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Review

Insights into eisosome assembly and organization

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Eisosomes, large protein complexes that are predominantly composed of BAR-domain-containing proteins Pil1 and its homologs, are situated under the plasma membrane of ascomycetes. A successful targeting of Pil1 onto the future site of eisosome accompanies maturation of eisosome. During or after recruitment, Pil1 undergoes self-assembly into filaments that can serve as scaffolds to induce membrane furrows or invaginations. Although a consequence of the invagination is likely to redistribute particular proteins and lipids to a different location, the precise physiological role of membrane invagination and eisosome assembly awaits further investigation. The present review summarizes recent research findings within the field regarding the detailed structural and functional significance of Pil1 on eisosome organization.

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1. Introduction: Central organizers of eisosome

The plasma membrane is a diverse structure that manages the traffic of materials in and out of the cell. It consists of dynamic compartments where different functions take place. These compartments exist over a wide range of spatial and temporal scales (Lingwood and Simons 2010). The budding yeast plasma membrane has been subdivided into three distinct membrane compartments: the membrane compartment of Can1 (MCC), the membrane compartment of TORC2 (MCT) and the membrane compartment of Pmal (MCP) (Young *et al.* 2002; Malinska *et al.* 2003; Grossmann *et al.* 2007; Berchtold and Walther 2009; Brach *et al.* 2011). MCC and MCT are found as distinct domains, whereas the MCP is found throughout the membrane except where a MCC or a MCT exists (Grossmann *et al.* 2007; Berchtold and Walther 2009). The cytoplasmic side of MCC appears to be closely associated with the protein cluster containing thousands of copies of Pil1 and its homolog Lsp1 (Grossmann *et al.* 2007; Frohlich *et al.* 2009). The cytosolic cluster was so named 'eisosome' (meaning portal for body in Greek) by Walther and coworkers because of its implication in endocytosis (Walther *et al.* 2006). It has been reported that in total 22 proteins (9 transmembrane and 13 cytoplasmic proteins) are members of MCC-associated proteins (Grossmann *et al.* 2008; Deng *et al.* 2009). Since the fluorescence microscope does not provide sufficient spatial resolution to differentiate between MCC and eisosome, Stradalova *et al.* (2009), using electron microscopy approaches, showed the ultrastructure of MCC that structurally resembles a furrow-like endocytic invagination. They showed that the transmembrane MCC marker Sur7 was found to localize to the superficial parts of the invaginated plasma membrane, while the cytosolic eisosome marker Pil1 was detected in the deeper parts of the furrow-like invagination mainly around the curved bottom of the structure (Stradalova *et al.* 2009). Upon deletion of budding yeast *PIL1,* GFP-fused eisosome (Lsp1, Slm1, Pkh1 and Pkh2) and MCC (Sur7, Nce102 and Can1) markers are localized to a few bright peripheral clusters called eisosome remnants, instead of localizing to the cell cortex evenly in a punctuated pattern (Walther *et al.* 2006, 2007; Frohlich *et al.* 2009; Grossmann *et al.* 2008; Kamble *et al.* 2011), indicating Pil1 is essential for the structural integrity of eisosome and MCC. Although Pil1 exhibits high levels of sequence homology among fungi, it seems that the function of Pil1 has not been completely conserved between budding yeast and other fungal species (Vangelatos *et al.* 2010; Kabeche *et al.* 2011; Reijnst *et al.* 2011; Seger *et al.* 2011). For instance, unlike budding yeast Pil1 (*Sc*Pil1) implicated in endocytosis (Walther *et al.* 2006; Murphy *et al.*

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2011), *Ashbya gossypii* Pil1 (*Ag*Pil1) and *Candida albican*s Pil1 (*Ca*Pil1) are required for polar growth and essential for cell growth, respectively (Reijnst *et al.* 2011; Seger *et al.* 2011). The fact that the fission yeast orthologs of Slm1 and Sur7 do not colocalize with the MCC/eisosome marker Pil1 or depend on Pil1 for their localization in *S. pombe* also suggests the functional divergence of Pil1 in organizing MCC/eisosome between budding yeast and fission yeast (Kabeche *et al.* 2011).

2. Up-to-date model for Pil1 assembly onto eisosome

Recent studies discovered that both Pil1 and Lsp1 contain a BAR domain, structurally most similar to N-BAR domain which is also found in amphiphysins (Olivera-Couto *et al.* 2011; Ziolkowska *et al.* 2011). BAR domains consist of a coiled-coil of three long alpha-helices that dimerize to form a banana-shaped domain with a positively charged concave surface. Cationic residues on the positive surface interact with anionic membrane lipids via electrostatic interactions (Frost *et al.* 2009). Owing to the rigid concave shape of the surface, the BAR domain can induce membrane curvature, required for a wide range of cellular processes such as endocytic invagination and cell motility (Gallop and McMahon 2005; Suetsugu *et al.* 2010). The BAR-domain-containing eisosome proteins Pil1 and Lsp1 are capable of self-assembly, binding lipid membranes, preferably those containing PI(4,5)P_2 (or PIP_2), and deforming them into long tubules (Kabeche *et al.* 2011; Karotki *et al.* 2011; Olivera-Couto *et al.* 2011). As expected, the positive surface patch of theoretical Pil1 homology modelling on the structural template of Lsp1 turned out to be important for its membrane binding and normal eisosome organization (Karotki *et al.* 2011; Olivera-Couto *et al.* 2011; Ziolkowska *et al.* 2011). Karotki *et al.* (2011) further proposed that, in addition to the positive residues on the concave surface, an N-terminal segment of Pil1 or Lsp1 might facilitate their efficient membrane binding and/or bending.

3. Other protein factors implicated in organizing eisosome

Beside the essential eisosomal organizing factor Pil1, according to a genome-wide screen for deletion mutants that show an alteration of Pil1 localization, a wide range of genes (a total of 88 genes), functioning ranging from endocytosis and vesicle trafficking to metabolism, appear to be involved in the organization of the eisosome (Frohlich *et al.* 2009). Additionally, a similar genome-wide screen for deletion mutants that shows an altered Can1 (MCC marker) pattern identified 27 genes (Grossmann *et al.* 2008). Given eisosome and MCC are tightly associated with each other, it is striking to note that there was such a low level of consistency between those screens that in both screens *NCE102, SUR4* and *MNN10* genes were the only overlapping ones that affect both MCC and eisosome organizations (Grossmann *et al.* 2008; Frohlich *et al.* 2009). One straightforward explanation for this would be that the recruitment of MCC and eisosome markers is differently regulated by non-overlapping factors in general, perhaps pointing out a subtle functional difference between MCC and eisosome. Except for Mnn10, the two other gene products, Sur4 and Nce102, are likely to influence eisosome organization through sphingolipid signalling by altering the level of sphingolipids and serving as a sphingolipid sensor in the plasma membrane, respectively (Han *et al.* 2002; Paul *et al.* 2006; Frohlich *et al.* 2009). Interestingly, it was found that the recruitment of Nce102, a bona fide MCC transmembrane protein, depends on the availability of sphingolipids at MCC where Nce102 acts as a negative regulator of Pkh kinases in Pil1 phosphorylation (Frohlich *et al.* 2009). Although more than 100 genes/proteins, to date, have been presented by the genetic screens to be important for MCC/eisosome organization, many more await further identification. This is because all essential genes (~18% of the yeast genome) and a considerable number of proteins that show functional redundancy with other proteins in the yeast genome were excluded from the lists. For the latter case, Slm1 and its homolog Slm2 share the same function in organizing the eisosome as shown by Kamble *et al.* (2011), but Slm2 is not included on either list of genome-wide screens. Slm1/2 were originally characterized as PIP_2 binding proteins through their C-terminal PH domain (Audhya *et al.* 2004; Fadri *et al.* 2005) and later found to be eisosome components (Grossmann *et al.* 2008; Kamble *et al.* 2011). A first clue to the targeting of Slm proteins to eisosome was provided by a recent microscopic study that clearly showed the central Slm (showing some sequence similarity to F-BAR) and PH domains are essentially required for eisosome targeting, but not by PH or F-BAR alone (Olivera-Couto *et al.* 2011), and thus underlining the significance of BAR domain on eisosome targeting.

4. Reversible phosphorylation and its consequences on eisosome organization

Another set of redundant genes that are not included in the genome list but important for eisosome organization are *PKH1* and *PKH2* (Walther *et al.* 2007), encoding the two mammalian PDK1 homologs Pkh1 and Pkh2 (Casamayor *et al.* 1999). The serine/threonine kinases Pkh1/2 are physically associated with the eisosome, and Pil1 and Lsp1 have been shown to be Pkh substrates *in vitro* (Zhang *et al.* 2004; Walther *et al.* 2007). Pil1 phosphorylation *in vivo* has been proposed to be an important regulator that affects eisosome assembly, since change in the phosphorylation level of Pil1

leads to defects in eisosome organization (Walther *et al.* 2007; Luo *et al.* 2008). However, controversy lies in the precise role of Pkh1/2-mediated phosphorylation of Pil1 on eisosome assembly. Walther *et al.* (2007) originally showed that hyperphosphorylation of Pil1 by elevated Pkh1 and Pkh2 protein levels leads to a severe defect in Pil1 assembly on eisosome (Walther *et al.* 2007). In support, they observed that the phospho-mimicking *pil1(4D)* mutant in which serines 45, 59 and 230, and threonine 233 were changed to Aspartic acid (D) was dispersed mainly into the cytoplasm, thereby concluding that Pil1 is dephosphorylated when eisosome-bound and released upon phosphorylation to the cytoplasm (Walther *et al.* 2007) (figure 1A). The purified recombinant *pil14(D)* protein indeed was less competent in self-assembly compared to wild-type Pil1, pointing to the role of Pil1 phosphorylation that leads to impairment of Pil1 assembly on eisosome (Karotki *et al.* 2011). In agreement, Deng *et al.* (2009) reported that the decreased Pil1-GFP fluorescence level in the cytoplasm correlates with the dephosphorylation of Pil1, especially at the sites of Ser-230 and Thr-233. However, two lines of evidence argue against the notion that the dephophorylated Pil1 associates with eisosome. First, according to Luo *et al.* (2008), a non-phosphorylable Pil1 mutant in which multiple phosphorylation sites (up to six) were mutated to Alanine was mislocalized to the cytoplasm, suggesting that eisosome formation requires phosphorylation of Pil1 (figure 1B). Consistent with this observation, the addition of KP-372-1, which inhibits Pkh1/2 kinases, caused the increase of the pool of dephosphorylated Pil1, primarily situated in the cytoplasm, supporting the view that Pil1 phosphorylation is required for eisosome assembly (Baxter *et al.* 2011). At the moment it is not clear how different groups of researchers obtained two opposing results using similar Pil1 mutants. Nonetheless, the notion of reversible Pil1 phosphorylation and dephosphorylation controlling structural integrity of eisosome is highly acceptable. The existence of a phosphatase system involved in Pil1 dephosphorylation was suggested by Deng *et al.* (2009) as they observed an abrupt decrease in Pil1 and Lsp1 phosphorylation level during cell cycle. One potential candidate phosphatase for dephosphorylation of Pil1 would be calcineurin, which is known to be directly dephosphorylate another eisosome members Slm1/2 (Bultynck *et al.* 2006). If so, the consequence of Pil1 and Lsp1 dephosphorylation by calcineurin or other phosphatases yet to be identified on eisosome assembly should be further investigated to establish the biochemical recruitment mechanisms of Pil1.

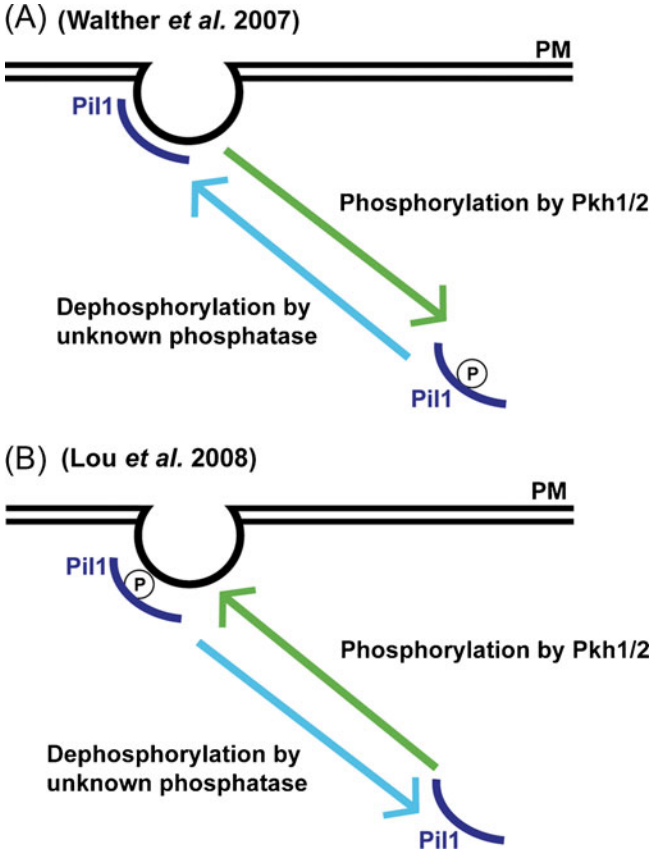

Figure 1. The controversy of the roles of reversible phosphorylation of Pil1 in eisosome organization. (**A**) The model suggests that Pil1 dephosphorylation promotes its recruitment to the eisosome. (**B**) The other model proposes that dephosphorylation of Pil1 is required for its disassembly from the eisosome.

5. Suggested roles of membrane lipids on eisosome

The plasma membrane is made up of several types of lipids, and these lipids play an important role in the recruitment of proteins. Sphingolipids consist of long saturated acyl chains that allow them to pack together tightly in the membrane (Brown and London, 2000). The micro-domain of the plasma membrane enriched in sphingolipids and cholesterol (Lemaire-Ewing *et al.* 2011) is often called a lipid raft, which is essential in cell signalling and protein trafficking (Staubach and Hanisch 2011). An early detergent lipid raft extraction assay showed that Can1, an integral MCC protein, localizes in lipid rafts and that a reduction in sphingolipids leads to the disruption of Can1 targeting (Malinska *et al.* 2003). The recruitment of another integral MCC protein Nce102 was also found to be dependent on a higher level of sphingolipids (Frohlich *et al.* 2009). Likewise, decreased content of sphingolipids in *lcb1-100* mutant (Zanolari *et al.* 2000) or treatment of myriocin that inhibits sphingolipid synthesis resulted in a loss of the MCC-associating cytoplasmic eisosome carrying Pil1 (Walther *et al.* 2007; Luo *et al.* 2008; Frohlich *et al.* 2009). These results apparently support the notion that membrane lipids are required for correct intracellular targeting of raft-associated proteins (Hearn *et al.* 2003). PI(4,5)P_2 (PIP_2) is a minor yet dynamic phospholipid

component of the plasma membrane which assists in the recruitment of a wide range of proteins to the plasma membrane (Strahl and Thorner 2007). Indeed, this was the case for Can1 targeting to the periphery of the plasma membrane; in *mss4*^{ts} temperature sensitive mutant that has ~10% of normal amount of PIP_2 (Desrivieres *et al.* 1998), the targeting of MCC protein Can1 was found to be impaired (Daquinag *et al.* 2007). However, the phenotypic defect in Can1 targeting in the mutant appears to be a secondary effect, perhaps caused by an actin cytoskeleton defect. More recently, Karotki *et al.* (2011) established a direct role of PIP_2 in organizing eisosome *in vivo*. In lower levels of PIP_2 (in *mss4*^{ts}), Pil1 progressively dissociated from the plasma membrane, mainly due to the fact that the probability of its direct interaction with PIP_2 significantly decreases while in an opposing condition (in *sjl1*Δ*sjl2*Δ) with higher chance, thus forming enlarged Pil1 puncta (Karotki *et al.* 2011) (E Murphy, unpublished). The fungal-specific sterol, ergosterol, enriched in lipid rafts, is another major lipid that plays a role in aiding the targeting of lipid–raft associating protein factors such as Gas1 and Pma1 (Bagnat *et al.* 2000). At least notable, but not complete, mistargeting of the MCC marker Can1 was observed in the condition where ergosterol levels were reduced (*erg6*Δ and *erg24*Δ) (Malinska *et al.* 2003). In contrast, unpublished data from Walther laboratory (Frohlich *et al.* 2009), including ours, strongly argue for a negligible role of ergosterol in targeting of the eisosome protein Pil1, since GFP fused Pil1 in those sterol mutant strains was still properly targeted to the plasma membrane.

6. Physiological functions of eisosome

It is curious that the liquid phase endocytic marker FM4-64 shows partial colocalization with Pil1 (Walther *et al.* 2006) (E Murphy, unpublished), and that the extent of spatial overlap between the eisosome and FM4-64 appears to increase as aberrant eisosome aggregates are formed by the loss of Pil1. However, it is now generally accepted that the eisosome does not mark receptor-mediated endocytic (RME) sites, on the basis of several lines of evidence: (1) the plasma membrane MCC protein Sur7, which colocalizes to the eisosome (Malinska *et al.* 2004; Walther *et al.* 2006), does not colocalize with RME sites carrying Rvs161 and Ede1 (Grossmann *et al.* 2008); (2) none of Abp1- and Sla1-GFP endocytic sites colocalized with Pil1-mCherry (Brach *et al.* 2011) and (3) Slm1, an eisosome marker, displayed only rare colocalization with Abp1-GFP, raising the possibility of random colocalization between them (Kamble *et al.* 2011). Nevertheless, what has emerged clearly is that a stable eisosome structure at the cell cortex is required for efficient receptor-mediated endocytosis occurring in the vicinity of the eisosome. This notion is supported by the observation that the rate of Ste3-mediated (a factor receptor) endocytosis to the vacuole in *pil1*Δ and *lsp1*Δ cells decreased significantly when compared to that of WT cells (Walther *et al.* 2006). Notably, the efficacy of receptor-mediated endocytosis dropped significantly in *PIL1*-lacking cells in which the synaptojanins (Sjl1/2) were severely mislocalized to the cytoplasm (Murphy *et al.* 2011). In particular, Sjl2 is a major plasma membrane phosphoinositide phosphatase that hydrolyses phosphates of PIP_2 (Guo *et al.* 1999), and a transient reduction of PIP_2 or the change of PIP_2 levels in a temporal manner via Sjl2 at endocytic sites is known to be critical for the efficiency of endocytosis (Sun *et al.* 2007; Toret *et al.* 2008). Thus, the failure of Sjl2 targeting to endocytic sites, most likely a side effect caused by loss of Pil1 (Murphy *et al.* 2011), might lead to a cascading failures in which an unsuccessful spatiotemporal regulation of PIP_2 levels, triggers the failure of endocytosis. At the moment it is plausible to propose that Pil1 is directly or indirectly involved in fine-tuning to regulate membrane phospholipid homeostasis. Furthermore, it appears that MCC/eisosome is a protective area that provides stability for the proteins localized there; the MCC component Can1 (transmembrane arginine transporter) was dissipated throughout the cell membrane and endocytosed at a much faster rate in *pil1*Δ cells than it is in WT cells, most likely due to the lack of the protective barrier in the mutant strain (Grossmann *et al.* 2008). It is yet important to note that the protective role of eisosome for its protein component from endocytosis seems not to be unanimously supported, based on the finding that the endocytosis rates of Can1 in *pil1*Δ and WT cells were essentially the same (Brach *et al.* 2011).

7. Concluding remarks

Work over the last 5–6 years has been focused on the contributions of Pil1 and its homologs in ascomycetes to MCC/eisosome organization. As discussed, a new, and we believe potentially very important piece of information regarding the structure and function of Pil1, has come with the recent finding that Pil1, containing a BAR domain, is able to self-assemble into filaments that serve as scaffold to reorganize membrane into an invagination. While evidence is mounting for a dynamic Pil1 assembly required for eisosome/MCC organization, precisely how these Pil1 fibres are used to help provide the force required for the invagination is not understood fully. In light of finding that inactivation of the PKC kinases Pkh1 and Pkh2 leads to the formation of extended net-like eisosome carrying Pil1, one can postulate that there must be a primary signal pathway with Pkh1/2 kinases that serve as a negative regulator of Pil1 assembly, as well as certain factors, including Nce102 that plays opposing regulatory roles. Therefore, in the future it is of great interest to understand the new and detail functions of already-known and yet-to-be-identified factors that influence Pil1 assembly

in vitro, and to further elucidate the physiological relevance of the factors in eisosome assembly *in vivo*. Along with at least learning more details about Pil1-lipids interaction, it is highly likely in the next few years to gain a better understanding of how the interaction of Pil1 with transmembrane MCC and cytosolic eisosome proteins regulates MCC/eisosome organization. All together, these studies during the coming years should provide even greater insights to the understanding of biological membrane organization and function.

References

Audhya A, Loewith R, Parsons AB, Gao L, Tabuchi M, Zhou H, Boone C, Hall MN, and Emr SD 2004 Genome-wide lethality screen identifies new PI4,5P2 effectors that regulate the actin cytoskeleton. *EMBO J.* **23** 3747–3757

Bagnat M, Keranen S, Shevchenko A and Simons K. 2000 Lipid rafts function in biosynthetic delivery of proteins to the cell surface in yeast. *Proc. Natl. Acad. Sci. USA* **97** 3254–3259

Baxter BK, Didone L, Oga D, Schor S and Krysan DJ 2011 Identification, in vitro activity and mode of action of phosphoinositide-dependent-1 kinase inhibitors as antifungal molecules. *ACS Chem. Biol.* **20** 502–510

Berchtold D and Walther TC 2009 TORC2 plasma membrane localization is essential for cell viability and restricted to a distinct domain. *Mol. Biol. Cell* **20** 1565–1575

Brach T, Specht T and Kaksonen M 2011 Reassessment of the role of plasma membrane domains in the regulation of vesicular traffic in yeast. *J. Cell Sci.* **124** 328–337

Brown DA and London E 2000 Structure and function of sphingolipid- and cholesterol-rich membrane rafts. *J. Biol. Chem.* **275** 17221–17224

Bultynck G, Heath VL, Majeed AP, Galan JM, Haguenauer-Tsapis R and Cyert MS 2006 Slm1 and slm2 are novel substrates of the calcineurin phosphatase required for heat stress-induced endocytosis of the yeast uracil permease. *Mol. Cell Biol.* **26** 4729–4745

Casamayor A, Torrance PD, Kobayashi T, Thorner J and Alessi DR 1999 Functional counterparts of mammalian protein kinases PDK1 and SGK in budding yeast. *Curr. Biol.* **9** 186–197

Daquinag A, Fadri M, Jung SY, Qin J and Kunz J 2007 The yeast PH domain proteins Slm1 and Slm2 are targets of sphingolipid signaling during the response to heat stress. *Mol. Cell Biol.* **27** 633–650

Deng C, Xiong X and Krutchinsky AN 2009 Unifying fluorescence microscopy and mass spectrometry for studying protein complexes in cells. *Mol. Cell. Proteom.* **8** 1413–1423

Desrivieres S, Cooke FT, Parker PJ and Hall MN 1998 MSS4, a phosphatidylinositol-4-phosphate 5-kinase required for organization of the actin cytoskeleton in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **273** 15787–15793

Fadri M, Daquinag A, Wang S, Xue T and Kunz J 2005 The pleckstrin homology domain proteins Slm1 and Slm2 are required for actin cytoskeleton organization in yeast and bind phosphatidylinositol-4,5-bisphosphate and TORC2. *Mol. Biol. Cell* **16** 1883–1900

Frohlich F, Moreira K, Aguilar PS, Hubner NC, Mann M, Walter P and Walther TC 2009 A genome-wide screen for genes affecting eisosomes reveals Nce102 function in sphingolipid signaling. *J. Cell Biol.* **185** 1227–1242

Frost A, Unger VM and De Camilli P 2009 The BAR domain superfamily: membrane-molding macromolecules. *Cell* **137** 191–196

Gallop JL and McMahon HT 2005 BAR domains and membrane curvature: bringing your curves to the BAR. *Biochem. Soc. Symp.* 223–231

Grossmann G, Malinsky J, Stahlschmidt W, Loibl M, Weig-Meckl I, Frommer WB, Opekarova M and Tanner W. 2008 Plasma membrane microdomains regulate turnover of transport proteins in yeast. *J. Cell Biol.* **183** 1075–1088

Grossmann G, Opekarova M, Malinsky J, Weig-Meckl I and Tanner W. 2007 Membrane potential governs lateral segregation of plasma membrane proteins and lipids in yeast. *EMBO J.* **26** 1–8

Guo S, Stolz LE, Lemrow SM and York JD 1999 SAC1-like domains of yeast SAC1, INP52 and INP53 and of human synaptojanin encode polyphosphoinositide phosphatases. *J. Biol. Chem.* **274** 12990–12995

Han G, Gable K, Kohlwein SD, Beaudoin F, Napier JA and Dunn TM 2002 The Saccharomyces cerevisiae YBR159w gene encodes the 3-ketoreductase of the microsomal fatty acid elongase. *J. Biol. Chem.* **277** 35440–35449

Hearn JD, Lester RL and Dickson RC 2003 The uracil transporter Fur4p associates with lipid rafts. *J. Biol. Chem.* **278** 3679–3686

Kabeche R, Baldissard S, Hammond J, Howard L and Moseley JB 2011 The filament-forming protein Pil1 assembles linear eisosomes in fission yeast. *Mol. Biol. Cell* **22** 4059–4067

Kamble C, Jain S, Murphy E and Kim K 2011 Requirements of Slm proteins for proper eisosome organization, endocytic trafficking and recycling in the yeast Saccharomyces cerevisiae. *J. Biosci.* **36** 79–96

Karotki L, Huiskonen JT, Stefan CJ, Ziolkowska NE, Roth R, Surma MA, Krogan NJ, Emr SD, Heuser J, Grunewald K, *et al.* 2011 Eisosome proteins assemble into a membrane scaffold. *J. Cell Biol.* **195** 889–902

Lemaire-Ewing S, Lagrost L and Neel D 2011 Lipid rafts: A signalling platform linking lipoprotein metabolism to atherogenesis. *Atherosclerosis* **221** 303–310

Lingwood D and Simons K. 2010 Lipid rafts as a membrane-organizing principle. *Science* **327** 46–50

Luo G, Gruhler A, Liu Y, Jensen ON and Dickson RC 2008 The sphingolipid long-chain base-Pkh1/2-Ypk1/2 signaling pathway regulates eisosome assembly and turnover. *J. Biol. Chem.* **283** 10433–10444

Malinska K, Malinsky J, Opekarova M and Tanner W 2003 Visualization of protein compartmentation within the plasma membrane of living yeast cells. *Mol. Biol. Cell* **14** 4427–4436

Malinska K, Malinsky J, Opekarova M and Tanner W 2004 Distribution of Can1p into stable domains reflects lateral protein segregation within the plasma membrane of living *S. cerevisiae* cells. *J. Cell Sci.* **117** 6031–6041

Murphy ER, Boxberger J, Colvin R, Lee SJ, Zahn G, Loor F and Kim K 2011 Pil1, an eisosome organizer, plays an important role in the recruitment of synaptojanins and amphiphysins to facilitate receptor-mediated endocytosis in yeast. *Eur. J. Cell Biol.* **90** 825–833

Olivera-Couto A, Grana M, Harispe L and Aguilar PS 2011 The eisosome core is composed of BAR domain proteins. *Mol. Biol. Cell* **22** 2360–2372

Paul S, Gable K, Beaudoin F, Cahoon E, Jaworski J, Napier JA and Dunn TM 2006 Members of the Arabidopsis FAE1-like 3-ketoacyl-CoA synthase gene family substitute for the Elop proteins of Saccharomyces cerevisiae. *J. Biol. Chem.* **281** 9018–9029

Reijnst P, Walther A and Wendland J 2011 Dual-colour fluorescence microscopy using yEmCherry-/GFP-tagging of eisosome components Pil1 and Lsp1 in *Candida albicans*. *Yeast* **28** 331–338

Seger S, Rischatsch R and Philippsen P 2011 Formation and stability of eisosomes in the filamentous fungus *Ashbya gossypii*. *J. Cell Sci.* **124** 1629–1634

Staubach S and Hanisch FG 2011 Lipid rafts: signaling and sorting platforms of cells and their roles in cancer. *Expert Rev. Proteom.* **8** 263–277

Stradalova V, Stahlschmidt W, Grossmann G, Blazikova M, Rachel R, Tanner W and Malinsky J 2009 Furrow-like invaginations of the yeast plasma membrane correspond to membrane compartment of Can1. *J. Cell Sci.* **122** 2887–2894

Strahl T and Thorner J 2007 Synthesis and function of membrane phosphoinositides in budding yeast *Saccharomyces cerevisiae*. *Biochim. Biophys.* Acta **1771** 353–404

Suetsugu S, Toyooka K and Senju Y. 2010 Subcellular membrane curvature mediated by the BAR domain superfamily proteins. *Semin. Cell Dev. Biol.* **21** 340–349

Sun Y, Carroll S, Kaksonen M, Toshima JY and Drubin DG 2007 PtdIns(4,5)P2 turnover is required for multiple stages during clathrin- and actin-dependent endocytic internalization. *J. Cell Biol.* **177** 355–367

Toret CP, Lee L, Sekiya-Kawasaki M and Drubin DG 2008 Multiple pathways regulate endocytic coat disassembly in *Saccharomyces cerevisiae* for optimal downstream trafficking. *Traffic* **9** 848–859

Vangelatos I, Roumelioti K, Gournas C, Suarez T, Scazzocchio C and Sophianopoulou V. 2010 Eisosome organization in the filamentous ascomycete *Aspergillus nidulans*. *Eukaryot. Cell* **9** 1441–1454

Walther TC, Aguilar PS, Frohlich F, Chu F, Moreira K, Burlingame AL and Walter P 2007 Pkh-kinases control eisosome assembly and organization. *EMBO J.* **26** 4946–4955

Walther TC, Brickner JH, Aguilar PS, Bernales S, Pantoja C and Walter P 2006 Eisosomes mark static sites of endocytosis. *Nature* **439** 998–1003

Young ME, Karpova TS, Brugger B, Moschenross DM, Wang GK, Schneiter R, Wieland FT and Cooper JA 2002 The Sur7p family defines novel cortical domains in *Saccharomyces cerevisiae*, affects sphingolipid metabolism and is involved in sporulation. *Mol. Cell Biol.* **22** 927–934

Zanolari B, Friant S, Funato K, Sutterlin C, Stevenson BJ and Riezman H 2000 Sphingoid base synthesis requirement for endocytosis in Saccharomyces cerevisiae. *EMBO J.* **19** 2824–2833

Zhang X, Lester RL and Dickson RC 2004 Pil1p and Lsp1p negatively regulate the 3-phosphoinositide-dependent protein kinase-like kinase Pkh1p and downstream signaling pathways Pkc1p and Ypk1p. *J. Biol. Chem.* **279** 22030–22038

Ziolkowska NE, Karotki L, Rehman M, Huiskonen JT and Walther TC 2011 Eisosome-driven plasma membrane organization is mediated by BAR domains. *Nat. Struct. Mol. Biol.* **18** 854–856

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