ER-driven membrane contact sites: Evolutionary conserved machineries for stress response and autophagy regulation?

Diana Molino\textsuperscript{a,b}, Anna Chiara Nascimbeni\textsuperscript{a,b}, Francesca Giordano\textsuperscript{c}, Patrice Codogno\textsuperscript{a,b} and Etienne Morel\textsuperscript{e,ab}

\textsuperscript{a}Institut Necker-Enfants Malades (INEM), INSERM U1151-CNRS UMR; \textsuperscript{b}Universit\'e Paris Descartes-Sorbonne Paris Cit\'e, Paris, France; \textsuperscript{c}Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris-Sud, Universit\'e Paris-Saclay, Gif-sur-Yvette, France

ABSTRACT
Endoplasmic Reticulum (ER), spreading in the whole cell cytoplasm, is a central player in eukaryotic cell homeostasis, from plants to mammals. Beside crucial functions, such as membrane lipids and proteins synthesis and outward transport, the ER is able to connect to virtually every endomembrane compartment by specific tethering molecular machineries, which enables the establishment of membrane-membrane contact sites. ER-mitochondria contact sites have been shown to be involved in autophagosome biogenesis, the main organelle of the autophagy degradation pathway. More recently we demonstrated that also ER-plasma membrane contact sites are sites for autophagosomes assembly, suggesting that more generally ER-organelles contacts are involved in autophagy and organelle biogenesis. Here we aim to discuss the functioning of ER-driven contact sites in mammals and plants and more in particular emphasize on their recently highlighted function in autophagy to finally conclude on some key questions that may be useful for further research in the field.

ER membrane dynamics and associated contact sites in autophagy

Intracellular communication and exchanges between organelles has long been thought to depend on vesicular transport and diffusion of molecules in the cytosol. However growing evidence push membrane contact sites in the front of stage of organelle dialogs and signaling regulation. The Endoplasmic Reticulum (ER), which is the most extended intracellular membrane system, has contact sites with most of the cell organelles including the plasma membrane (PM).\textsuperscript{1} Macroautophagy (hereafter referred to as autophagy), is a major lysosomal degradative pathway for intracellular components conserved in eukaryotic cells. Autophagy which is stimulated upon stress induction, requires ER membrane for the biogenesis of autophagosome, the main organelle of autophagy, which will later fuse with lysosome.\textsuperscript{2} Autophagy and autophagosome biogenesis are initiated by the recruitment of evolutionary conserved ATG (autophagy-related, such as ATG1, ATG5, ATG8, ATG12, ATG16L1, etc.) proteins and non-ATG proteins (such as Vps34, Vps15, AMBRA-1) engaged at the ER membrane in a region named omegasome. These regions are characterized by accumulation of PI3P\textsuperscript{3} and consequent recruitment of specific PI3P binding proteins such as DFCP1 and WIPIs. DFCP1 labels at early stage the ER cradle known as omegasome and WIPI2, yeast orthologue of ATG18, is recruited to the phagophore,\textsuperscript{4} a cup shaped structure that ultimately closes-up to form the double membrane bound autophagosome. The ER-mitochondria contact sites have been shown to be a place for the recruitment of the ATG machinery and autophagosome assembly.\textsuperscript{5} However, our recent work shows that ER-PM is another site for the recruitment of early actors in autophagosome biogenesis.\textsuperscript{6} Indeed, we show that the extended synaptotagmins (E-Syts) proteins, key-regulators of ER-PM tethering,\textsuperscript{7,9} are directly involved in autophagosome biogenesis. More particularly, we show that E-Syts are engaged, after nutritional stress sensing, in the local targeting of PI3P synthetizing complex via VMP1 (vacuole membrane protein 1) mobilization, a step known to be responsible for autophagosome biogenesis initiation (Fig. 1A and B). Interestingly, the VMP1 protein, originally identified as a regulator of PI3P synthesis during autophagosome biogenesis initiation\textsuperscript{10,12} was recently reported to be physically associated with ER-driven contact sites at mitochondria, endosomes and lipid droplets,\textsuperscript{13} making sense with our own observations showing the targeting of VMP1 at...
ER-PM contact sites (ER-PMcs) engaged in autophagosome biogenesis. Importantly, it has recently been shown that VMP1 controls the formation of these ER contacts by controlling SERCA (Sarco/Endoplasmic Reticulum Calcium ATPase) activity. Loss of VMP1 greatly increases these contact sites and causes stable association of isolation membranes with the ER. This in turn affects autophagy, impeding the dissociation of ATG proteins from isolation membranes upon closure, thus blocking autophagosome formation. Notably, this dissociation process appears to require clearance of PI3P, thus suggesting a role for VMP1 and contact sites in the fine-tuning of the key phospholipid in autophagosome biogenesis.

Plants and mammalian ER-driven contact sites as models: Open questions

Cytoplasmic spreading and “exploring” by ER are common feature across eukaryotic cells. The importance of ER engagement in membrane contact sites stems out from the evolutionary conserved capability of the ER to promote tethers with other endomembranes, from yeast to plants and mammals. Some functions of ER-PMcs have been identified and are conserved among metazoan, as for example in Ca²⁺ signaling, some others have been evoked more recently and so far now ascertained only in few models, like the ER-PMcs functioning in autophagosome biogenesis in mammalian cells.
However, even if in plants cells ER-PMcs were not formally identified as crucial platforms for autophagosome biogenesis, the presence of pre-autophagosomal markers on ER membrane at the immediate vicinity of PM is in favor of a similar situation. In the light of our recent results, it would be definitely worth deeply searching for a potential role of plant ER-PMcs in autophagy.

One way to unveil the different conserved functions of the ER-PMcs is to analyze the different proteins that have been found localized in there, mostly categorized as ER-proteins. For example the plant PM proteins STIM1 and STIMATE, regulating Ca$^{2+}$ signaling, have been found in mammalian cells. STIM1 proteins, belonging to the family of proteins known as calbin1/2/3 in yeast and VAP (Scs2/22 in yeast) proteins, are the E-Syts (Tri-dria lipid binding protein (SMP) domain, shared among integral ER membrane proteins involved in varied cellular functions, including lipid transport and homeostasis, membrane trafficking and neurotransmitter release. Plants VAP, VAP27 also localize to ER-PMcs, however differently from metazoans, the two proteins (Syt1 and VAP27) label two different ER subdomains, suggesting that different type of ER-PMcs exist, at least in plants. So do they are different ER-PMcs with different functions? Do ER-PMcs play different roles in different organisms?

In plant cells the ER network appears very different from animal ones (Fig. 1C), mainly because a huge vacuole squeezes the cytoplasm close to the cell cortex obliging a great proximity between organelles, so for instance both Golgi bodies and ER appear very close to the PM. Special structures, found only in plant PM, are plasmodesmata (PD), a kind of pore mainly used as cell-cell communication structures. SYT1 and VAP27 are also found here and they both interact with plasmodesmata resident reticulon. Furthermore, they both interact with viral proteins and mediate trafficking of viral repli-cal complex. Thus, considering the intrinsic roles of autophagy in viral infection and the link between both ER-PMcs and ER-MTcs with autophagy, as already mentioned, one may wonder if the relationship between autophagy and viral infection passes by ER contact sites, at mitochondria and/or plasma membrane.

Finally, some other key questions would be worth to assess are: are the autophagosome produced at ER-MTcs vs ER-PMcs different? Are they engulfing different cargos? Is the engagement of different sets of ER-driven contact sites in the biogenesis of autophagosome a possibility for the cell, in plants and in mammals, to adapt to different types of stresses?

Interestingly, a role for the ER stress sensor PERK in the regulation of ER-PM appositions, through the modulation of the actin cytoskeleton, has been recently discovered. However, whether this mechanism correlates with the autophagy pathway was not explored. Can ER-PMcs and ER-MTcs, beyond the recruitment of the autophagy machinery, be specialized domains for lipid transfer and signaling to the autophagy pathway? What are the functions of ER-other organelles contact sites? Should VMP1 protein be considered as a molecular linker between contact sites and autophagosome biogenesis machineries? In this context, it is interesting to note that different Osh/ORP proteins have been found localizing to different organelles. The meaning of these
ORP-VAP interactions at ER-organelles contacts is the lipid transfer between organelle membranes,\(^\text{36}\) possibly for signaling pathways activation, but probably also to control membrane compositions. Indeed, ORP1L localizes to late autophagosomes and it has been proposed to regulate transport and positioning of late autophagosomes upon cholesterol and—under low-cholesterol conditions—contacts the ER protein VAP-A, forming thus ER-autophagosome contact sites.\(^\text{37}\) Whether other ER-driven contact sites are engaged in autophagy to initiate and/or terminate the process are open questions in an emerging and fascinating aspect of ER membrane dynamics during stress-response(s).

**ORCID**

Etienne Morel [http://orcid.org/0000-0002-4763-4954]

**References**

[1] Hoffmann PC, Kukulski W. Perspective on architecture and assembly of membrane contact sites. Biol Cell 2017 [cited 2017 Oct 13]; Available from: [http://doi.wiley.com/10.1111/boc.201700031](http://doi.wiley.com/10.1111/boc.201700031).

[2] Molino D, Zemirli N, Codogno P, et al. The journey of the autophagosome through mammalian cell organelles and membranes. J Mol Biol 2017. [cited 2017 Feb 14];429:497–514. Available from: [http://www.ncbi.nlm.nih.gov/pubmed/27986571](http://www.ncbi.nlm.nih.gov/pubmed/27986571).

[3] Axe EL, Walker SA, Maniwa M, et al. Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. J Cell Biol 2008;182:685–701. Available from: [http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18725538](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18725538).

[4] Wilson MII, Dooley HCC, Tooze SAA. WIP1b and Atg16L1: setting the stage for autophagosome formation. Biochem Soc Trans. 2014;42:1327–34. Available from: [http://www.biochemsoctrans.org/bst/042/bst0421327.htm](http://www.biochemsoctrans.org/bst/042/bst0421327.htm).

[5] Hailey DW, Rambold AS, Satpute-Krishnan P, et al. Mitochondrial membrane components for autophagosome biogenesis during starvation. Cell. 2010 [cited 2015 Sep 14];141:656–67. Available from: [http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3059894&tool=pmcentrez&rendertype=abstract](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3059894&tool=pmcentrez&rendertype=abstract). doi:10.1016/j.cell.2010.04.009.

[6] Nascimbeni AG, Giordano F, Dupont N, et al. ER+plasma membrane contact sites contribute to autophagosome biogenesis by regulation of local PI3P synthesis. EMBO J [Internet]. 2017;36. [cited 2017 Jun 28];e201797006. Available from: [http://www.ncbi.nlm.nih.gov/pubmed/28550152 doi:10.15252/embj.201797006](http://www.ncbi.nlm.nih.gov/pubmed/28550152 doi:10.15252/embj.201797006).

[7] Taylor P, Liou J, Chang C, et al. synaptotagmins Unveiling physiological functions of extended synaptotagmin. Cell Cycle. 2015;14:23–5.

[8] Pérez-Lara Á, Jahn R. Extended synaptotagmins (E-Syts): Architecture and dynamics of membrane contact sites revealed. Proc Natl Acad Sci [Internet] 2015;112:4837. Available from: [http://www.pnas.org/lookup doi:10.1073.pnas.1504487112. doi:10.1073/pnas.1504487112](http://www.pnas.org/lookup doi:10.1073.pnas.1504487112. doi:10.1073/pnas.1504487112).

[9] Giordano F, Saheki Y, Idevall-Hagren O, et al. PI(4,5)P(2)-dependent and Ca(2+)-regulated ER-PM interactions mediated by the extended synaptotagmins. Cell [Internet]. 2013;153:497–509. Available from: [http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=23791178. doi:10.1016/j.cell.2013.05.026](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=23791178. doi:10.1016/j.cell.2013.05.026).

[10] Molejon MI, Ropolo A, Re AL, et al. The VMP1-Bed1 interaction regulates autophagy induction. Sci Rep [Internet]. 2013;3:1055. Available from: [http://www.nature.com/srep/2013/130111/srep01055/full/srep01055.html. doi:10.1038/srep01055](http://www.nature.com/srep/2013/130111/srep01055/full/srep01055.html. doi:10.1038/srep01055).

[11] Calvo-Garrido J, Carilla-Latorre S, Escalante R. Vacuole membrane protein 1, autophagy and much more. Autophagy [Internet]. 2008. [cited 2016 Jun 16];4:835–7. Available from: [http://www.ncbi.nlm.nih.gov/pubmed/18641456. doi:10.4161/auto.6574](http://www.ncbi.nlm.nih.gov/pubmed/18641456. doi:10.4161/auto.6574).

[12] Calvo-Garrido J, King IS, Munoz-Braceras S, et al. Vmp1 regulates PtdIns3P signaling during autophagosome formation in Dictyostelium discoideum. Traffic [Internet]. 2014 [cited 2017 Oct 13];15:1235–46. Available from: [http://doi.wiley.com/10.1111/tra.12210. doi:10.1111/tra.12210](http://doi.wiley.com/10.1111/tra.12210. doi:10.1111/tra.12210).

[13] Tábara L-C, Escalante R. VMP1 establishes ER-microdomains that regulate membrane contact sites and autophagy. PLoS One [Internet]. 2016. [cited 2017 Feb 16];11:e0166499. Available from: [http://dx.plos.org/10.1371/journal.pone.0166499. doi:10.1371/journal.pone.0166499](http://dx.plos.org/10.1371/journal.pone.0166499. doi:10.1371/journal.pone.0166499).

[14] Zhao YG, Chen Y, Miao G, et al. The ER-localized transmembrane protein EPG-3/VMP1 regulates SERCA activity to control ER-isolation membrane contacts for autophagosome formation. Mol Cell [Internet]. 2017. [cited 2017 Oct 16];67:974–989.e6. Available from: [http://www.ncbi.nlm.nih.gov/pubmed/28890335. doi:10.1016/j.molcel.2017.08.005](http://www.ncbi.nlm.nih.gov/pubmed/28890335. doi:10.1016/j.molcel.2017.08.005).

[15] Lamb CA, Yoshimori T, Tooze SA. The autophagosome: origins unknown, biogenesis complex. Nat Rev Mol Cell Biol [Internet]. 2013;14:759–74. Available from: [http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=23791178. doi:10.1038/nrm3696](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=23791178. doi:10.1038/nrm3696).

[16] Okeke E, Dingsdale H, Parker T, et al. Endoplasmic reticulum-plasma membrane junctions: structure, function and dynamics. J Physiol [Internet]. 2016. [cited 2017 Oct 13];594:2837–47. Available from: [http://doi.wiley.com/10.1113/JP271142. doi:10.1113/JP271142](http://doi.wiley.com/10.1113/JP271142. doi:10.1113/JP271142).

[17] Wu MM, Buchanan J, Luik RM, et al. Ca\(^{2+}\) store depletion causes STIM1 to accumulate in ER regions closely associated with the plasma membrane. J Cell Biol. 2006 [cited 2017 Oct 13];174:803–13. Available from: [http://www.ncbi.nlm.nih.gov/pubmed/16966422. doi:10.1083/jcb.200604014](http://www.ncbi.nlm.nih.gov/pubmed/16966422. doi:10.1083/jcb.200604014).

[18] Le Bars R, Marion J, Le Borgeux R, et al. ATG5 defines a phagophore domain connected to the endoplasmic reticulum during autophagosome formation in plants. Nat Commun [Internet]. 2014;5:4121. Available from: [http://www.ncbi.nlm.nih.gov/pubmed/24947672](http://www.ncbi.nlm.nih.gov/pubmed/24947672).
[19] Maass K, Fischer MA, Seiler M, et al. A signal comprising a basic cluster and an amphipathic alpha-helix interacts with lipids and is required for the transport of Ist2 to the yeast cortical ER. J Cell Sci [Internet]. 2009 [cited 2017 Oct 13];122:625–35. Available from: http://jcs.biologists.org/cgi/doi/10.1242/jcs.036012. doi:10.1242/jcs.036012.

[20] Skirpan AL, Dowd PE, Sijacic P, et al. Identification and characterization of PiORP1, a Petunia oxysterol-binding-protein related protein involved in receptor-kinase mediated signaling in pollen, and analysis of the ORP gene family in Arabidopsis. Plant Mol Biol [Internet]. 2006 [cited 2017 Oct 13];61:553–65. Available from: http://link.springer.com/10.1007/s11103-006-0030-y. doi:10.1007/s11103-006-0030-y.

[21] Stefan CJ, Manford AG, Baird D, et al. Osh proteins regulate phosphoinositide metabolism at ER-plasma membrane contact sites. Cell [Internet]. 2011 [cited 2017 Oct 13];144:389–401. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0092867410015266. doi:10.1016/j.cell.2010.12.034.

[22] Lehto M, Laitinen S, Chinetti G, et al. The OSBP-related protein family in humans. J Lipid Res. 2001 [cited 2017 Oct 13];42:1203–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11483621.

[23] Moser von Filseck J, Opi A, et al. Phosphatidylserine transport by ORP/Osh proteins is driven by phosphatidylinositol 4-phosphate. Science [Internet]. 2015 [cited 2017 Oct 13];349:432. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26206936. doi:10.1126/science.1264014.

[24] Südhof TC, Jahn R. Proteins of synaptic vesicles involved in exocytosis and membrane recycling. Neuron [Internet]. 1991 [cited 2017 Oct 13];6:665–77. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1673848. doi:10.1016/0896-6273(91)90165-V.

[25] Saheki Y, De Camilli P. The extended-syntaptotagmins. Biochim Biophys Acta - Mol Cell Res [Internet]. 2017 [cited 2017 Oct 13];1864:1490–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28363589. doi:10.1016/j.bbamcr.2017.03.013.

[26] Wong LH, Levine TP. Tubular lipid binding proteins (TULIPs) growing everywhere. Biochim Biophys Acta - Mol Cell Res [Internet]. 2017 [cited 2017 Oct 20];1864:1439–49. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28554774. doi:10.1016/j.bbamcr.2017.05.019.

[27] Siao W, Wang P, Voigt B, et al. Arabidopsis SYT1 maintains stability of cortical endoplasmic reticulum networks and VAP27-1-enriched endoplasmic reticulum-plasma membrane contact sites. J Exp Bot [Internet] 2016 [cited 2017 Oct 13];67:6161–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27811083. doi:10.1093/jxb/erw381.

[28] Hughes L, Hawes C, Monteith S, Vaughan S. Serial block face scanning electron microscopy—the future of cell ultrastructure imaging. Protoplasma [Internet]. 2014 [cited 2017 Oct 13];251:395–401. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24240569. doi:10.1007/s00709-013-0580-1.

[29] Peña EJ, Heinlein M. Cortical microtubule-associated ER sites: organization centers of cell polarity and communication. Curr Opin Plant Biol [Internet]. 2013 [cited 2017 Oct 13];16:764–73. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1369526613001477. doi:10.1016/j.pbi.2013.10.002.

[30] Kriechbaumer V, Botchway SW, Slade SE, et al. Reticulomics: Protein-protein interaction studies with two plasmodesmata-localised reticulon family proteins identify binding partners enriched at plasmodesmata, ER and the plasma membrane. Plant Physiol [Internet]. 2015 [cited 2017 Oct 13];169:1933–45, pp.01153.2015. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26353761.

[31] Barajas D, Xu K, de Castro Martín IF, et al. Co-opted Oxy-sterol-binding ORP and VAP proteins channel sterols to RNA virus replication sites via membrane contact sites. PLoS Pathog [Internet]. 2014 [cited 2017 Oct 13];10:e1004388. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25329172. doi:10.1371/journal.ppat.1004388.

[32] Lewis JD, Lazarowitz SG. Arabidopsis syntaptotagmin SYTA regulates endocytosis and virus movement protein cell-to-cell transport. Proc Natl Acad Sci U S A [Internet] 2010, [cited 2017 Oct 13];107:2491–6. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.0909080107. doi:10.1073/pnas.0909080107.

[33] Williamson CD, Colberg-Poley AM. Access of viral proteins to mitochondria via mitochondria-associated membranes. Rev Med Virol [Internet]. 2009 [cited 2017 Oct 13];19:147–64. Available from: http://doi.wiley.com/10.1002/rmv.611. doi:10.1002/rmv.611.

[34] Münz C. Autophagy proteins in viral exocytosis and anti-viral immune responses. Viruses [Internet]. 2017 [cited 2017 Oct 13];9:288. Available from: http://www.mdpi.com/1999-4915/9/10/288. doi:10.3390/v9100288.

[35] van Vliet AR, Giordano F, Gerlo S, et al. The ER Stress Sensor PERK coordinates ER-plasma membrane contact site formation through interaction with Filamin-A and F-Actin remodeling. Mol Cell [Internet]. 2017 [cited 2017 Oct 16];65:885–899.e6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28238652. doi:10.1016/j.molcel.2017.01.020.

[36] Weber-Boyvat M, Kentala H, Peräna EJ, Heinlein M. Cortical microtubule-associated ER sites: organization centers of cell polarity and communication. Curr Opin Plant Biol [Internet]. 2013 [cited 2017 Oct 13];16:764–73. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1369526613001477. doi:10.1016/j.pbi.2013.10.002.

[37] Wijdeven RH, Janssen H, Nahidiazar L, et al. Cholesterol and ORP1L-mediated ER contact sites control autophagosome transport and fusion with the endocytic pathway. Nat Commun [Internet]. 2016 [cited 2017 Oct 16];7:11808. Available from: http://www.nature.com/doifinder/10.1038/ncomms11808. doi:10.1038/ncomms11808.