Functional patchworking at the plasma membrane

Sébastien Léon¹ & David Teis²

Lipids and proteins are not evenly distributed within the plasma membrane (PM), but instead segregate laterally into many specialized microdomains whose functional relevance is not clear. In this issue, Busto *et al* (2018) demonstrate that substrate flux through a nutrient transporter drives the lateral relocation of the transporter between specific microdomains at the yeast PM, suggesting that regulating the lateral plasma membrane compartmentalization for individual proteins could be a general process for cellular response to environmental conditions.

The EMBO Journal (2018) 37: e100144

See also: JV Busto *et al* (August 2018)

The heterogeneity of lateral compartments in the plasma membrane (PM) of all cells has been recognized for a long time. Their size and lifetime range from highly dynamic nano-domains that exist for only a few seconds to stable macroscopic domains. Yet, we have a limited understanding of the function, structure, and organization of PM domains, because their characterization is technically challenging. Important progress was made using quantitative imaging techniques to chart the landscape of the PM of the budding yeast *Saccharomyces cerevisiae* (Spira *et al*, 2012). Yeast is an excellent model system to systematically study the molecular mechanisms underlying lateral segregation since diffusion rates of membrane proteins in the yeast PM are much slower than those seen in mammalian cells. In addition, fluorescent tags can be easily introduced at the endogenous loci of studied genes, leading to wild-type expression levels.

Early observations indicated that the arginine permease Can1 clusters in patch-like compartments named MCCs (membrane compartment occupied by Can1), whereas the H⁺-ATPase Pma1 organizes in a network-like domain, referred to as MCP (membrane compartment of Pma1; Malinska *et al*, 2003). MCCs are associated with eisosomes, which are furrow-like membrane invagination that are formed by BAR domain-containing proteins (Lsp1, Pil1; Stradalova *et al*, 2009; Walther *et al*, 2006). The MCC/eisosomes were initially proposed to have an important role in mediating endocytic uptake from the yeast PM (Walther *et al*, 2006). However, it turned out that MCCs in fact protect a subset of plasma membrane proteins from endocytosis (Grossmann *et al*, 2008). Additional PM domains were described in yeast, including the MCT (containing the target of rapamycin complex 2, TORC2; Berchtold & Walther, 2009), the MCL (containing the sterol transporters Ltc3/4; Murley *et al*, 2017), and the MCW (containing the cell wall stress sensor Wsc1; Heinisch *et al*, 2010). Importantly, a systematic investigation of 46 different PM proteins revealed an even higher complexity in protein distribution, ranging from discrete nano-scale patches to almost continuous networks with various levels of overlap between individual proteins (Spira *et al*, 2012). How this PM patchwork contributes to the function of the proteins that are partitioned into the respective domains had not been studied so far.

In this issue, Busto and co-workers carefully characterize the changes in lateral PM segregation exhibited by the methionine transporter Mup1 in response to nutrient availability (Fig 1; Busto *et al*, 2018). They reveal how segregation of Mup1 is

controlled and how its lateral distribution contributes to endocytosis and function of the transporter. Mup1 is an ideal model protein to study these processes, because it is tightly regulated by availability of its substrate, methionine. In the absence of methionine, Mup1 is highly expressed and concentrated at the cell surface, while addition of methionine enables the α -arrestin Art1 to rapidly recruit the Nedd4-like HECT type ubiquitin ligase Rsp5 to Mup1 (Lin *et al*, 2008). The ensuing ubiquitination is then required for Mup1 endocytosis.

Interestingly, in the absence of methionine, the majority of Mup1 partitions into MCCs. This localization depends on eisosomes, sphingolipids, the putative sphingolipid sensor Nce102, and TORC2 signaling. Inside MCCs, Mup1 appeared to be protected from endocytosis. However, upon addition of methionine to the growth medium, Mup1 moved out of MCCs into a unique network-like domain at the PM. This relocation only occurred when methionine passed through the transporter into the cell. Substrate transport requires conformational changes of the transporter from the outward open to the inward open state. Mutations in Mup1 that block substrate transport impaired exit from the MCCs, suggesting that conformational changes in the transporter during the substrate transport cycle drive its lateral relocation at the PM. Importantly, exit from MCCs and entry into the network-like domain were essential for Art1/Rsp5-dependent ubiquitination of Mup1 and subsequent endocytosis. These findings are consistent with the idea that transporter activity is tightly coupled to ubiquitination and that ubiquitin initiates endocytosis by triggering the assembly of the endocytic machinery. A general use of

1 UMR 7592 Centre National de la Recherche Scientifique/Université Paris-Diderot, Institut Jacques Monod, Sorbonne Paris Cité, Paris, France

2 Division of Cell Biology, Biocenter, Medical University of Innsbruck Austria, Innsbruck, Austria. E-mail: david.teis@i-med.ac.at

DOI 10.15252/emboj.2018100144 | Published online 30 July 2018

Figure 1. The cartoon represents the functional patchworking at the plasma membrane for the methionine transporter Mup1.

(1) Mup1 is sequestered in the MCC in its open outward conformation and protected from ubiquitination and endocytosis. The lateral diffusion (arrows) out of the MCC and into the network-like domain is slow. (2) Upon transport of substrate (methionine) through Mup1, the change to the open inward conformation drives the efficient relocation from the MCC into the network-like domain. (3) In the network-like domain, Mup1 is ubiquitinated by Art1/Rsp5 (not shown) which results in the recruitment of the endocytic machinery, actin, and subsequent endocytosis.

dynamic lateral segregation might allow tight coupling between PM localization and activity of nutrient transporters with the availability of their substrates.

This conclusion is echoed by recent studies, in which substrate transport was found to induce partitioning of the arginine permease Can1 and the uracil permease Fur4 out of MCCs prior to endocytosis (Gournas *et al*, 2018; Moharir *et al*, 2018). While conformational changes induced by substrate transport again seem to be directly involved in

lateral relocation of these transporters, altered interactions of the transporters with specific lipids may also contribute to dynamic partitioning. Moreover, these findings can also be extended to other types of membrane protein and into plants and metazoans. In human cells, the clustering of the potassium channel Kv1.3 in PM microdomains of T cells protects it from ubiquitination and subsequent endocytosis (Martinez-Marmol *et al*, 2017). This process ultimately tunes the immunological response.

Additionally, a transport-dependent ubiquitination mechanism was proposed for the glutamate receptor, GLT-1, involving the beta-arrestin-1/Nedd4-2 ubiquitination complex (Ibanez *et al*, 2016). Placed in the context of the study by Busto *et al* (2018), these examples collectively suggest that lateral compartmentalization of PM proteins plays an important role in controlling cellular physiology of all living cells.

As often the case in science, this study provides the basis for exciting future studies. Among the most interesting questions is the molecular nature of the network-like domain that is populated by Mup1 and Can1 upon transport-driven exit from MCCs. Do they go into the same domain? Which proteins and lipids organize these networks? Do the networks always exist or are they only formed by the active transporters that exit the MCCs. How does the network domain promote transporter ubiquitination by the α -arrestins/Rsp5 complex and subsequent endocytosis?

Not only nutrient excess, but also nutrient limitation can induce endocytosis. This 'starvation-induced' endocytosis results in the selective down-regulation of many different transporters. Interestingly, a fraction of these transporters remains segregated in MCCs and is protected from endocytosis (Gournas *et al*, 2018). The molecular events leading to endocytosis in response to nutrient limitation have not yet been defined but may as well include lateral partitioning and/or global lipid changes.

Finally, the identification and characterization of factors that are required to organize the functional patchwork of the PM will provide a better molecular understanding of how cells communicate with their environment, protect their contents, and maintain homeostasis.

References

- Berchtold D, Walther TC (2009) TORC2 plasma membrane localization is essential for cell viability and restricted to a distinct domain. *Mol Biol Cell* 20: 1565–1575
- Busto JV, Elting A, Haase D, Spira F, Kuhlman J, Schäfer-Herte M, Wedlich-Söldner R (2018) Lateral plasma membrane compartmentalization links protein function and turnover. *EMBO J* 37: e99473
- Gournas C, Gkionis S, Carquin M, Twyffels L, Tyteca D, Andre B (2018) Conformation-dependent partitioning of yeast nutrient transporters into starvation-protective membrane domains. *Proc Natl Acad Sci USA* 115: E3145–E3154

- Grossmann G, Malinsky J, Stahlschmidt W, Loibl M, Weig-Meckl I, Frommer WB, Opekarova M, Tanner W (2008) Plasma membrane microdomains regulate turnover of transport proteins in yeast. *J Cell Biol* 183: 1075–1088
- Heinisch JJ, Dupres V, Wilk S, Jendretzki A, Dufrene YF (2010) Single-molecule atomic force microscopy reveals clustering of the yeast plasma-membrane sensor Wsc1. *PLoS One* 5: e111104
- Ibanez I, Diez-Guerra FJ, Gimenez C, Zafra F (2016) Activity dependent internalization of the glutamate transporter GLT-1 mediated by beta-arrestin 1 and ubiquitination. *Neuropharmacology* 107: 376–386
- Lin CH, MacGurn JA, Chu T, Stefan CJ, Emr SD (2008) Arrestin-related ubiquitin-ligase adaptors regulate endocytosis and protein turnover at the cell surface. *Cell* 135: 714–725
- Malinska K, Malinsky J, Opekarova M, Tanner W (2003) Visualization of protein compartmentation within the plasma membrane of living yeast cells. *Mol Biol Cell* 14: 4427–4436
- Martinez-Marmol R, Styrzewska K, Perez-Verdaguer M, Vallejo-Gracia A, Comes N, Sorkin A, Felipe A (2017) Ubiquitination mediates Kv1.3 endocytosis as a mechanism for protein kinase C-dependent modulation. *Sci Rep* 7: 42395
- Moharir A, Gay L, Appadurai D, Keener J, Babst M (2018) Eisosomes are metabolically regulated storage compartments for APC-type nutrient transporters. *Mol Biol Cell* <https://doi.org/10.1091/mbc.E17-11-0691>
- Murley A, Yamada J, Niles BJ, Toulmay A, Prinz WA, Powers T, Nunnari J (2017) Sterol transporters at membrane contact sites regulate TORC1 and TORC2 signaling. *J Cell Biol* 216: 2679–2689
- Spira F, Mueller NS, Beck G, von Olshausen P, Beig J, Wedlich-Soldner R (2012) Patchwork organization of the yeast plasma membrane into numerous coexisting domains. *Nat Cell Biol* 14: 640–648
- Stradalova V, Stahlschmidt W, Grossmann G, Blazikova M, Rachel R, Tanner W, Malinsky J (2009) Furrow-like invaginations of the yeast plasma membrane correspond to membrane compartment of Can1. *J Cell Sci* 122: 2887–2894
- Walther TC, Brickner JH, Aguilar PS, Bernales S, Pantoja C, Walter P (2006) Eisosomes mark static sites of endocytosis. *Nature* 439: 998–1003