Membrane contact sites—an interesting species, an interesting mix

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Eukaryotic cells are defined by the presence of membrane-bound organelles that divide the cell into distinct compartments, with specialized micro-environments optimized for the reactions taking place in each cell. However, membranes pose a barrier to free diffusion throughout the cell. Transfer between compartments can occur through vesicles or by using specialized transfer proteins such as phospholipid transfer proteins. Another solution has evolved in the form of a network of contact sites that link different organelles [1]. These contact sites bring membranes into close proximity (within approximately 30nm), tethering them without fusion. Several forms of membrane contact sites have been identified, predominantly connecting the endoplasmic reticulum (ER) with mitochondria, the Golgi, endosomes and the plasma membrane (PM). Membrane contact sites allow for transfer between two compartments, primarily of either calcium ions or lipids, but other functional roles are emerging. In this issue of *EMBO reports*, Loewen and colleagues reveal a role for PM–ER contact sites in regulating phosphatidylcholine (PC) synthesis in budding yeast [2].

Lipid synthesis at contact sites

In *Saccharomyces cerevisiae* much of the ER lies in close proximity to the PM. This PM-associated ER (pmaER) is enriched with lipid-synthesizing enzymes [3], and the contacts between the PM and the ER are thought to allow for cross-talk and regulation of lipid composition of the membranes between the two compartments. Six proteins required for PM–ER contacts in yeast have been identified: the vesicle-associated membrane protein-associated protein orthologues Scs2 and

Scs22, Ist2 (a member of the TMEM16 ion channel family) and the tricalbin proteins (Tcb1, Tcb2, and Tcb3, orthologues of the extended synaptotagmin family; [4]). Deletion of the genes that encode these proteins results in elevated phosphoinositide levels in the PM and induction of the unfolded protein response in the ER, indicating a role for PM–ER contacts in cell signalling and ER function, as well as organelle morphology [4].

SCS2 interacts genetically with *ICE2*—an integral ER protein of unknown function [5]. $\Delta scs2\Delta ice2$ cells lack pmaER and have reduced growth, suggesting that the contact sites are important for normal cellular function. In their paper [2], Loewen and colleagues found that $\Delta scs2\Delta ice2$ cells are choline auxotrophs and showed that PM–ER contacts sites are required for PC synthesis. PC can be synthesized through the Kennedy pathway from lipid precursors including choline. Addition of choline or overexpression of Opi3—the enzyme that catalyses the last methylation steps of PC biosynthesis—rescued growth of $\Delta scs2\Delta ice2$ cells. Overexpression of the preceding enzyme Cho2 in the pathway could not rescue the defect, indicating a fault in the final methylation steps of PC biosynthesis. Strikingly, although the expression level of the Opi3 protein was similar to wild-type cells, Opi3 no longer localized to pmaER in $\Delta scs2\Delta ice2$ cells, suggesting a crucial role for PM–ER contacts in regulating the activity of the protein. Scs2 directs localization of the oxysterol-binding protein homologues Osh2 and Osh3 to pmaER [6], and Loewen and colleagues showed that Osh3 rescues choline auxotrophy in $\Delta scs2\Delta ice2$ cells but did not restore pmaER. Changes in the abundance of PM–ER contacts are shown

to affect PM stability by using a detergent-sensitivity assay against the non-ionic detergent NP-40, susceptibility to which is partly rescued by growth on choline-containing media. These data suggest an important role for pmaER in maintaining the structural integrity of the PM.

The authors searched for regulators of pmaER by screening for proteins that suppress the choline requirement of the $\Delta scs2\Delta ice2$ mutant, and identified the phosphatidic acid phosphohydrolase Pah1. Surprisingly, a catalytically inactive form of Pah1 rescued the $\Delta scs2\Delta ice2$ choline auxotrophy and, unlike Opi3, Pah1 also rescued the PM–ER contact sites. Thus, it seems that Pah1 is involved in establishing pmaER. How this relates to the other structural components of these contact sites remains to be established.

The authors used an elegant *in vitro* methylation assay to demonstrate that Opi3 can methylate phosphatidylethanolamine (PE) to PC *in trans*—a mechanism that had been proposed previously [7] and that offers a pathway for the cell to make rapid changes to membrane composition. As the authors point out, all eukaryotic PE methyltransferases are located on the cytosolic face of the ER whilst the prokaryotic equivalents are all soluble enzymes. Thus, it is probable that all PE methyltransferases act *in trans* to control PC synthesis in the plasma membrane.

Functions of membrane contact sites

Contact sites between different organelles have become the source of considerable interest, with roles in controlling organelle morphology [8], regulation of growth factor [9], phosphoinositide [4,6] and calcium signalling (reviewed in [11]). Others have shown that phospholipids at

Fig 1 | Phospholipid synthesis at membrane contact sites. (A) PC synthesis mediated by the PE *N*-methyltransferase Opi3, acting *in trans* at PM-ER contact sites. (B) Sac1 catalyses the dephosphorylation of PI4P to generate PI also at ER-PM contact sites. (C) In metazoans, Rab10 mediates the localization of CEPT1 and PIS to dynamic ER domains that act in phospholipid synthesis and control ER morphology. Evidence also suggests a role for this domain at ER-PM contact sites. CEPT1, choline/ethanolamine phosphotransferase 1; ER, endoplasmic reticulum; FFAT, two phenylalanines in an acidic tract; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PH, plekstrin homology; PI, phosphatidylinositol; PI4P, phosphatidylinositol-4-phosphate; PM, plasma membrane; PIS, phosphatidylinositol synthase; PME, phosphatidylmethylethanolamine.

contact sites can be modified, for example the dephosphorylation of PI4P by Sac1 [6]. Interestingly, Sac1 acts at membrane contact sites that are maintained through binding of Scs2 to the FFAT (two phenylalanines in an acidic tract) motif within Osh3. Scs2 maintains pmaER contact in both cases and Osh3 acts as a common adaptor to both Sac1 and Opi3 at pmaER (Fig 1). It is expected that there are common architectural determinants of pmaER that are then used by diverse enzymes to control phospholipid metabolism within the PM.

Localized lipid synthesis at membrane contact sites also probably occurs elsewhere in the cell. Recent work [1] showed that the small GTP-binding protein Rab10 organizes a subdomain of the ER membrane, which is rich in the phospholipid synthesis enzyme choline/ethanolamine phosphotransferase 1 (CEPT1) and phosphatidylinositol synthase (PIS; Fig 1). This domain controls the morphology of the ER, notably through homotypic fusion. Previous work provided evidence for a highly mobile, ER-derived compartment rich in PIS in which PI is synthesized before delivery to other membranes through membrane contact sites [10]. Whether these two phenomena are in fact one and the same, and whether local phospholipid synthesis contributes to the resolution of homotypic fusion events at the ER, remains to be defined. As there is no evidence that PIS or CEPT1 can act *in trans* in the way that Opi3 does, another possibility is that these enzymes could participate in the delivery through flippase activity (C. McMaster, personal communication).

Perspective

The most interesting aspect of this work demonstrates the capability for *de novo* lipid synthesis at membrane contact sites. The structural components of these membrane contact sites and the enzymes that

localize to them are only beginning to be defined, and the purpose of localized lipid synthesis remains unclear. This report has opened up many new avenues of investigation. Remaining questions concern the role of Pah1 in establishing pmaER, the mechanisms that regulate Opi3 activity at these sites and the relationship to other phospholipid synthesis pathways. Further work is likely to define the role of pmaER in the control of ER stress. Clearly, the nature and function of contact sites between cellular membranes is a hot topic with many questions that remain to be answered.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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