or state. Further work is needed to fully apprehend the molecular organization of interorganelle contacts scaffolded by VAP-A, VAP-B, or MOSPD2 and their numerous functions in the cell physiology.

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References

- De Vos, K. J., Morotz, G. M., Stoica, R., Tudor, E. L., Lau, K.-F., Ackerley, S.,... Miller, C. C. J. (2012). VAPB interacts with the mitochondrial protein PTPIP51 to regulate calcium homeostasis. *Human Molecular Genetics*, *21*, 1299–1311.
- Di Mattia, T., Wilhelm, L. P., Ikhlef, S., Wendling, C., Spohner, D., Nominé, Y.,... Alpy, F. (2018). Identification of MOSPD2, a novel scaffold for endoplasmic reticulum membrane contact sites. *EMBO Reports*, *19*, e45453.
- Dong, R., Saheki, Y., Swarup, S., Lucast, L., Harper, J.W., & De Camilli, P. (2016). Endosome-ER contacts control actin nucleation and retromer function through VAP-dependent regulation of PI4P. *Cell*, *166*, 408–423.
- Eden, E. R., Sanchez-Heras, E., Tsapara, A., Sobota, A., Levine, T. P., & Futter, C.E. (2016). Annexin A1 tethers membrane contact sites that mediate ER to endosome cholesterol transport. *Developmental Cell*, *37*, 473–483.
- Eisenberg-Bord, M., Shai, N., Schuldiner, M., & Bohnert, M. (2016). A tether is a tether is a tether: Tethering at membrane contact sites. *Developmental Cell*, *39*, 395–409.
- Gatta, A. T., & Levine, T. P. (2017). Piecing together the patchwork of contact sites. *Trends in Cell Biology*, *27*, 214–229.
- Gomez-Suaga, P., Paillusson, S., Stoica, R., Noble, W., Hanger, D. P., & Miller, C. C. J. (2017). The ER-mitochondria tethering complex VAPB-PTPIP51 regulates autophagy. *Current Biology*, *27*, 371–385.
- Hamasaki, M., Furuta, N., Matsuda, A., Nezu, A., Yamamoto, A., Fujita, N.,... Amano, A. (2013). Autophagosomes form at ER-mitochondria contact sites. *Nature*, *495*, 389–393.
- Helle, S. C. J., Kanfer, G., Kolar, K., Lang, A., Michel, A. H., & Kornmann, B. (2013). Organization and function of membrane contact sites. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, *1833*, 2526–2541.
- Kawano, M., Kumagai, K., Nishijima, M., & Hanada, K. (2006). Efficient trafficking of ceramide from the endoplasmic reticulum to the Golgi apparatus requires a VAMP-associated protein-interacting FFAT motif of CERT. *Journal of Biological Chemistry*, *281*, 30279–30288.
- Levine, T. (2004). Short-range intracellular trafficking of small molecules across endoplasmic reticulum junctions. *Trends in Cell Biology*, *14*, 483–490.
- Liou, J., Kim, M. L., Do Heo, W., Jones, J. T., Myers, J. W., Ferrell, J. E., & Meyer, T. (2005). STIM is a Ca²⁺ sensor essential for Ca²⁺-store-depletion-triggered Ca²⁺ influx. *Current Biology*, *15*, 1235–1241.
- Loewen, C. J. R., & Levine, T. P. (2005). A highly conserved binding site in vesicle-associated membrane protein-associated protein (VAP) for the FFAT motif of lipid-

- binding proteins. *Journal of Biological Chemistry*, 280, 14097–14104.
- Loewen, C. J. R., Roy, A., & Levine, T. P. (2003). A conserved ER targeting motif in three families of lipid binding proteins and in Opi1p binds VAP. *The EMBO Journal*, 22, 2025–2035.
- Mikitova, V., & Levine, T. P. (2012). Analysis of the key elements of FFAT-like motifs identifies new proteins that potentially bind VAP on the ER, including two AKAPs and FAPP2. *PLoS One*, 7, e30455.
- Moser von Filseck, J., Vanni, S., Mesmin, B., Antonny, B., & Drin, G. (2015). A phosphatidylinositol-4-phosphate powered exchange mechanism to create a lipid gradient between membranes. *Nature Communications*, 6, 6671.
- Murphy, S. E., & Levine, T. P. (2016). VAP, a versatile access point for the endoplasmic reticulum: Review and analysis of FFAT-like motifs in the VAPome. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, 1861, 952–961.
- Nascimbeni, A. C., Giordano, F., Dupont, N., Grasso, D., Vaccaro, M. I., Codogno, P., & Morel, E. (2017). ER-plasma membrane contact sites contribute to autophagosome biogenesis by regulation of local PI3P synthesis. *The EMBO Journal*, 36, e201797006.
- Phillips, M. J., & Voeltz, G. K. (2015). Structure and function of ER membrane contact sites with other organelles. *Nature Reviews Molecular Cell Biology*, 17, 69–82.
- Rocha, N., Kuijl, C., van der Kant, R., Janssen, L., Houben, D., Janssen, H.,... Neefjes, J. (2009). Cholesterol sensor ORP1L contacts the ER protein VAP to control Rab7–RILP–p150Glued and late endosome positioning. *The Journal of Cell Biology*, 185, 1209–1225.
- Rowland, A. A., Chitwood, P. J., Phillips, M. J., & Voeltz, G. K. (2014). ER contact sites define the position and timing of endosome fission. *Cell*, 159, 1027–1041.
- Sha, B., Phillips, S. E., Bankaitis, V. A., & Luo, M. (1998). Crystal structure of the *Saccharomyces cerevisiae* phosphatidylinositol-transfer protein. *Nature*, 391, 506.
- Stoica, R., De Vos, K. J., Paillusson, S., Mueller, S., Sancho, R. M., Lau, K.-F.,... Dickson, D. W. (2014). ER–mitochondria associations are regulated by the VAPB–PTPIP51 interaction and are disrupted by ALS/FTD-associated TDP-43. *Nature Communications*, 5, 3996.
- Szabadkai, G., Bianchi, K., Várnai, P., De Stefani, D., Wieckowski, M. R., Cavagna, D.,... Rizzuto, R. (2006). Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca²⁺ channels. *The Journal of Cell Biology*, 175, 901–911.
- Vihervaara, T., Uronen, R.-L., Wohlfahrt, G., Björkhem, I., Ikonen, E., & Olkkonen, V. M. (2011). Sterol binding by OSBP-related protein 1L regulates late endosome motility and function. *Cellular and Molecular Life Sciences*, 68, 537–551.
- Wijdeven, R. H., Janssen, H., Nahidiazar, L., Janssen, L., Jalink, K., Berlin, I., & Neefjes, J. (2016). Cholesterol and ORP1L-mediated ER contact sites control autophagosome transport and fusion with the endocytic pathway. *Nature Communications*, 7, 11808.
- Wilhelm, L. P., Wendling, C., Védie, B., Kobayashi, T., Chenard, M., Tomasetto, C.,... Alpy, F. (2017). STARD3 mediates endoplasmic reticulum-to-endosome cholesterol transport at membrane contact sites. *The EMBO Journal*, 36, 1412–1433.