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Abstract: It is now clear that many intracellular signals result from multiple membrane-bound compartments acting in concert. Membrane contact sites, regions of close apposition between organelles, have emerged as major points of convergence during signaling, as these are places where material is exchanged. The material exchanged can be either water-insoluble molecules such as membrane lipids that are passed directly between membranes, or ions such as Ca2+. Here we highlight new insights into the role of contacts in regulating several aspects of signaling by second messengers, including lipid traffic that underpins generation of IP3 during intense PLC stimulation, NAADP generated Ca2+ signals, store-operated Ca2+ entry, and cyclic AMP signaling.

Signalling at Membrane Contact Sites: two membranes come together to handle second messengers

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Abstract

It is now clear that many intracellular signals result from multiple membrane-bound compartments acting in concert. Membrane contact sites, regions of close apposition between organelles, have emerged as major points of convergence during signaling, as these are places where material is exchanged. The material exchanged can be either water-insoluble molecules such as membrane lipids that are passed directly between membranes, or ions such as Ca^{2+}. Here we highlight new insights into the role of contacts in regulating several aspects of signaling by second messengers, including lipid traffic that underpins generation of IP_3 during intense PLC stimulation, NAADP generated Ca^{2+} signals, store-operated Ca^{2+} entry, and cyclic AMP signaling.

Abbreviations

A-kinase anchoring protein – AKAP

diacylglycerol – DAG

endoplasmic reticulum – ER

extended synaptotagmin – E-Syt

nucleus-vacuole junction – NVJ

phosphatidic acid – PA

phosphatidylcholine – PC

phosphatidylinositol – PI

phospholipase C – PLC

PI phosphate – PIP

store-operated Ca2+ entry – SOCE

Introduction

The events of most signaling pathways are classically modelled as taking place in one subcellular compartment, for example the plasma membrane. However, even classical signaling pathways involve components of other organelles such as the endoplasmic reticulum (ER). This necessitates interorganellar or intermembrane cross-talk. While this could theoretically take place at a distance, it is now clear that coordination is facilitated by membrane contact sites, which we define as any place inside cells where organelles come close to each other (see Box 1) [1,2].

The more prominent membrane contact sites, such as those between the ER and mitochondria or between the ER and plasma membrane, have been observed since the earliest ultrastructural studies of intracellular architecture [3]. They have long been posited to be locations of important cellular functions [4,5], and they were eventually found to harbor specific proteins, such as Ca^{2+} channels and lipid biosynthetic enzymes. The field of contact sites has blossomed in the last decade, with many more contact site components being identified, although specific tethers that structure membrane contact sites remain mostly unknown [1,2,6], with notable exceptions [7].

The presence of a membrane contact site has two major effects: (i) Enhancing speed of communication. A 30-fold reduction of distance (from 1 micron to 30 nm) produces a 1000-fold reduced diffusion time (from 200 ms to 200 μs); therefore, membrane contact sites are ideal places where signalling pathways can pass information and material between organelles. (ii) Creating biochemically distinct subdomains in each organelle where a unique set of functions co-exist; controlling entry/exit into these subdomains could be a way to regulate signalling. Here, we highlight a selection of the recent developments that link membrane contact sites to signalling by different second messengers.

1. Ca^{2+} signaling at the plasma membrane and the phosphatidylinositol (PI) cycle.

Ca^{2+} is a ubiquitous signalling ion that is well established in regulating cell function. Its versatility is underpinned by spatiotemporal complexity of the Ca^{2+} signal; in essence cells can sense (decode) where and when Ca^{2+} signals occur [8]. Ca^{2+} signal genesis can be thought of as a chain of events which start and end at the plasma membrane but involving Ca^{2+} fluxes from storage organelles, including the ER, acidic organelles, and mitochondria. At several steps, membrane contact sites between different Ca^{2+} sources are likely critical for communication. Ca^{2+} flux across the ER-mitochondria interface has been extensively studied [9]; here, we focus on a new aspect of Ca^{2+} signaling at ER-plasma membrane contacts.

A classical mechanism for generation of Ca^{2+} signals at the plasma membrane is through receptor-mediated activation of phospholipase C (PLC), which hydrolyzes $PI(4,5)P_2$ to IP_3 and diacylglycerol (DAG), leading to release of Ca^{2+} from ER Ca^{2+} stores by IP_3 receptors. One long-suggested role for contact sites is the resupply of PI to the plasma membrane during intense PLC signaling [10], which occurs during prolonged stimulation with hormones and neurotransmitters. Under these conditions not only is all the $PI(4,5)P_2$ hydrolyzed, but there is not enough PI in the plasma membrane to resynthesize the amount of $PI(4,5)P_2$ that is consumed [11]. Therefore, the plasma membrane needs PI to be resupplied. This occurs by recycling the lipid moiety of $PI(4,5)P_2$. PLC makes DAG and this undergoes five modifications to be returned to $PI(4,5)P_2$ (Figure 1). But the second and third of these modifications are carried out by ER membrane enzymes [12,13], the active sites of which are located in short (≤20 aa) loops on their cytosolic faces. Therefore, plasma membrane lipids ≥15 nm away [14•] cannot be substrates. This led to the proposal towards the end of the last millennium that the precursor lipid for these enzymes (phosphatidic acid, PA) and their product (phosphatidylinositol, PI) exchange at plasma membrane-ER contact sites [10]. Vindicating that prediction, it has now been shown that a single protein transfers both PA and PI in a countercurrent (Figure 1).

Massive and sustained $PI(4,5)P_2$ hydrolysis accompanies phototransduction in flies, but not in vertebrates. The retinal degeneration–type B (RdgB) protein is localised to contacts between the ER and plasma membrane in fly photoreceptors. It was known to transfer both PI and phosphatidylcholine (PC) like all other lipid transfer proteins in the same family, so it could carry out half of the necessary traffic in $PI(4,5)P_2$ resynthesis [15]. Nir2, a human homolog of RdgB, was

elegantly shown to have a similar role during intense Ca^{2+} signaling [16] . However, no means for traffic of PA, the other half of the reaction, was known. Things started to change when RdgB was found to have a weak ability to solubilise PA [17]. Now the PI transfer domains in both RdgB and Nir2, have been shown to exchange PA for PI at plasma membrane-PM contacts during PLC activation (Figure 1) [18••,19••]. The C-terminal domains in RdgB/Nir2 bind the PA headgroup, enhancing contact site targeting in response to PIP hydrolysis [20]. In addition Nir3, a close homolog of Nir2, has a higher affinity for the PA headgroup and slower transfer of PA, so that it can exchange lipid after low level PIP hydrolysis [21•]. Thus, there is a series of PA/PI exchange proteins fine-tuned to be recruited and act during different intensities of PLC activation to resupply lipid for IP_3 generation.

The mechanistic details of lipid transfer during PA/PI exchange has not yet been confirmed. It has been proposed that lipid modifying enzymes use lipid transfer proteins as co-factors to present (and activate) lipid [22]. This might apply for PI to PI(4)P conversion, but PI(4)P cannot bind RdgB/Nir, so this lipid must be present in the plasma membrane (Figure 1). The possibility of lipid transfer proteins acting as enzyme cofactors could be tested, for example by looking for direct binding of lipid transfer protein to enzyme. Overall, the important take-home message from the new work is that subtle variation in lipid specificity [17] and affinity [21•] allows closely related lipid transfer proteins to perform specific functions.

2. Ca^{2+} signaling and membrane contact sites between acidic Ca^{2+} stores and the ER.

In addition to IP_3, the second messenger NAADP plays an important role in generating highly localised Ca^{2+} signals [23]. This messenger is unusual as it activates Ca^{2+} release from acidic organelles such as lysosomes. Its likely targets are two-pore channels (TPCs), ancient members of the voltage-gated ion channel super family [24]. Importantly, the Ca^{2+} signal from these stores is amplified and propagated by Ca^{2+} release channels on the ER such as the IP_3 receptor, even when NAADP is bypassed by releasing lysosomal Ca^{2+} directly through osmotic stress [25]. Despite much evidence for interorganellar communication, we know little concerning how signals are transmitted across the acidic organelle-ER interface. Indirect evidence supports an intimate connection. For example, functional coupling is lost in is several unrelated cell types when cells are broken open; presumably the geometry of Ca^{2+}stores is disrupted. TPC localization is also critical, as TPCs do not trigger ER Ca^{2+} release when rerouted to the plasma membrane [26]. Cross-talk at membrane contact sites has been an attractive explanation for "tight" functional coupling [27], but little is known about these sites.

What is the hard evidence in support of membrane contacts between lysosomes and the ER? They have only recently been studied at the ultrastructural level [25,28,29]. However, unlike their endosome-ER counterparts slightly earlier in the endocytic pathway, their molecular composition remains undefined. Thus, a formal demonstration that lysosome-ER contacts support lysosome-ER Ca^{2+} communication is at present lacking. Drawing on the morphological similarity between the nucleus-vacuole junction (NVJ) in yeast and lysosome-ER junctions in animal cells, one might anticipate similarities in composition [27]. The founding NVJ tethers (vacuolar Vac8p and nuclear Nvj1p) are not conserved in animals [7]. However, other NVJ components are conserved widely: Nvj2p [30], Lam6p/Ltc1p [31] and Mdm1p and Nvj3p [32••]. Although it has been proposed that Lam6p is also a component of mitochondrial-vacuolar contacts [33], as we have argued elsewhere Lam6p is an ER membrane protein; therefore Lam6p near mitochondrial-vacuolar contacts must be in an ER tubule very close to the vacuole [31,33]. A similar peri-vacuolar (but non-NVJ) distribution has been found for Mdm1p and Nvj3p [32••]. This raises the possibility that some of the Lam6p/Mdm1p/Nvj3p-positive structures correspond to direct contacts between the yeast vacuole and ER tubules. Such contacts have never been described before in yeast, and so we suggest the name **Va**cuolar **n**on-NVJ **c**ytoplasmic **E**R (VancE) contacts. These may well be orthologous to lysosome-ER membrane contact sites in animal cells, which would indicate ways in which the yeast model system can be used to study acidic Ca^{2+} stores. The finding that SNX14, a human homologue of Mdm1p and Nvj3p in yeast ,is required for normal neuronal cell function [34] indicates that studies characterizing its roles in lysosomal-ER contact formation and function in animal cells are urgently required.

3. Membrane contact sites and the regulation of store-operated Ca^{2+} entry.

Store-operated (or capacitative) Ca^{2+} entry (SOCE) is a well established phenomenon linking depletion of ER Ca^{2+} stores to entry of Ca^{2+} across the PM. Its debut in the membrane contact site world rested on molecular identification of the ER Ca^{2+} sensor STIM and the plasma membrane Ca^{2+} entry channel Orai as the core components. Notably, live-cell imaging of STIM upon ER Ca^{2+} store depletion showed marked clustering of STIM in the ER and that it physically interacted with Orai at contact sites [35]. Work in the timeframe of this review has furthered the view that SOCE is subject to complex regulation. Septins emerged as key coordinators of SOCE [36]. Septins are highly regulated filament forming proteins laid down on membranes and in cytoplasmic meshes to create intracellular boundaries that can act as diffusion barriers. An siRNA screen for regulators of NFAT translocation to the nucleus (which is dependent of SOCE), identified septins 3, 4 and 5. Depletion of septins inhibits SOCE by preventing Orai clustering. Both septins and $PI(4,5)P_2$, which septins bind, redistribute during store depletion, with septin forming a boundary around a region rich in $PI(4,5)P_2$. To turn off SOCE, it has now been shown that Orai-STIM1 clusters move into this physically demarcated signaling microdomain, and there Ca^{2+} entry through Orai is inhibited by recruitment of SARAF, an integral ER protein [37]. Yet more organizers of SOCE micro-domains such as STIMATE/TMEM110 [38•,39•] are emerging.

Also there is new insight into the function of extended-synaptotagmins (E-Syts), ER-plasma membrane contact site proteins that respond to Ca^{2+} entry. E-Syt1-3 (yeast homologs called tricalbins) are integral ER proteins that bridge across contacts to the plasma membrane, where they bind anionic lipids and Ca^{2+} with multiple C2 domains [30,40,41•]. The expression level of E-Syts correlates with contact site size, but E-Syts also contain a lipid transfer domain that may exchange lipids between the ER and plasma membrane, so they are not merely tethers. E-Syt1 has two unique functions not shared by E-Syt2 and E-Syt3 that have now been studied in detail. The first is that the third of the five C2 domains in E-Syt1 binds the plasma membrane only when cytosolic Ca^{2+} levels rise, such as happens during induction of SOCE; this may narrow the contact site gap [14•,42]. The second is that tethering of micro-domains rich in both $PI(4,5)P_2$ and Orai-STIM1 requires only E-Syt1, not E-Syt2 or E-Syt3 [37].

Summating all these findings, it appears that there is lateral compartmentalisation of different stages of the SOCE pathway within contact sites, with Ca^{2+} entry through $PI(4,5)P_2$–poor micro-domains regulating dynamics of $PI(4,5)P_2$–rich micro-domains in close proximity (Figure 2). The functional consequence of such regulation remains to be established.

4. Cyclic AMP at contacts

The classical second messenger cyclic AMP (cAMP) has been proposed to signal at precise intracellular locations [43], but as yet membrane contact sites have not been implicated. cAMP is synthesized by ten adenylate cyclases, some soluble, others membrane bound, in response to G-protein coupled receptor activation. Compartmentalization is achieved by A-kinase anchoring proteins (AKAPs) which scaffold large complexes that include combinations of cAMP-dependent kinases (PKA), their targets, and both protein phosphatases and phosphodiesterases to terminate signaling [44]. The relationship of cAMP signaling with membrane contacts is that two AKAPs have been shown to bind to VAP [45,46•], which is a common target on the ER for membrane contact site proteins [2]. AKAP220 (alternative name AKAP11) mediates crosstalk between PKA and GSK3β [47]. AKAP110 (alternative names AKAP3, SKIP, SPKAP) is in various locations including the mitochondrial matrix [48], where it may mediate the effects of intra-mitochondrial cyclic AMP on ATP production [49]. The interactions of these AKAPs with VAP positions cAMP signaling at membrane contact sites with the ER, opening up a new aspect of second messenger signaling at contact sites.

5. Outlook

Membrane contact sites are the only places where molecular machines on two different compartmental membranes can directly interact. They are possibly involved in any signaling event

that involves multiple compartments. The involvement of multiple compartments has turned out to be important in many unexpected places (for example, [50]). Here we define contact sites as having two constituent membranes (Box 1), and we have focussed on contacts of either plasma membrane or acidic organelles (lysosome or degradative vacuole) with the ER. However, a new theme is that contacts may be more than two-way. A good example is the role of mitochondria, which form contacts not only with the ER [3-5], possibly more than one type [51], but also directly with both the plasma membrane [52] and with acidic organelles [53]. This indicates that multiple contacts on three or more compartments may interact with one another.

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BOX 1: Definitions

Membrane Contact Sites

One definition for membrane contact sites might include only those with an intermembrane gap within a defined range, assumed to be 10-30 nm, and if specific proteins and functions are known. However, we suggest a much wider definition of contacts as places where organelles are found so close together (without ever fusing) that proteins in each membrane form complexes that bridge the gap to create a functionally significant relationship. The inter-membrane gap is not constrained by the 10-30 nm norm. In the particular, the upper limit for gaps is not yet known. Some *bona fide* membrane contact site proteins can reach ≥100 nm, for example Ist2p and STIM proteins [54], so this is the current upper limit of contact site width, but it could yet be outdone. Also, the combinations of organelles that might form contact sites is far wider than first thought, and recent work suggests that they may be found wherever two organelles share biochemical functions [50].

Lipid transfer proteins

Lipid transfer proteins have the *in vitro* ability to solubilise lipids and can move them from donor to acceptor liposomes [55]. Typically they have the form of a box with a mobile lid, both with a largely hydrophobic lining [2]. The lipids that are solubilised *in vivo* appear to be dominated by one or two species, but close examination reveals highly complex mixtures [56•], the significance of which is not yet known. It is considered likely that lipid binding will always affect the outside conformation of the domain, even with the lid re-closed, so it is accepted that all lipid transfer domains can generate signals by acting as lipid sensors. Some lipid transfer proteins have such strong effects on signaling pathways away from contact sites that lipid sensing is proposed to be more significant than lipid transfer [57]. This has led to a more revolutionary idea that presumed lipid traffic *in vivo* does not occur according to current hypotheses describing lipid transfer proteins, all of which are based on over-literal interpretations of the *in vitro* activity [55]. The solution to this controversy lies in testing how lipids are actually handled inside cells [18••,19••], and if lipid transfer proteins interact with proposed downstream components in signaling pathways, such as PI kinases.

LEGENDS

Figure 1. Proposed actions of RdgB/Nir proteins to resupply lipid during PIP hydrolysis

Signaling is initiated by $PI(4,5)P_2$ hydrolysis by PLC to diacylglycerol (DAG) in the plasma membrane (black arrow), with release of IP_3. Intense signaling can only be maintained by resynthesis of $PI(4,5)P_2$ by the pathway DAG→phosphatidic acid (PA)→CDP-DAG→phosphatidylinositol (PI)→PI 4-phosphate (^4P)→ $PI(4,5)P_2$ ($^{45}P_2$). Among the enzymes (yellow arrows) for each of the five steps, three are on the plasma membrane: DAG kinase, PI 4-kinase and PIP 5-kinase. Two enzymes are in the ER: CDP-DAG synthase and PI synthase. The lipid solubilizing domains of RdgB and Nir2 transfer both PA from plasma membrane to ER, and PI in the opposite direction (blue and purple arrows respectively). The domains also can transfer PC (not shown), but how this integrates with PI/PA transfer is not yet known. RdgB/Nir2 also targeting both membranes: (i) a FFAT motif "f" that binds VAP "V", which is an integral ER protein; (ii) its C-terminal domains bind PA [20] and a second plasma membrane determinant (*) that has been identified as DAG [19••]. Since both PA and DAG accumulate on the plasma membrane soon after PLC is activated, proteins in this family are recruited specifically when they are needed [21•]. Because PA is a building block contained within all other glycerophospholipids, including PI and PC, it is likely that PA can fit into all PI/PC transfer proteins. However, only a minority of such proteins efficiently transfer PA, possibly because they specifically interact with the shared 1-phosphate. A proposed variation in this cycle is that RdgB/Nir2 and related PI transfer proteins present resynthesised PI directly to PI 4-kinase (dotted blue arrow).

Figure 2. Regulation of store-operated Ca^{2+} entry (SOCE) at ER-plasma membrane contact sites

Depletion of ER stores results in interactions between STIM1 in the ER and Orai1 in the plasma membrane (PM) resulting in substantial Ca^{2+} influx (left, indicated by yellow fill). This is regulated by translocation (large dotted black arrow) of STIM1/Orai1 from a $PI(4,5)P_2$-poor region into a $PI(4,5)P_2$-rich region of the PM demarcated by Septins (right, level of $PI(4,5)P_2$ indicated by red fill). Here, STIM1

interacts with SARAF which in turn promotes Ca^{2+}-dependent inactivation of Orai1 thereby tempering Ca^{2+} influx. This plasma membrane microdomain is stabilized by E-Syt1, which targets here by coincidence detection of $Ca^{2+}$and $PI(4,5)P_2$ that are bound by its third and fifth C2 domains (indicated by yellow and red edges respectively).

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Electron microscopic tomography of vitrified specimens shows that membrane contact sites in neurons are of two different types, one corresponding to contacts seen in tissue culture cells driven

to have high levels of contacts containing STIM1 and Orai1, and another corresponding to contacts in cells over-expressing E-Syts. Each type of contact has a characteristic width and a characteristic appearance of proteins within the 15-27 nm contact site gap, and there were changes upon cell stimulation that correspond to activation of an extra C2 domain within E-Syt1, leading to binding at a closer range.

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Yadav et al. (2015) and Kim et al. (2015) [19] both came to similar conclusions that RdgB in flies and its human homolog Nir2 respectively carry out PI/PA exhcange at plasma membrane-ER contacts. Yadav et al. showed that the RdgB domain also transfers PA in vitro. Kim et al. demonstrated that Nir2 is required to avoid precursor build up for both lipid transfer steps, and also that the C-terminal domains mediate targeting to plasma membrane-ER contacts. These papers together with the known locations of the enzymes required for PIP consumption and resynthesis establish an absolute requirement for this lipid transfer protein to transfer lipids in vivo. This provides part of the answer to a question raised elsewhere [55]: these PI transfer proteins (PITPs) do move lipids from one place to another. Also see Chang et al. [21] for a nuanced study of Nir3.

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This paper can be seen as a companion to Yadav et al. (2015) [18] and Kim et al. (2015) [19]. Nir3, a paralog of Nir2 found only in vertebrates, was found to work alongside it, but to have different sensitivities: translocation to contacts by Nir3 is much more sensitive because its C-terminal domains have a higher affinity for PA; also its PI transfer domain is lable to transfer PA, but is slower than Nir2. These modulations allow Nir3 to function after low level PIP hydrolysis, while Nir2 is adapted to faciliate maximal stimulation.

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A genome-wide screen for new genes involved in traffic to the yeast vacuole was used to idnetify Mdm1p, the yeast homolog of human Snx13/14/15, and its previously unstudied homolog Ydr179w-ap (Nvj3p) as novel components of the nucleus-vacuole junction. One domain is common to all these proteins: the PXA domain, which has no known function. Mdm1 and human Snx13/14/15 have extra domains: transmembrane helices that are embedded in the outer nuclear envelope, and PX domains that target the vacuole by binding PI(3)P. Loss of Mdm1p function (as per Snx14 mutations that cause complex and severe human neurological problems) cause defects in sphinoglipid metabolism, which is one of the proposel functions of the NVJ. Both of the newly studied yeast components are seen at contacts between cytoplasmic ER tubules and the vacuole, in particular when Nvj1p is deleted. This second class of contacts between elements of the ER and the degradative vacuole has never been described formally before, and the name Vacuolar non-NVJ cytoplasmic ER (VancE) contacts has been proposed [40].

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This paper uses an elegant spatially restricted proteomic approach to identify a physical interaction between TMEM110 (renamed STIMATE) and STIM1 which is mapped to the coiled-coil region of STIM1. This region is known to mediate a conformational switch that releases the SOAR/CAD domain of STIM1 to allow gating of Orai. STIMATE may therefore promote Ca2+ entry by stabilizing short term transition states upon store depletion as well long term stabilization of ER-PM junctions - see [52].

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This paper and [51] provide insight into the mechanism of action of TMEM110, a protein previously identified in a siRNA screen for regulators of NFAT translocation. TMEM110 is identified as a positive regulator of SOCE. Knockdown/knockout studies reveal a substantial reduction in cortical ER, ER-PM junctions and the formation of STIM1 clusters upon store depletion.

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A crystal structure of E-Syt2 showed that it contains the tube-forming head-to-head dimeric lipid transfer domains predicted by Kopec et al. (Bioinformatics, 2010, vol 26, pp1927-31), but with two important additional discoveries: (1) Four phospholipids are bound to each protein dimer, and lipids extracted from mammalian cells are representative of the inner leaflet of the plasma membrane, confirming a prediction that E-Syts bind only acyl chains, with headgroups projecting out of the protein so that they are not subject to any selection. (2) The overall length of the lipid carrying tube is <10 nm and its ends are hydrophilic; this severly undermines a model where E-syts and related proteins at contacts form tubular bridges for lipids to flow through across contact sites, and an alternative model is suggested where lipids are shuttled across contacts four at a time. It is intriguing to question how any phospholipid gradient between ER and plasma membrane is maintained if E-Syts can exchange them non-specifically.

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A high-throughput interomics project was reported oringinally with 26,000 interactions, and then later expanded with an on-line update that includes ≥56,000 interactions from >5000 tagged proteins. The quality of the data was sufficiently high to identify likely functions and locations for proteins of unknown function. A detailed study was carried out for the interactions of VAP (standing for VAMP-associated protein). This is a key membrane contact site protein as it is the ER receptor for many proteins that target contact sites, and is important for many ER functions, as well as being implicated in motor neurone disease (also called amyotrophic lateral sclerosis, ALS) [57]. AKAP110 was among the interactors, as previously predicted from its conserved FFAT motif [55], along with several PKA regulatory subunits that likely bind to it. This places cAMP signalling at contact sites.

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Heterologous lipid transfer domains of the StART family were expressed in yeast as fusions placed in between a GAL4 DNA binding domain from yeast and the activation domain of herpes VP16. The resulting constructs were transcriptional stimulators in a StART-dependent fashion. Although the details of how StART domains activate this construct are not clear, the natural ligands for a number of active constructs were studied by mass spectrometry of protein recovered from cells. Interestingly, a wide range of lipidic ligands (5-40) was found in all cases, with hierarchical clustering required to identify the classes of ligands specific for each domain.

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Figure 1

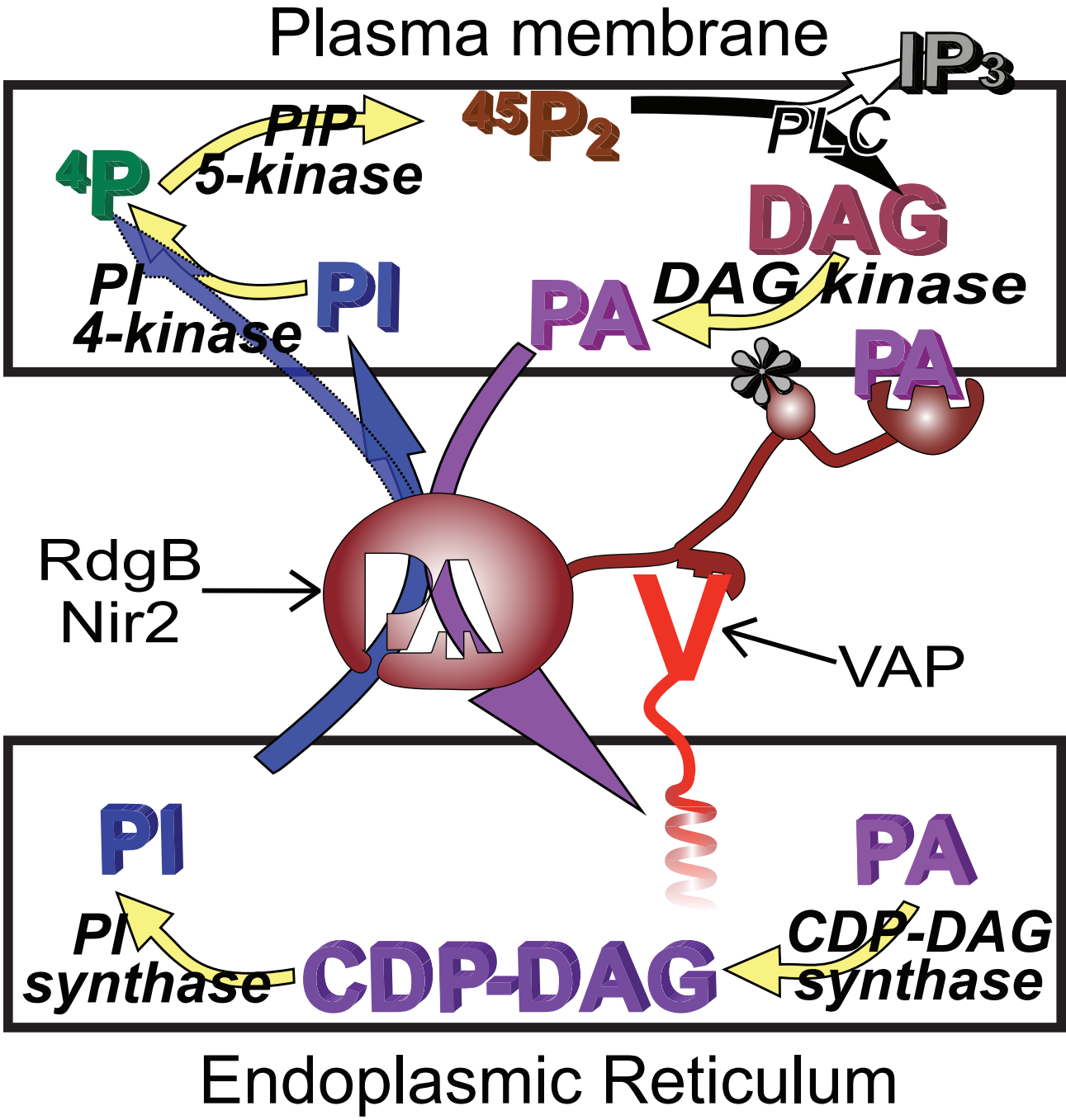
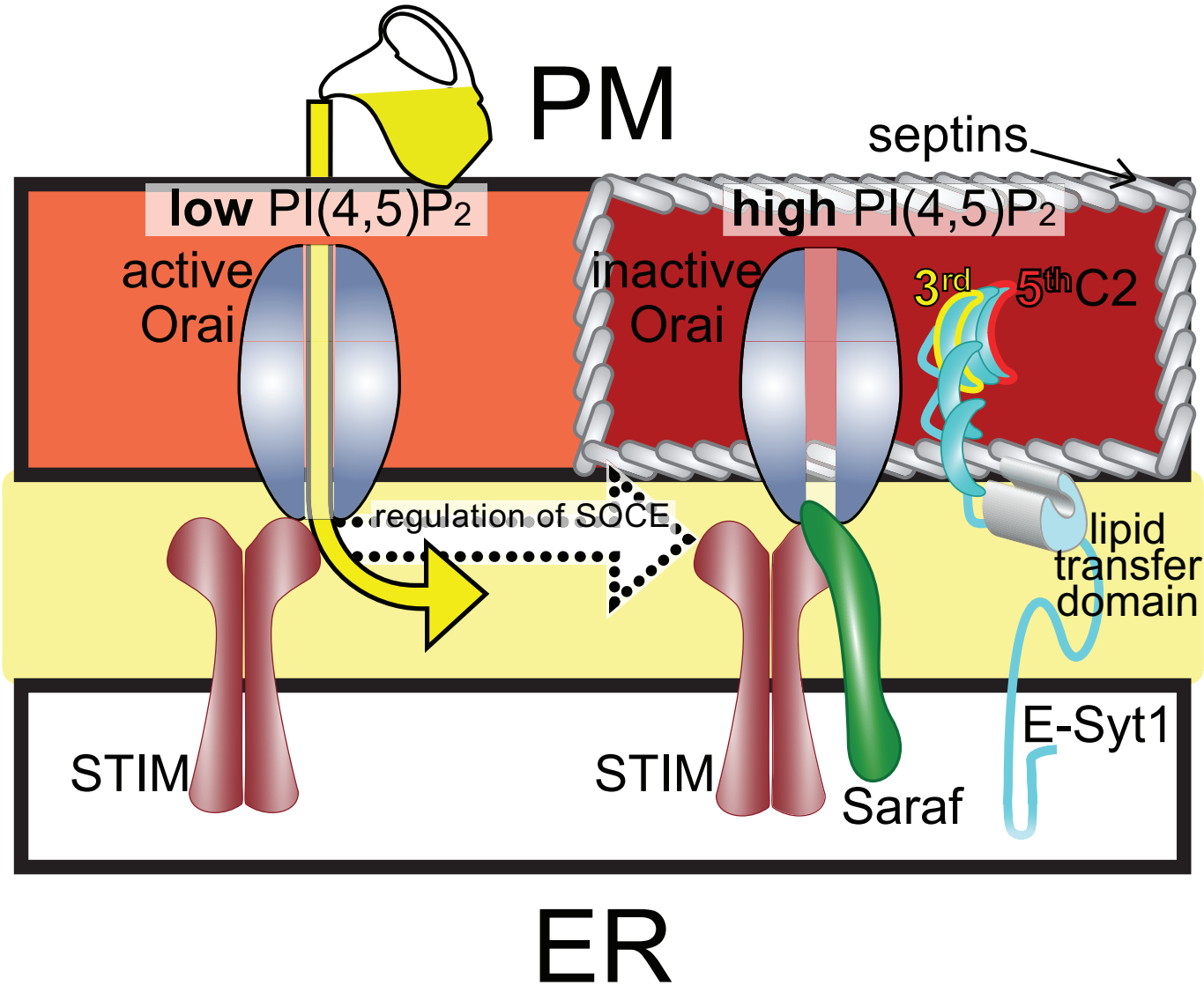

Plasma membrane
IP3
PIP
5-kinase
4P
45P2
PLC
DAG
PI
4-kinase
PI
PA
DAG kinase
PA
RdgB
Nir2
PA
V
VAP
PI
PA
PI
synthase
CDP-DAG
CDP-DAG
synthase
Endoplasmic Reticulum

Figure 2


PM
septins
low PI(4,5)P2
high PI(4,5)P2
active Orai
inactive Orai
3rd
5th C2
regulation of SOCE
lipid transfer domain
STIM
STIM
Saraf
E-Syt1
ER