Extended synaptotagmins (E-Syts): Architecture and dynamics of membrane contact sites revealed

Ángel Pérez-Lara and Reinhard Jahn[1]

Department of Neurobiology, Max Planck Institute for Biophysical Chemistry, 37077 Göttingen, Germany

Eukaryotic cells are compartmentalized into membrane-enclosed organelles with defined structure and functions. These organelles are separated from each other, and for a long time it was thought that communication between them is confined to vesicular traffic or to exchange of membrane lipids by cytoplasmic transfer proteins. Recently, however, it has become apparent that organelles hitherto not suspected of being intimate friends can align very closely with each other, forming membrane contact sites (MCSs) of distinct structure and composition. Such sites are now known to play pivotal roles in the exchange of molecules and ions, thus being of considerable functional importance. In particular, the widespread network of the endoplasmic reticulum (ER), origin of most membrane proteins and lipids, forms multiple contacts with a variety of organelles such as mitochondria and the plasma membrane (PM). At ER–PM contacts the membranes are closely aligned, being separated by gaps of only 10–30 nm. Different proteins have been shown to be instrumental for contact formation such as junctophilins, stromal interaction molecule (STIM), tricalbins, and, more recently, a small group of ER membrane proteins termed extended synaptotagmins (E-Syts) (1, 2). The name E-Syts reflects their similarity to synaptotagmins, a family of proteins involved in Ca^{2+}-dependent secretion. Although rapid progress has being made concerning the role of E-Syt domains in establishing ER–PM contacts, information about the architecture of the contact sites has hitherto been lacking. In PNAS, Fernández-Busnadiego et al. (3) used cryoelectron tomography to close this gap in our understanding.

E-Syts bear an amino-terminal ER-membrane anchor, a synaptotagmin-like mitochondrial-lipid-binding protein domain (SMP), and multiple C2 domains. C2 domains are widespread and independently folded domains of about 130 residues that mediate Ca^{2+}/phospholipid binding and/or protein–protein interactions. Both the ER-membrane anchor and the C2 domains are required for establishing ER–PM contacts. The E-Syt family has three members: E-Syt1 with five C2 domains and E-Syt2/E-Syt3, each with three C2 domains (2). The C2C domain of E-Syt2 and E-Syt3 form membrane contacts by binding to the PM-specific phospholipid PtdIns(4,5)P_2, which resembles the mechanism of ER–PM contacts mediated by the STIM1 protein. In contrast to E-Syt-2/3, E-Syt1 only translocates to the ER–PM junctions after an increase in intracellular Ca^{2+}, which is mediated by its C2C domain (4).

Using a clever combination of mutant and wild-type protein overexpression, Fernández-Busnadiego et al. (3) report that contacts mediated by E-Syts are distinguished by a prominent electron-dense layer between (and parallel to) the membranes. Comparison of various E-Syts and E-Syt mutants indicates that the layer depends on the presence of the C2A, C2B, and SMP domains. The conspicuous layer structure of the E-Syt–mediated contacts is clearly different from the contacts formed by STIM that are characterized by filaments connecting (and thus perpendicular to) the two membranes.

Whereas E-Syt2 and E-Syt3 form contacts independent of Ca^{2+}, E-Syt1 is recruited to the contact sites upon a rise of the Ca^{2+} level. Intriguingly, the authors show that, at least in certain cell types, a small pool of E-Syt1 is localized at the contact sites even in the absence of Ca^{2+}. These sites, however, have a larger and more variable distance than those mediated by E-Syt3. An increase in intracellular Ca^{2+} is associated with a shortening of the gap between the membranes. An analysis of E-Syt1 mutants shows further that whereas the C2E domain is required for the Ca^{2+}-dependent interactions with the PM, the C2C domain is needed for distance shortening (3).

Together, these results reveal fascinating parallels between E-Syts, ferlins, and synaptotagmins (5–7). In all cases, membrane tethering is mediated by C2-domain-mediated interactions with acidic phospholipids [most

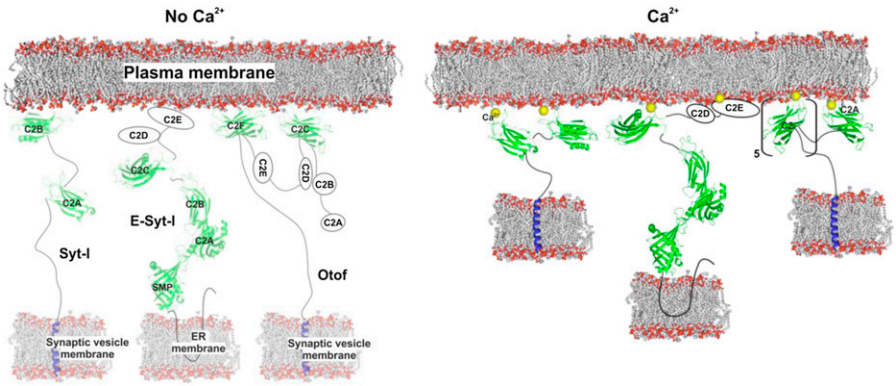

Fig. 1. Membrane contact mediated by C2 domain-containing membrane proteins. In the absence of Ca^{2+}, synaptotagmin 1 (Syt-I), extended synaptotagmin 1 (E-Syt-I), and otoferlin (Otof) tether the different organelle membranes to the PM via interaction of some of their C2 domains with phospholipids. An increase in cytosolic Ca^{2+} triggers the Ca^{2+}-dependent binding of these and/or additional C2 domains to the plasma membrane, shortening the distance between the targeted membranes (3, 7, 10). Note that E-Syt-1 forms dimers via homophilic interaction of its SMP domains (8), which has been omitted from the figure.

Author contributions: Á.P.-L. and R.J. wrote the paper.

The authors declare no conflict of interest.

See companion article on page E2004.

[1]To whom correspondence should be addressed. Email: rjahn@gwdg.de.

prominently PtdIns(4,5)P2] that are enhanced by Ca^{2+}. In the case of synaptotagmin 1 and otoferlin, the proteins mediate membrane apposition between synaptic vesicles and the PM, with loose tethering being observable in the absence of Ca^{2+}. Furthermore, it seems to be a common theme that Ca^{2+} not only enhances binding but also controls the distance between opposite membranes (5, 7) (Fig. 1), even though it needs to be established in which way distance shortening is related to the rather distinct functions of these molecules.

There is still a lot to learn about how exactly E-Syts connect membranes and how the architecture of the contact site is related to their function. Structural studies have revealed that the SMP domains form dimers that exhibit a hydrophobic groove capable of harboring phospholipids (8), supporting the view that E-Syts mediate phospholipid exchange between the membranes. Furthermore, the C2A and C2B domains seem to be connected rather rigidly, in a scaffold-like manner (9), which may explain the defined architecture of the electron-dense layer between the connected membranes. Evidently, for phospholipids to be transferred the hydrophobic side chains need to be protected during the entire transfer reaction across the gap between the two membranes. It will require further work to clarify how this is achieved (e.g., whether the SMP domains form channel-like connections or operate as dynamic shuttles).

1 Helle SC, et al. (2013) Organization and function of membrane contact sites. *Biochim Biophys Acta* 1833(11):2526–2541.
2 Min SW, Chang WP, Südhof TC (2007) E-Syts, a family of membranous Ca2+-sensor proteins with multiple C2 domains. *Proc Natl Acad Sci USA* 104(10):3823–3828.
3 Fernández-Busnadiego R, Saheki Y, De Camilli P (2015) Three-dimensional architecture of extended synaptotagmin-mediated endoplasmic reticulum–plasma membrane contact sites. *Proc Natl Acad Sci USA* 112:E2004–E2013.
4 Giordano F, et al. (2013) PI(4,5)P(2)-dependent and Ca(2+)-regulated ER-PM interactions mediated by the extended synaptotagmins. *Cell* 153(7):1494–1509.
5 Lek A, Evesson FJ, Sutton RB, North KN, Cooper ST (2012) Ferlins: Regulators of vesicle fusion for auditory neurotransmission, receptor trafficking and membrane repair. *Traffic* 13(2): 185–194.
6 Chapman ER (2008) How does synaptotagmin trigger neurotransmitter release? *Annu Rev Biochem* 77:615–641.
7 Jahn R, Fasshauer D (2012) Molecular machines governing exocytosis of synaptic vesicles. *Nature* 490(7419):201–207.
8 Schauder CM, et al. (2014) Structure of a lipid-bound extended synaptotagmin indicates a role in lipid transfer. *Nature* 510(7506): 552–555.
9 Xu J, et al. (2014) Structure and Ca^{2+}-binding properties of the tandem C_2 domains of E-Syt2. *Structure* 22(2):269–280.
10 Padmanarayana M, et al. (2014) Characterization of the lipid binding properties of Otoferlin reveals specific interactions between PI(4,5)P2 and the C2C and C2F domains. *Biochemistry* 53(30):5023–5033.