SNAP iN, SNAP oUT—SNAREs at ER-PM Contact Sites

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Abstract

Inter-organelle communication is essential for the exchange of cellular content in eukaryotes, particularly at membrane contact sites between the endoplasmic reticulum (ER) and the plasma membrane (PM). Accomplishing this critical task requires close positioning of the involved membranes via tether proteins and associated complexes. One such complex involves the SNAREs Sec22b and Syntaxin 1. Discovered to be interacting at the ER-PM membrane contact site (MCS), Sec22b-Stx1 forms a unique non-fusogenic bridge tethering the two membranes. Contrarily, SNAP25 was shown to be absent from the Sec22b-Stx1 complexes.

Two recent studies focused on this interplay of SNAREs and Lipid transfer proteins at MCSs. The Longin domain of Sec22b appeared to be the reason behind SNAP25’s exclusion from Sec22b-Stx1 assembly, and inclusion of E-Syts. It was also shown that yeast Sec9p and mammalian SNAP25 regulate ER-PM contact sites via their interaction with LTP OSBP-homologous proteins (ORP/OSH). In this following short review, we will take a closer look at the protein complexes involving SNAREs at MCSs and potential regulation by the Longin domain of Sec22b.

Keywords

Sec22b-Stx1 complex, SNAP25, E-Syt, ORP/OSH, membrane contact site

Inter-organelle contact sites are key sites for non-vesicular lipid transport, and intracellular signalling regulations (Eisenberg-Bord et al., 2016). Similar to other MCS’s, the ER-PM junction is essential for establishing and maintaining membrane lipid dynamics within and across the ER and PM. The formation of such ER-PM contact sites is reliant on membrane tethers, bringing together two bilayer organelles without triggering membrane fusion (Gallo et al., 2016). Two recently published papers explored the interplay between Lipid Transfer Proteins (LTPs) and Soluble N-ethylmaleimide-sensitive factor Attachment Receptors (SNAREs). The classical view about SNAREs role relates to their function in promoting fusion between membranes following tethering and docking. Nearly forty members of this protein family have been identified in mammalian cells (Wang et al., 2017), where they are distinguished by the presence in their shared α-helical coiled-coil domain (SNARE motif) of an arginine (R) residue in the vesicle-SNARE (v-SNARE) and a glutamine (Q) residue in the target membrane-SNARE (t-SNARE).

The current paradigm on the function of SNAREs relates to their specific association in molecular machinery governing, via conformational changes, the sequential, quasi-irreversible steps of membrane docking and fusion. This is the mechanism whereby neurotransmitter release from synaptic vesicles occurs following fusogenic ternary complexes formation between the v-SNARE VAMP2 and its t-SNARE partners SNAP25 and Stx1, present in the PM (Figure 1A). The hypothesis, that SNAREs could also be used as scaffolds for partner proteins acting in membrane lipid modification and/or transfer is now further supported by two recent articles published by Gallo et al. (2020) and Weber-Boyvat et al. (2020). This is in line with previous studies showing that, in yeast and the brain, ER-resident R-SNARE Sec22b interacts with the PM Sso1 and Syntaxin 1 (Stx1) respectively, in the absence of

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SNAP23/25/29/47. Since Sec22b participates in neuritogenesis, it was previously hypothesized that it would mediate membrane expansion independently of exocytosis, via non-vesicular lipid transfer from the ER to the PM (Petkovic et al., 2014).

A first study investigated the composition of the Sec22b-based complexes, in an attempt to detect the ER-resident LTPs Extended Synaptotagmins (E-Syt), thought to mediate lipid transfer at ER–PM contact sites (Giordano et al., 2013; Saheki et al., 2016), and determine whether its co-existence with Stx1, would exclude that of SNAP25, a partner of Stx1 (Gallo et al., 2020) (Figure 1B). Using co-immunoprecipitation experiments and cell imaging, they established that E-Syt2 efficiently co-precipitated with Stx1/Stx3 and Sec22b. Comparison of material co-immunoprecipitating with full-length (FL) Sec22b or Sec22b lacking the longin domain (Sec22b\L), revealed a differential behaviour of E-Syt2 and SNAP25. Sec22b\L was noted to be precipitating more SNAP25 and less E-Syt2, in comparison to FL Sec22b (Gallo et al., 2020) (Figure 1B). Using co-immunoprecipitation experiments and cell imaging, they established that E-Syt2 efficiently co-precipitated with Stx1/Stx3 and Sec22b. Comparison of material co-immunoprecipitating with full-length (FL) Sec22b or Sec22b lacking the longin domain (Sec22b\L), revealed a differential behaviour of E-Syt2 and SNAP25. Sec22b\L was noted to be precipitating more SNAP25 and less E-Syt2, in comparison to FL Sec22b (Gallo et al., 2020). Further supporting these findings, Sec22b\L was detected at the PM; insinuating fusion with the PM in the absence of the longin domain, likely via the formation of Stx1-SNAP25-Sec22b\L ternary complexes. The longin domain is an N-terminal globular region present in proteins such as Sec22b, VAMP7, and Ykt6 (Daste et al., 2015; Rossi et al., 2004), this finding suggests that the Sec22b longin domain excludes SNAP25 allowing for the formation of non-fusogenic Sec22b-Sxt1 complexes. Additionally, the decreased ability Sec22b\L to co-precipitate E-Syt suggests that the Sec22b longin domain acts as a main binding site for E-Syt and that it could interfere with the Stx1-SNAP25 association. This surprising apparent release of SNAP25 (in the presence of E-Syt) in a cellular context remains to be further investigated because there is evidence that when a tripartite Sec22b-Stx1-SNAP25 forms in vitro, it can lead artificial membranes to fuse (McNew et al., 2000; Petkovic et al., 2014). Furthermore, the direct binding of E-Syt to Sec22b remains to be demonstrated yet, as the possibility of longin domains molecular partner mediating association with E-Syt cannot be dismissed. The role of potential additional actors in the Sec22b-Stx-E-Syt complexes such as members of the Rab family also deserves further investigation, since there is now evidence that Rab11 and Rab8 bind to the VAMP7 longin domain (Kandachar et al., 2018). Earlier studies have shown that E-Syt facilitates the transfer of glycerolipids between two populations of liposomes, mimicking the ER and the PM, supporting ER-PM tethering (Chang et al., 2013). E-Syt has a tripartite structure including a membrane anchoring N-terminal domain, a synaptotagmin-like mitochondrial lipid-binding protein (SMP) domain along with multiple PM lipids binding acidic phospholipids C2 domains. It is abundantly present at the ER-PM contact sites, either through its membrane tethering activity or after recruitment together with Sec22b-Stx1/3 complexes. As the Sec22b-E-Syt association persists following the deletion of the SMP domain, it plausibly involves its E-syt C2 domains region as Sec22b longin domain binding site. Interestingly, this interaction...
between E-Syt and Sec22b could be a factor promoting and/or stabilizing the association of Sec22b with Stx1/3, as demonstrated by proximity ligation assay (Gallo et al., 2020). Moreover, E-Syt2 and Sec22b were shown by STED microscopy to be present in the growth cones of developing neurons, where they populate ER-PM contact sites; interacting with each other in very close proximity, beneath the PM. E-Syt overexpression was shown to be responsible for a significant membrane expansion in the form of filopodia, an effect which depended on Stx1 and Sec22b but not SNAP-25 based on experiments using Sec22b mutants and clostridial neurotoxins (Gallo et al., 2020). It is thus proposed that E-Syt is engaged in a tripartite complex with Sec22b and Stx1/3, regulating lipid transfer to the PM within ER-PM contact sites. This is in accordance with the lipid transfer catalysed by dE-Syts (ortholog of E-Syt) in photoreceptors (Nath et al., 2020) and the modulation of synaptic growth (Kikuma et al., 2017) in the fly.

Besides E-Syts, another class of LTP associated with the Sec22p-Sso1/2 complex was previously identified in yeast, namely the cytosolic ORP/Osh proteins (Petkovic et al., 2014). With two distinct membrane recognition mechanisms, ORP/Oshp are tethering proteins, likely functioning at MCSs. ORP/Oshp family members are capable of binding and transferring oxysterols, cholesterol, phosphoinositides-4-phosphate[Pi(4)P], and Phosphoinositides. In yeast and mammals, ORPs/Oshs can transfer phospholipids such as phosphatidylycerine in an exchange reaction with PI4P (D’Ambrosio et al., 2019; Maeda et al., 2013; Venditti et al., 2019). ORP/Oshp family members share two phenylalanines in an acid tract (FFAT) and can be targeted to the ER via integral membrane protein VAP (VAMP-associated protein). Additionally, a pleckstrin homology (PH) domain mediates ORP/Oshp binding to non-ER organelles via recognition of phosphoinositides. Recently, Weber-Boyvat et al. (2020) demonstrated that ORP/Osh is a novel interacting partner of SNAP25 (Sec9p in yeast) both in yeast and mammalian cells (Weber-Boyvat et al., 2020). The study focused on the interaction between Sec9p and Osh1-3, and their cross-talk with Ssc2p(VAP), Sso1/2p(Stx1), at the ER-PM MCSs. Using co-immunoprecipitation and BiFC technique, Weber-Boyvat et al. (2020) established the direct interaction between Osh and SNARE domain of Sec9p, along with Sec9p interaction with Sso1; occurring together as part of the same complex and exhibiting similar molecular interaction in mammals (Figure 1C). Furthermore, there is a highly integrated interplay between these proteins. Not only does Oshp stability rely on the presence of intact Sec9p, but a single mutation in Sec9p can alter Ssc2p oligomerization and Sec22p/Sso1p interaction. Moreover, the presence of Oshp is required for Sec22p (VAP-A) oligomerization, whereas, the loss of Osh1-3p does not affect Sec22p-Sso1p interaction. Experiments conducted in hippocampal neurons confirmed that part of this cross-talk was conserved in eukaryotes since ORP2 can modulate the oligomerization state of VAP-A. Importantly, knocking-down ORP2 in neurons using an shRNA strategy reduced neurite growth, as well as synapse formation further emphasizing the importance of lipid transfer in neurite growth. More generally, this indicates that in addition to E-Syts, several classes of LTPs such as ORP/Osh can participate in membrane expansion processes. The intriguing information presented by Gallo and Weber-Boyvat’s publications establishes that LTPs have membrane tethering activity in MCS, and interact with SNAREs without promoting membrane fusion. A key motif for the function of ORP appears to be the FFAT sequence. Weber-Boyvat et al. (2020) observed that FFAT-containing Osh overexpression reduces secretion from yeast cells. Contrarily, in the absence of the FFAT sequence in Osh (ORP), which prevents binding to Sec2p (VAP-A), secretion was unaffected. In this scenario, it can be expected that Sec9p (SNAP25) is recruited by an Osh-Ssc2p complex, which might then induce tethering between ER and PM. As no secretion occurs in this case, negative regulation of exocytosis would be due to Osh, a soluble LTP, acting by routing Sec9p (SNAP25) in a ternary complex; with Osh-Scs2p-Sec9p allowing only lipid transfer/modification. Topologically, a similar situation would be encountered with E-Syt interacting with the longin Sec22b, engaging in a tethering complex with Stx1, devoid of SNAP25. Therefore, two complexes involving a subset of SNARE proteins can be hypothesized as illustrated in Figure 1B with E-Syt binding to Stx1-Sec22b complexes (via the sec22b longin domain) and in Figure 1C with SNAP25-interacting ORP binding to the ER membrane through VAP-A (via FFAT sequence).

Together these observations support the notion that LTPs across ER-PM MCS act through non-fusogenic tethering bridges, facilitated the case of E-Syt2 by its binding to Sec22b via the longin domain and absence of SNAP25. Based on the aforementioned observations, it will be promising to explore the intricate events leading to the formation of the Sec22b-Stx1 complexes with E-Syt, especially its interaction with the longin domain. These studies can be used to hypothesize and further investigate how and why SNAP25 is removed/replaced or deactivated from the complexes, along with giving an insight into the potential central regulatory function of the longin domain. Keeping SNAP-25 out of the Sec22b-Stx1 complexes to prevent membrane fusion (Figure 1B), allowing SNAP-25 in the Osh/ORP-VAP-A complexes (Figure 1C) might be a key molecular mechanism at ER-PM contact sites. SNAP-25 was originally named Super
protein (Hess et al., 1992) because of its extremely fast axonal transport. Its potential role in regulating lipid transfer vs membrane fusion might require to call it again by its original name.

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