Fungal Plasma Membrane Domains

This review presents a domain-oriented view of the lateral organisation of the fungal plasma membrane, emphasizing on recent advances in the partitioning mechanisms of proteins into distinct compartments and their physiological significance

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Abstract

The plasma membrane (PM) performs a plethora of physiological processes, the coordination of which requires spatial and temporal organization into specialized domains of different sizes, stability, protein/lipid composition and overall architecture. Compartmentalization of the PM has been particularly well studied in the yeast *Saccharomyces cerevisiae*, where five non-overlapping domains have been described: The Membrane Compartments containing the arginine permease Can1 (MCC), the H^+-ATPase Pma1 (MCP), the TORC2 kinase complex (MCT), the sterol transporters Ltc3/4 (MCL), and the cell wall stress mechanosensor Wsc1 (MCW). Additional cortical foci at the fungal plasma membrane are the sites where clathrin-dependent endocytosis

occurs, the sites where the external pH sensing complex PAL/Rim localizes, and sterol-rich domains found in apically-grown regions of fungal membranes.

In this review we summarize knowledge from several fungal species regarding the organization of the lateral PM segregation. We discuss the mechanisms of formation of these domains, and the mechanisms of partitioning of proteins there. Finally, we discuss the physiological roles of the best-known membrane compartments, including the regulation of membrane and cell wall homeostasis, apical growth of fungal cells, and the newly emerging role of MCCs as starvation-protective membrane domains.

1. Introduction

The plasma membrane (PM) delineates the cell, provides mechanical support and is the place where multiple cellular processes of central importance take place, including selective solute transport, establishment of cell polarity, interactions with extracellular components and other cells, organisation of membrane fluxes via endocytosis and exocytosis, and initiation of signalling responses in response to extracellular cues [Reviewed in (Malinsky *et al.* 2013; Schuberth and Wedlich-Söldner 2015; Gaubitz *et al.* 2016; Sezgin *et al.* 2017)]. During the last four decades, it has increasingly become evident that the complexity of the functions fulfilled by the PM correlates with its lateral organisation into distinct membrane domains differing in their biophysical properties and biochemical composition (Karnovsky *et al.* 1982; Simons and Ikonen 1997; Edidin 2003; Simons and Sampaio 2011; Carquin *et al.* 2016; Sezgin *et al.* 2017). The first membrane domains that have been extensively studied are the lipid rafts in mammalian cells (also referred to as membrane rafts, detergent-resistant membranes -DRMs- or detergent-insoluble membranes) (Simons and Ikonen 1997), reviewed in ((Lichtenberg, Goñi and Heerklotz 2005; Simons and Sampaio 2011; Sonnino and Prinetti 2012; Malinsky *et al.* 2013; Maryanovich and Gross 2013; Bernardino de la Serna *et al.* 2016; Carquin *et al.* 2016; Cheng and Nichols 2016; Levental and Veatch 2016; Sezgin *et al.* 2017; Bieberich 2018).

Contrary to mammalian cells, membrane domains have long been underappreciated in fungi. Among several reasons is that there was quite an uncertainty about DRMs in *S. cerevisiae*, since several DRM-insoluble proteins show different lateral PM segregation patterns. Initial work suggested that, similarly to the situation in animal cells, PM proteins of *S. cerevisiae* can be classified as DRM-soluble and -insoluble (Bagnat *et al.* 2012). Subsequently, work using confocal microscopy suggested the existence of domains at the PM of budding yeast (Malínská *et al.* 2003; Grossmann *et al.* 2007, 2008), but the correlation with DRMs remained unclear. The situation was resolved later when it was shown that probably all PM proteins are DRM-insoluble, at least when present at the PM, while they become DRM-soluble once they get endocytosed (Lauwers and André 2006; Lauwers *et al.* 2007). Thus, extraction of fungal membrane proteins by detergents seems to not be a criterion for partitioning in specialised PM compartments. However, heterogeneities at the

plasma membrane of *S. cerevisiae* have been reported as early as 1963 (Moor and Mühletttaler 1963). Despite a large "lag-phase" since then, currently a significant fraction of our knowledge on lateral PM compartmentalization comes from studies using fungi, and this became a very active and rapidly evolving field of research. In *S. cerevisiae* in particular, it is now well established that several plasma membrane domains exist, ranging from static patches to more dynamic network-like domains (Mueller, Wedlich-Söldner and Spira 2012; Spira *et al.* 2012), leading to a patchwork organisation model for the PM (Figure 1).

Membrane domains identified in fungi (Table 1) and various non-fungal species differ significantly in four parameters: size, dynamics, composition, and mechanism of formation. In respect to size, PM heterogeneities have been reported to range from the nano- and submicrometric- to the micrometric scale. Nanoscale domains have a size of 2-10 nm for dimer/oligomers and membrane-associated protein complexes (Kusumi *et al.* 2011) and 20-100 nm for raft-like domains, e.g. lipid rafts, caveolae and tetraspanins (Parton and Simons 2007; Lingwood and Simons 2009; Yáñez-Mó *et al.* 2009). Submicrometric domains are >200 nm and <1 µm, e.g. fungal eisosomes (Moseley 2018) and several domains in mammalian cells [reviewed in (Carquin *et al.* 2016)]. Finally, large, several µm long, micrometric domains exist, like the ones formed *in vitro* in artificial membranes [reviewed in (Carquin *et al.* 2016)], or the Ceramide-rich platforms formed *in vivo* in response to stress (Grassmé *et al.* 2003; Stancevic and Kolesnick 2010). In respect to dynamics, membrane domains range from very dynamic (half-life of sec) and transiently formed heterogeneities of the PM to static and ever-present, well-defined structures that could even be considered as organelles. Regarding composition, membrane domains are considered to preferentially accumulate specific lipids and/or proteins, a feature that is related to their function. Finally, PM domains are formed by a variety of mechanisms. The initial idea of differential segregation of lipids being the forming mechanism of lipid rafts suggested the existence of lipid-driven membrane domains. On the other hand, several mechanisms of protein-driven membrane domains are well documented. Such protein-driven domains are membrane areas where lateral PM diffusion of proteins is restricted by cytoskeleton fences, while proteins can also be anchored to these fences (Sako and Kusumi 1995; Kusumi *et al.* 2011). Protein-driven domains are also organized by specialized scaffolding proteins, as is the case of eisosomes in fungi and caveolae in mammalian cells. Additional types of lateral PM compartmentalization are formed by systematic targeted vesicular sorting, like in the case of the apical growth of fungal hyphae (Peñalva 2010; Hernández-González *et al.* 2018), as well as by the juxtaposition of stable membranes forming specific Membrane Contact Sites (MCS), like the Endoplasmic Reticulum-Plasma Membrane (ER-PM) MCS (Prinz 2014). The above characteristics of membrane domains are interconnected and related to their function. For example, it is more likely that small, lipid-driven nanoscale domains are transiently formed and mainly consist of lipids (e.g. lipid rafts), while more stable domains are usually larger and their organization and composition is mainly dictated by proteins (Carquin et al., 2016, Kusumi et al., 2011).

In fungi, the characteristics of membrane domains are strongly influenced by two particularities of the PM, namely its particular lipid composition (Farnoud *et al.* 2015), and the fact that it is surrounded by a cell wall. Because of the cell wall, it is turgor pressure that directs the shape of the fungal PM. Consequently, there is no need for an underlying

cytoskeletal network to support the plasma membrane, like in mammalian cells . On the contrary, the extremely slow diffusion of membrane proteins at the fungal PM is independent of the actin cytoskeleton, and rather depends on the high content and the nature of sterols and sphingolipids (Valdez-Taubas and Pelham 2003; Malinsky and Opekarová 2016; Bianchi *et al.* 2018; Gournas *et al.* 2018). The main sterol of fungal cells is ergosterol, which modulates the fluidity, permeability and integrity of the fungal PM (Dufourc 2008; Lv, Yan and Jiang 2016). Compared to cholesterol (the main sterol in animal cells), ergosterol is significantly more efficient in promoting membrane domain formation (Xu *et al.* 2001) and has greater packing efficiency of lipid acyl chains compared to cholesterol (Cournia, Ullmann and Smith 2007; Abe and Hiraki 2009). Saturated sphingolipids are known to preferentially associate with ergosterol (Tanaka and Tani 2018), are present mostly in the outer leaflet of the PM and mainly organized in membrane domains by association with ergosterol and specific proteins. Fungal complex sphingolipids contribute to the formation of the highly ordered "gel-like" fungal membrane domain formation (Björkbom *et al.* 2010; Aresta-Branco *et al.* 2011; Vecer *et al.* 2014; Herman *et al.* 2015).

The present review deals with all so far described PM compartments in fungi (Table 1), with special emphasis on the recently expanded number of membrane domains in the yeast *S. cerevisiae*. In addition, we summarize and discuss the recent advances in the physiological significance of lateral compartmentalization of the fungal PM. Particular focus is given in the organisation, assembly and the mechanisms of protein partitioning in the Membrane Compartment of Can1 (MCC), the most intensively-studied membrane domain in fungi. The emerging complexity and the multitude of roles of the MCC can serve as an example for what to be expected for the more recently identified and less studied membrane domains.

2.1. The Membrane Compartment of Can1 / Eisosome

The more intensively-studied PM domain in fungi is the membrane compartment containing the arginine permease Can1 (MCC), initially identified by confocal microscopy as large, static patches at the plasma membrane of *S. cerevisiae* (Malínská *et al.* 2003). Using electron microscopy, MCCs were shown to correspond to furrow-like invaginations of the plasma membrane and to colocalize subcortically (Stradalova *et al.* 2009) with a previously identified protein scaffold termed "eisosome" (Walther *et al.* 2006). These structures have long ago been observed in *S. cerevisiae* and *Schizosaccharomyces pombe* (Moor and Mühletttaler 1963; Takeo, Shigeta and Takagi 1976; Walther, Müller and Schweingruber 1984), and have recently been found in several cell-walled organisms, like fungi, lichens and microalgae (Lee *et al.* 2015). Although some phylogenetic analysis of the conservation of eisosomal proteins exists (Olivera-Couto *et al.* 2011; Scazzocchio, Vangelatos and Sophianopoulou 2011), it remains to be shown experimentally that the invaginations found in these organisms indeed correlate with eisosomal proteins. In the literature the term MCC has been used to describe the region of the plasma membrane, along with integral membrane proteins, while the term eisosome refers to the subcortical protein complex. Given that functions of the constituents of MCC and eisosome are interdependent, the term MCC/eisosome has extensively been used in order to describe the whole domain. In *S.*

cerevisiae, MCC invaginations are 200-300 nm long, 50-100 nm deep and 30-50 nm wide (Figure 2A) (Stradalova *et al.* 2009; Moseley 2018), while in *S. pombe* they are much longer (1-2 μm) (Kabeche *et al.* 2011). MCCs host, in addition to the arginine permease Can1, several more membrane proteins, like several nutrient transporters (Grossmann *et al.* 2007; Brach, Specht and Kaksonen 2011) and members of the Sur7 (Young *et al.* 2002) and Nce102 families of tetraspan proteins (Grossmann *et al.* 2008; Fröhlich *et al.* 2009). Moreover, several cytoplasmic proteins are bound subcortically at eisosomes, like the major scaffolding BAR domain-containing proteins Pil1 and Lsp1 (Olivera-Couto *et al.* 2011; Ziółkowska *et al.* 2011), the coiled-coil protein Seg1 (Moreira *et al.* 2012), the Pkh1 and Pkh2 kinases, and the Slm1 and Slm2 proteins (Liu *et al.* 2005; Bultynck *et al.* 2006; Walther *et al.* 2007). Homologues of Pil1, Nce102 and Seg1 are essential for the formation of MCCs in several fungal species (see below). Additional proteins have been localized in MCCs [Detailed lists of proteins can be found in (Douglas and Konopka 2014)]. Although these proteins have no role in MCC formation/stability, they are related to the various physiological roles of the domain, analyzed below. An important part of the knowledge regarding MCC/eisosomes comes from studies in *S. cerevisiae*. Thus, the sections below have an *S. cerevisiae*-based description of eisosomes, frequently referring to work in other fungal systems when relevant.

2.1.1. MCC/eisosome **assembly and organization**

Subcellular distribution - A typical cell of *S. cerevisiae* in exponential phase contains around 20-50 invaginations/Pil1-foci (1.5-2.5 invaginations per μm^2 of cell surface) (Stradalova *et al.* 2009; Gournas *et al.* 2018), while this number increases at least two-fold in cells from stationary phase cultures (Takeo, Shigeta and Takagi 1976; Gournas *et al.* 2018). This increase in stationary phase is also observed in *S. pombe* (Walther, Müller and Schweingruber 1984; Kabeche *et al.* 2011) and *Candida albicans* (Douglas, Wang and Konopka 2013), and is analogous to the high number of eisosomes observed in the quiescent conidiospores and ascospores of *Aspergillus nidulans* (Vangelatos *et al.* 2010; Athanasopoulos *et al.* 2013). Contrary to the above, eisosomes are absent from the apical regions and growing membranes, like the newly formed buds of *S. cerevisiae* (Grossmann *et al.* 2008; Moreira *et al.* 2009), the tip in the hyphae of *A. nidulans* (Vangelatos *et al.* 2010; Athanasopoulos *et al.* 2013, 2015; Pinar and Peñalva 2017) and *Ashbya gossypii* (Seger, Rischatsch and Philippsen 2011), as well as the apical growth region in the pseudohyphae of *C. albicans* and in the cells of *S. pombe* (Kabeche *et al.* 2011). Thus, eisosome abundance is higher in non-growing membranes/conditions, which is in agreement with the newly-emerging role of MCCs/eisosomes as starvation-protective membrane domains [(Gournas *et al.* 2018), details below)]. In *A. nidulans* in particular, different types of eisosomes could exist, as eisosomes in different cell types have distinct protein composition. In conidia and the head of the germlings, high levels of PilA, PilB, SurG and Nce102 but not SlmA (sole orthologue of Slm1/2 in *A. nidulans*) have been detected. In the membranes of germtubes, eisosomes contain PilA and SlmA, but not PilB or SurG, which are cytosolic or targeted to the vacuole for degradation, respectively (Vangelatos *et al.* 2010; Athanasopoulos *et al.* 2015; Pinar and Peñalva 2017).

Eisosome assembly by Pil1 – In budding yeast, the cytoplasmic protein scaffold is composed mainly by two extremely abundant proteins, Pil1 and Lsp1 (Figure 2A) (Walther *et al.* 2006) and their homologues in *C. albicans* (Reijnst, Walther and Wendland 2011), fission yeast [Pil1-Pil2 (Kabeche *et al.* 2011)] and *A. nidulans* [PilA and PilB (Vangelatos *et al.* 2010)]. In the absence of this scaffold in *S. cerevisiae*, membrane proteins of the MCC become homogeneously distributed at the PM (Walther *et al.* 2006; Grossmann *et al.* 2008). Interestingly, in *A. nidulans* the absence of PilA leads to increased intracellular distribution of SurG and Nce102 in endosomes and to their vacuolar sorting for degradation (Vangelatos *et al.* 2010; Athanasopoulos *et al.* 2015). In most fungi where eisosomes have been studied to date, only one Pil1 homologue is important for MCC/eisosome organization, while the role of their paralogues remains elusive. Exception to this is *C. albicans*, where both Pil1 and Lsp1 contribute to the formation of furrows (Wang *et al.* 2016), and the entomoparasite *Beauveria bassiana* belonging to the Sordariomycetes, where the two paralogues (Pil1A, Pil1B) localize to static, punctate spots at cell periphery independently of each other (Zhang *et al.* 2017).

Pil1 and Lsp1 are banana-shaped N-BAR domain-containing proteins (for Bin1, Amphiphysin, and Rvs proteins), which can sense/induce membrane curvature (Ziółkowska *et al.* 2011). Indeed, they are able to self-assemble, they bind to Phosphatidylinositol 4,5-bisphosphate [PI(4,5)P_2]-containing membranes, tubulate membranes *in vitro*, and form helical tubules at the inner membrane of the invagination (Karotki *et al.* 2011). Pil1 in particular is important for sculpting the membrane and forming the invagination. Time-lapse and FRAP experiments in various fungal species have shown that Pil1 and Lsp1 cortical foci are not dynamic (Malinska *et al.* 2004; Walther *et al.* 2006; Kabeche *et al.* 2011; Karotki *et al.* 2011; Athanasopoulos *et al.* 2015). However, more detailed analyses, both in budding and fission yeast, have shown that Pil1 assemblies are not totally static. More specifically, using scanning techniques with autocorrelation analysis, it was shown that Pil1 and Lsp1 exhibit slow dynamics, consistent with a binding-dissociation equilibrium of eisosomal and cytoplasmic pools (Olivera-Couto *et al.* 2015). Similarly, in fission yeast it was recently shown, using single molecule approaches, that Pil1 molecules are partially dynamic and exchange specifically at the edges of eisosomes and not along the core (Okeke *et al.* 2016). These results suggest that polymerization/depolymerization of Pil1 assemblies occurs at the edges of the Pil1 filaments.

MCC/eisosome assembly is regulated by phosphorylation of Pil1. Both Pil1 and Lsp1 are known to be phosphorylated by the mitogen-activated protein kinase (MAPK) Slt2 and a pair of redundant protein kinases, Pkh1 and Pkh2, that localize in eisosomes (Zhang, Lester and Dickson 2004; Walther *et al.* 2007; Mascaraque *et al.* 2012). Although contradictory effects have been reported about the role of these modifications on eisosome assembly (Zhang, Lester and Dickson 2004; Walther *et al.* 2007; Luo *et al.* 2008; Deng, Xiong and Krutchinsky 2009; Fröhlich *et al.* 2009; Ziółkowska *et al.* 2011), a mutant of Pil1 resistant to Pkh1/2 phosphorylation-induced disassembly has been described. This mutant, Pil1(4A), carries Ala substitutions of 4 phosphate-accepting residues and is resistant to Pkh1/2 phosphorylation in response to depletion of sphingolipids

(see also below), suggesting that phosphorylation of Pil1 at these residues promotes the disassembly of eisosomes (Walther *et al.* 2007; Fröhlich *et al.* 2009).

MCC/eisosome assembly by Nce102 - Nce102 is the only transmembrane protein known to be required for eisosome assembly (Grossmann *et al.* 2008; Fröhlich *et al.* 2009). In *S. cerevisiae*, in the absence of Nce102 the furrow-like membrane invaginations are lost (Stradalova *et al.* 2009), while the number of Pil1 foci is reduced by 70% (Grossmann *et al.* 2008; Fröhlich *et al.* 2009). Similarly, the homologues of Nce102 in *S. pombe* [SpFhn1, (Kabeche *et al.* 2011)] and *A. nidulans* [AnNce102, (Athanasopoulos *et al.* 2015)] are required for proper MCC assembly. Deletion of SpFhn1 reduces Pil1 spots to 15%. Intriguingly, deletion of AnNce102 reduces to 33% the PilA spots only in the head of the germlings and not along hyphae, suggesting once more that eisosomes in *A. nidulans* have different composition (and roles potentially), in the various developmental stages (Athanasopoulos *et al.* 2015). Nce102 has well-conserved homologues in ascomycetes, as well as a paralogue in *S. cerevisiae*, Fhn1 (Loibl *et al.* 2010). Fhn1 is poorly expressed in aerobically grown cells and complements deletion of Nce102 when overexpressed (Loibl *et al.* 2010). Nce102 contains a four transmembrane helix domain termed MARVEL (MAL and related proteins for vesicle trafficking and membrane link) (Douglas, Wang and Konopka 2013) and its N and C termini are oriented toward the cytosol (Loibl *et al.* 2010). The C-terminal tail is well conserved and the last 10 residues are important for promoting MCC assembly (Loibl *et al.* 2010). Surprisingly, the homologue of Nce102 in *C. albicans* is not essential for eisosome organization and localizes in MCCs only in saturated cell cultures (Douglas, Wang and Konopka 2013). Since *C. albicans* has eight more MARVEL domain-containing members, more could contribute to MCC/eisosome organization. Although details on the molecular mechanisms by which Nce102 promotes eisosome assembly are missing, several lines of evidence suggest that Nce102, when in MCCs, inhibits Pil1 phosphorylation by the Pkh1/2 kinases (Fröhlich *et al.* 2009). The most prominent is that Pil1(4A)-containing eisosomes, where Pil1 molecules are devoid of Pkh1/2 phosphorylation sites (Walther *et al.* 2007), are resistant to deletion of Nce102 regarding the formation of MCC patches and the partitioning there of Sur7 and Can1 (Fröhlich *et al.* 2009; Gournas *et al.* 2018).

Eisosome assembly by Seg1 - In *S. cerevisiae, de novo* formed eisosomes are excluded from the tips of emerging buds, as mentioned above. Although the mechanism for this exclusion is not fully understood, it could, at least in part, be explained by the cell-cycle regulated expression of Pil1 and Nce102 and the time-consuming assembly of MCCs (Grossmann *et al.* 2008; Moreira *et al.* 2009). It has recently been suggested that a certain degree of membrane curvature could be required in order for Pil1 to form dense helical tubules and to, consequently, lead to the formation of PM invaginations (Bharat, Hoffmann and Kukulski 2018). This was based on the observation by Cryo-EM of shallow Pil1-containing eisosomes. Although these shallow eisosomes could alternatively be a response to increased membrane tension (Kabeche, Howard and Moseley 2015a; Riggi *et al.* 2018), their existence is

consistent with previous results showing that Pil1 is not the protein initiating the formation of eisosomes. This role seems to be played by Seg1, a protein important for proper MCC assembly and known to precede Pil1/Lsp1 during eisosome formation (Moreira *et al.* 2012). Importantly, Seg1 protein levels were shown to control the shape and length of eisosomes, while Seg1 is believed to form a platform that is subsequently stabilized by the binding of Pil1 and Lsp1 (Moreira *et al.* 2012). Similarly, in *S. pombe* the Seg1-like protein Sle1 was found to be necessary for generating and stabilizing the extremely long Pil1 filaments (Kabeche *et al.* 2011; Moreira *et al.* 2012; Kabeche, Howard and Moseley 2015b). Interestingly, formation of the long invaginations observed in *S. pombe* seems to require both SpSle1 and SpPil1, as their simultaneous heterologous expression in *S. cerevisiae* leads to formation of elongated furrows (Vaskovicova *et al.* 2015).

Given all the above, a potential mechanism for the formation of new MCCs/eisosomes is the following: Pil1/Lsp1 molecules first bind sparsely to Seg1-predefined, non-curved membranes and form shallow eisosomes. These shallow eisosomes deepen in a subsequent step, following a decrease in membrane tension and/or increase in the levels of available PM, a step potentially requiring Nce102. This would somehow lead to the close packing of Pil1/Lsp1 and the formation of the invagination (Bharat, Hoffmann and Kukulski 2018).

Besides the proteins that mediate MCC organization, additional proteins have been reported to indirectly affect eisosome assembly (Grossmann *et al.* 2008; Fröhlich *et al.* 2009). These proteins seem to affect the synthesis of lipids and sterols, and will be discussed along with the physiological roles of MCCs/eisosomes.

2.1.2. The physiological roles of MCCs/eisosomes

The physiological roles of MCCs have remained for years elusive, since in several fungi mutants lacking MCCs/eisosomes do not show significant growth defects, at least under the conditions tested. However, the last years it has started becoming evident that MCCs are important for optimal response to several stress conditions, and this is related to their connection with both TORC1 and TORC2 (Babst 2019). A still not well understood function of MCCs is likely related to the eisosome partitioning of the Pkh1/2 kinases (Walther *et al.* 2007; Luo *et al.* 2008). Signaling via the Pkh1/2 is required for proper response to several stresses. In all cases, the target of Pkh1/2 is found in another subcellular location, thus it seems that eisosomes act as negative regulators of Pkh1/2 via spatial control of their localization, e.g. the kinases are inactive when associated with eisosomes (Malinsky and Opekarová 2016). Protein targets of Pkh1/2 include the kinases Ypk1 and Ypk2 (Casamayor *et al.* 1999), Sch9 (Liu *et al.* 2005) and Pkc1 (Inagaki *et al.* 2015). Ypk1/2 are orthologues of the mammalian AGC kinases and are the main effectors of TORC2 that control lipid biosynthesis (Niles *et al.* 2014). Ypk1/2 localization is cytoplasmic, while they are recruited to the Membrane Compartment of TORC2 (MCT) by the Slm1/2 proteins (Berchtold *et al.* 2012; Niles *et al.* 2012) (Their role is discussed in chapter 2.3). Pkc1, a protein kinase C of *S. cerevisiae*, is the most upstream known kinase controlled by the Cell Wall Integrity (CWI) pathway (Verna *et al.* 1997). Sch9, the homologue of the mammalian AKT kinase and

effector of TORC1, localizes at the vacuolar membrane (Urban *et al.* 2007). Sch9 integrates signals about nutrient availability or the presence of stressful conditions and controls protein synthesis, longevity and stress tolerance (Eltschinger and Loewith 2016). Pkh1/2-dependent phosphorylation of Sch9 could be related to the presence of transporters in MCCs and their role as starvation-protective membrane domains (discussed in detail in paragraph 2.1.2.3).

2.1.2.1. Regulation of plasma membrane/lipid homeostasis

Early evidence in *S. cerevisiae* suggested that eisosomes are related to sphingolipid (SL) biosynthesis, as a mutant lacking three Sur7-like proteins (*sur7*Δ *fmp45*Δ *ynl194c*Δ) was reported to have altered levels of SLs (Young *et al.* 2002). In addition, Pil1 (Phosphorylation induced by long chain bases) and Lsp1 (Long chain bases stimulate phosphorylation) were initially identified to be differentially phosphorylated in response to sphingolipid precursors (Zhang, Lester and Dickson 2004). This phosphorylation was subsequently shown to be mediated by the Pkh1/2 kinases (Walther *et al.* 2007; Luo *et al.* 2008) and to control eisosome assembly (see paragraph 2.1.1). The Pkh1/2 kinases have been shown to partially localize in eisosomes, at least when overexpressed (Walther *et al.* 2007; Luo *et al.* 2008). Nce102, one of the two MCC organisers, was proposed to act as a sensor of the quantity of SLs at the PM (Fröhlich *et al.* 2009). According to the authors, in SL replete conditions, Nce102 resides in MCCs and inhibits the Phk1/2 kinases found there, thus promoting eisosome assembly. Upon SL depletion, Nce102 moves out of MCCs and becomes homogeneously distributed at the PM, leading to activation of Pkh1/2, the phosphorylation of Pil1 and the disassembly of eisosomes. (Walther *et al.* 2007; Fröhlich *et al.* 2009). This disassembly would result in the release of Slm1/2 from the MCC, and their relocation to the MCT in order to stimulate SL biosynthesis via TORC2 activation (Berchtold *et al.* 2012) (for details see chapter 2.6). Evidence in *A. nidulans* is in agreement with the above, as deletions of *nce102* or *pilA* partially suppress the genetic or pharmacological inhibition of SL biosynthesis (Athanasopoulos *et al.* 2015).

Eisosomes have also been reported to sense and control the levels of $PI(4,5)P_2$. This phosphoinositide is significantly enriched at the PM of fungal cells and its level is controlled by the PI4P 5-kinase (Mss4) and the synaptojanin-like lipid phosphatases (Inp *et al.* 1998; Odorizzi, Babst and Emr 2000). Pil1, in both *S. pombe* and *S. cerevisiae,* has been shown to be important for the recruitment at the PM of the synaptojanin-like lipid phosphatases, Syj1 and Inp51/Sjl1 (Fröhlich *et al.* 2014; Kabeche *et al.* 2014). Among several membrane proteins able to bind $PI(4,5)P_2$, Pil1 is known to bind this lipid *in vitro* and to require $PI(4,5)P_2$ for tubulating membranes (Karotki *et al.* 2011). Defects in $PI(4,5)P_2$ production affect the assembly of eisosomes, showing an interdependence of MCCs and $PI(4,5)P_2$ similar to the one of eisosomes and SLs (Fröhlich *et al.* 2014). In *S. pombe* in particular, a linear assembly pathway linking the hydrolysis of $PI(4,5)P_2$ with eisosome assembly has been identified through a genetic screen (Kabeche *et al.* 2014), while data from both yeasts suggest that this pathway functions in parallel to TORC2 signaling (Fröhlich *et al.* 2014; Kabeche *et al.* 2014).

2.1.2.2. Regulation of cell wall stress

An important but poorly understood function of MCCs/eisosomes is their relationship to the integrity of the cell wall. Although eisosome disassembly, via the release of the Pkh1/2 kinases, should affect the phosphorylation of Pkc1 and thus activate the CWI pathway (details in chapter 2.5), accumulating evidence suggests the existence of a direct link between eisosomes and the cell wall. First, furrow-like invaginations and Pil1-like BAR-domain containing proteins are found in several cell walled organisms, including fungi, lichens and microalgae (Lee *et al.* 2015). Second, in several fungal species, members of the MCC-resident Sur7-like transmembrane proteins are related to cell wall integrity. Indeed, defects of mutants carrying deletions of *sur7* have been reported in *C. albicans* (see below), and in *Trichoderma atroviride*, where deletion of the Sur7-like member Sfp2 causes defects in cell wall assembly, hyphal growth and endocytosis (Atanasova *et al.* 2018).

The role of Sur7 has been studied mostly in *C. albicans*, where, contrary to most fungi, *sur7*Δ cells have important growth defects (Foderaro, Douglas and Konopka 2017). More specifically, CaSur7 is important for growth in several stress-promoting conditions, like high copper levels and elevated temperature (Alvarez *et al.* 2008; Alvarez, Douglas and Konopka 2009; Wang *et al.* 2011; Douglas *et al.* 2012). This is related to altered organization of the cell wall, as *sur7*Δ cells have thicker but weaker cell wall and show extensive, aberrant invaginations of the cell wall (Alvarez, Douglas and Konopka 2009; Wang *et al.* 2011; Douglas *et al.* 2012; Foderaro, Douglas and Konopka 2017). The same phenotypes are observed in *pil1*Δ *lsp1*Δ cells, and interestingly they can be rescued by overexpression of Sur7, suggesting that for these phenotypes eisosomes act by promoting the proper localization and/or stability of Sur7 (Wang *et al.* 2011, 2016; Douglas and Konopka 2019).

The aberrant cell wall invaginations mentioned above correlate with altered organization of the plasma membrane, as evidenced by the mislocalization of cortical actin, septins, and the $PI(4,5)P_2$ lipid (Alvarez *et al.* 2008; Douglas *et al.* 2012; Wang *et al.* 2016). Consistently, it has recently been suggested that *sur7*Δ cells exhibit high levels of exposure of phosphatidylserine (PS) to the extracellular side of the membrane (Douglas and Konopka 2019), that could be the cause for the increased permeability of cells when exposed to copper. Importantly, *sur7*Δ cells share this phenotype with other *C. albicans* mutants affected for actin organization and endocytosis. Thus, defects in lateral PM organization and polar distribution of lipids seem to be newly-uncovered, additional mechanism related to copper toxicity (Douglas and Konopka 2019).

In other fungi, the defects of *sur7*Δ cells are less pronounced. In *S. cerevisiae* deletion of Sur7 only results in a minor phenotype, while a triple Sur7-like protein mutant, *sur7*Δ *fmp45*Δ *ynl194c*Δ, shows altered levels of SL biosynthesis and defects in sporulation (Young *et al.* 2002). Pun1, another Sur7-like protein, has been found to be important for pseudohyphal growth (Xu *et al.* 2010), while Fmp45 affects the recovery from stationary phase (Martinez *et al.* 2004). In *A. nidulans*, deletion of *surG* only leads to a minor increase in the resistance to itraconazole, suggesting that SurG could affect ergosterol metabolism/transport (Vangelatos *et al.* 2010).

It is important mentioning that Sur7 and Nce102 of *C. albicans* are required for the formation of invasive hyphae *in vitro* and for virulence in mice, while *sur7*Δ cells show poor growth in phagosomes of macrophages (Douglas *et al.* 2012; Douglas, Wang and Konopka 2013). Thus, MCCs seem to be important for pathogenicity. This is also the case in the mycoparasite *Trichoderma atroviride*, where Sfp2 is essential for infection of the prey fungus (Atanasova *et al.* 2018). Eisosomes are also essential for pathogenicity of the entomopathogenic fungus *Beauveria bassiana* to the larvae of *Galleria mellonella* (Zhang *et al.* 2017).

2.1.2.3. MCCs as starvation-protective transporter-reservoir domains: mechanisms of partitioning and protection from endocytosis

Background - Although Can1 was the first protein found to concentrate in MCCs, for many years there has been considerable controversy on the role of this partitioning. Eisosomes have initially been described as sites of endocytosis (Walther *et al.* 2006), based on data showing that eisosomes co-localize with FM4-64-labelled invaginations of the plasma membrane and experiments showing that the endocytosis of Ste3 occurred more slowly in *pil1*Δ cells lacking eisosomes. Subsequent results showing that MCCs are furrow-like invaginations of the plasma membrane (Stradalova *et al.* 2009), proposed another interpretation: the furrows keep the cortical ER away from specific sites of the PM, thus resulting in a specific PM domain devoid of cER where the endocytic foci form (Stradalova *et al.* 2012) (see Figure 1). This view was consistent with experiments showing that eisosomes do not co-localize with sites of actin-dependent endocytosis and are not required for the endocytosis of a series of protein cargos or lipids (see paragraph 2.7.1).

In line with the above, Grossmann et al., 2008 had already proposed that MCCs are sites negatively regulating the substrate-induced downregulation of Can1. However, Brach et al., 2011 showed that there was no difference in substrate-induced endocytosis of Can1 in *wt* and *pil1*Δ cells. Moreover Spira et al., 2012 proposed that Can1 MCC partitioning affected the function of the transporter, based on canavanine (a toxic analogue of Arg that is transported solely by Can1) sensitivity assays. The above in total suggested that MCCs are not related to endocytosis, but rather control the activity of transporters. Recently however, new genetic tools related to transporter endocytosis, better resolution in confocal/super-resolution microscopy, and detailed biochemical measurements of the kinetic characteristics of the transporter in different conditions made possible to re-evaluate the role of MCCs in transporter downregulation in *S. cerevisiae*. These recent studies pointed out to eisosomes as endocytosis-protective sites for at least three MCC-resident transporters: Can1, the methionine transporter Mup1 and the uracil transporter Fur4 (Bianchi *et al.* 2018; Busto *et al.* 2018; Gournas *et al.* 2018; Moharir *et al.* 2018). Three of these studies made use of genetic tools to uncouple the endocytosis of the transporter from its lateral PM segregation. As these tools proved essential for this work, a brief description of the mechanisms of transporter endocytosis is included below.

Downregulation of membrane proteins occurs by endocytosis and sorting to the vacuole for degradation. This sorting in *S. cerevisiae* and other fungi requires the covalent fusion on

transporters of K63-linked poly-Ub chains (Lauwers, Jacob and Andre 2009; Lauwers *et al.* 2010) by the single member of the Nedd4-like HECT Ubiquitin (Ub)-ligase in *S. cerevisiae*, Rsp5 (Hein *et al.* 1995), HulA in *A. nidulans* (Gournas *et al.* 2010). The recognition of transporters by the Ub-ligase is mediated by specific proteins, the α-arrestins, which act as adaptors between the transporter and Rsp5 (Lin *et al.* 2008; Becuwe *et al.* 2012a). 14 α-arrestin proteins exist in *S. cerevisiae*, and provide the necessary specificity to Rsp5-dependent ubiquitylation and endocytosis of the target membrane proteins. This is achieved by the tight regulation of α-arrestins in response to distinct physiological signals (e.g. preferable carbon or nitrogen source availability, stress conditions, presence of substrate), as they are under control of several signaling pathways (MacGurn *et al.* 2011; Becuwe *et al.* 2012b; Merhi and André 2012; Karachaliou *et al.* 2013; O'Donnell *et al.* 2013; Crapeau, Merhi and André 2014). Art1 is the major α-arrestin required for the substrate-induced ubiquitylation and endocytosis of several highly specific nutrient transporters in *S. cerevisiae*, including Can1 and Mup1 (Lin *et al.* 2008; Nikko and Pelham 2009; Ghaddar *et al.* 2014b). Art1 is under the control of TORC1 (MacGurn *et al.* 2011), well known to sense nitrogen availability (Eltschinger and Loewith 2016). We and others have recently shown that an additional level for the selectivity of Art1 towards its target transporters exists (Guiney, Klecker and Emr 2016; Gournas *et al.* 2017). Briefly, despite the fact that all amino acids can activate TORC1 upon uptake into nitrogen-deprived cells, TORC1-stimulated Art1 is only able to recognize the transporter whose substrate is available. This happens because the sequence that Art1 recognizes in Can1 and Mup1 (Art1 binding site, Art1BS) seems to be hidden when the transporter is catalytically inert, and exposed only when the transporter actively transports its substrate and transiently shifts to an Inward-Facing (IF) state of the transport cycle (Gournas *et al.* 2017). Fur4 is also endocytosed by the presence of uracil (Séron *et al.* 1999), and in this case several arrestins seem to contribute to Fur4 substrate-induced endocytosis (Nikko and Pelham 2009; Keener and Babst 2013). Similar to the Art1BS, Fur4 was previously reported to contain a PEST or "degron sequence" at its N-tail that is phosphorylated by Casein Kinase 1 and required for ubiquitylation (Marchal, Haguenauer-Tsapis and Urban-Grimal 2000), as well as a LID domain close to TM1 and proposed to sense conformational changes (Keener and Babst 2013). Thus, the degron sequence is most probably recognized by several α-arrestins and only when exposed by significant conformational rearrangements sensed by the LID domain.

The mechanism of transporter partitioning in MCCs- Not all molecules of Can1, Mup1 and Fur4 are in MCCs, as observed using super-resolution microscopy and Single Particle Tracking (Bianchi et al. 2018) or methodologies uncoupling lateral segregation and endocytosis of these nutrient transporters (Busto et al., 2018; Gournas et al., 2018a; Moharir et al., 2018). Mup1 molecules, in particular, have been shown to not be homogeneously distributed even outside the eisosomes, but to reside in a network-like compartment that does not colocalize with the MCP (Busto *et al.* 2018), thus potentially being yet another membrane domain (see also Paragraph 2.7.2). Within the eisosome, super-resolution microscopy and TIRF experiments on Can1 and Mup1 showed that the majority (76 % for Can1) of MCC-resident transporter molecules remain at the outer MCC region and do not reach the center of the furrow (eisosome centroid) (Bianchi *et al.* 2018; Busto *et al.* 2018). This is similar to the situation reported for Sur7 using electron microscopy and immunogold

labelling (Stradalova *et al.* 2009), and suggest that membrane proteins cannot easily diffuse at the Pil1/Lsp1 rich coat of the centroid of eisosomes. Indeed, FRAP experiments of Can1 have shown that the transporter population diffuses 3-fold more slowly in MCCs than outside MCCs (Brach, Specht and Kaksonen 2011; Gournas *et al.* 2018), while Single Particle Tracking experiments indicate that a significant percentage of single Can1 molecules can even be transiently immobilized to the eisosome centroid region (Bianchi *et al.* 2018). The above results point out to a mechanism of transient anchoring of transporters to a limited number of positions within eisosomes (Figure 3) (Bianchi *et al.* 2018). Although the nature of the anchoring suggests protein-protein interactions, MCC/eisosomal proteins interacting with the transporters and required for their partitioning there have up to now not been identified. Nce102 was shown to be required for the partitioning of Mup1 in MCCs (Busto *et al.* 2018), however, the absence of Nce102 only weakly affected the MCC partitioning of Can1 (Gournas *et al.* 2018).

Nce102, the most upstream MCC factor proposed to participate in the sensing of sphingolipids (Fröhlich *et al.* 2009), could alternatively regulate the MCC partitioning of transporters by affecting the availability of sphingolipids, directly at the eisosome or in general at the plasma membrane. Indeed, it has been shown that an intact complex sphingolipid biosynthesis pathway is essential for both Can1 and Mup1 MCC partitioning (Busto *et al.* 2018; Gournas *et al.* 2018). For Can1 in particular, it has been shown that SLs are required for the slower diffusion of the permease in MCCs (Gournas *et al.* 2018). For Mup1, intact TORC2 signaling has additionally been shown to be required, as Mup1 MCC partitioning is lost upon TORC2 inhibition (Busto *et al.* 2018). Given that both the inhibition of TORC2 and of SL biosynthesis lead to the depletion of complex SLs (on the contrary, inhibition of SL biosynthesis results in stimulation of TORC2 activity) (Roelants *et al.* 2011; Berchtold *et al.* 2012), these results suggest an essential role of complex SLs in the MCC partitioning of transporters. A potential mechanism could involve a direct role of certain complex SL species, specifically enriched in MCCs and contributing to the conformation-dependent anchoring of the transporters there. Such a conformation-dependent binding of a lipid (cholesterol) to a transporter has been recently reported, using Cryo-EM, for the human Serotonin transporter SERT (Coleman *et al.* 2019), which, like fungal amino acid transporters, also belongs to the APC superfamily. Interestingly, the cholesterol molecule was found in proximity to TM1, which is shown to undergo major conformational rearrangements when SERT shifts from the Inward- to the Outward-Facing conformation (Coleman *et al.* 2019), a situation very similar to that modeled for Can1 (Gournas *et al.* 2017). Consequently, it would be tempting to consider that Can1 would bind to specific lipids (e.g. SLs) only when found in an OF conformation, and this would mediate its transient anchoring at MCCs (Figure 3). Alternatively, SL depletion could cause a general defect in the organization of the plasma membrane and indirectly affect transporter MCC partitioning. A third scenario is that a depletion of SLs would also reduce the level of sterols, the functional partners of SLs, in eisosomes. Filipin staining suggests that ergosterol is indeed enriched in MCCs (Grossmann *et al.* 2007), while intact ergosterol biosynthesis is required for proper sorting of the heterologous plant transporter Hup1 in MCCs (Grossmann *et al.* 2008). Since up to now it is a matter of debate whether SLs are enriched in MCCs [as suggested by the SL-sensing role of eisosomal proteins, (Fröhlich *et al.* 2009; Berchtold *et al.* 2012)] or are

preferentially distributed at the MCP, as suggested by the SL requirement for the DRM partitioning and the plasma membrane stability of Pma1 and Gap1 (Wang and Chang 2002; Lauwers and André 2006; Lauwers *et al.* 2007), further experiments are required to understand how SLs contribute to the MCC partitioning of transporters.

The situation is clearer regarding the effect of substrate on the MCC partitioning of transporters. Can1, Mup1, Fur4 and Tat2 (tryptophan transporter) lose preferential MCC segregation in the presence of their substrate (Brach, Specht and Kaksonen 2011; Bianchi *et al.* 2018; Busto *et al.* 2018; Gournas *et al.* 2018; Moharir *et al.* 2018). In the presence of Arg, the immobile population of Can1 in MCCs diminishes, while the whole population shifts towards outside the MCC (Bianchi *et al.* 2018). Consistently, in the presence of substrate and in conditions of deficient Ub-dependent endocytosis, the slower diffusion of Can1 in MCCs is abolished (Gournas *et al.* 2018). Several lines of evidence suggest that this occurs when the transporter shifts to an Inward-Facing (IF) state of the transport cycle (Gournas *et al.* 2018). But how could the conformation of the transporter affect its MCC partitioning? As mentioned above, a change in conformation could alter the affinity of the transporter for specific lipids, like in the case of the human SERT (Coleman *et al.* 2019). Alternatively, a conformational change would alter other physicochemical properties of the TMs, like their length spanning the lipid bilayer or their surface area. These properties have recently been reported to be important for partitioning of mammalian membrane proteins in lipid rafts (Lorent *et al.* 2017). Since also in *S. cerevisiae* it is the TMs that define the patchwork domain segregation of membrane proteins (Spira *et al.* 2012), it is probable that one or all of the above control the conformation-dependent domain partitioning of transporters.

A mechanism for the partitioning of transporters in MCCs (Figure 3) consistent with all data is the following: In the absence of their substrate, transporters are predominantly found in an Outward-Facing conformation of the transport cycle, and preferentially segregate in MCCs due to (indirect or direct) SL-dependent transient trapping there. A substrate-induced shift of the transporter to an IF state somehow abolishes this conditional trapping (loss of interaction with an MCC/eisosome protein and/or lipid) and transporter molecules now freely diffuse at the PM. This model is also compatible with the fact that the membrane potential (ΔΨ) is important for the MCC partitioning of Can1 (Grossmann *et al.* 2007; Herman *et al.* 2015). Can1, like all membrane proteins, is adapted to the ΔΨ and has an asymmetric distribution of polar residues, with negatively and positively charged residues being more abundant on the extracellular side and cytoplasmic sides of the membrane, respectively (Ghaddar *et al.* 2014a). Consequently, disruption of the ΔΨ is expected to affect the conformation of Can1, thus also affecting its MCC partitioning.

An additional mechanism for the steric exclusion of membrane proteins from MCCs has also been proposed (Bianchi *et al.* 2018). According to this model, large cytosolic domains of membrane proteins prohibit them from entering the MCC due to steric hindrance. In support of this model, removal of the large cytosolic tail of Pma1 resulted in failure of exclusion from MCC, while the presence of additional cytoplasmic domains to the C-tail of Can1 reduced its MCC localization (Bianchi *et al.* 2018). Moreover, the flexibility of the cytoplasmic domains could affect the MCC partitioning of membrane proteins, since the absence of a linker between Can1/Lyp1 and the C-terminally tagged fluorescent protein

could reduce the MCC partitioning of the transporters (Bianchi *et al.* 2018). However, as Bianchi et al. 2018 discussed, although a steric hindrance-based mechanism could have a complementary role in excluding very large proteins from MCCs, it cannot be sufficient to explain the MCC partitioning of several transporters, as the differences in size between them are rather small. For example, even within the Yeast Amino acid Transporter (YAT) family of proton symporters (Gournas, Athanasopoulos and Sophianopoulou 2018) - member of the APC superfamily (Gournas *et al.* 2016)- where the proteins share the same structure and more or less the same size, several transporters are homogeneously distributed at the PM [e.g. Tat1, Dip5 and Gap1 (Abe and Iida 2003; Lauwers *et al.* 2007; Hatakeyama *et al.* 2010; Spira *et al.* 2012)]. Gap1 in particular does not preferentially segregate in MCCs (Lauwers *et al.* 2007), even in the absence of its substrate (Gournas *et al.* 2018), despite having N- and C-tails that are nearly identical in size with Can1 and Lyp1 (Ghaddar *et al.* 2014a, 2014b; Gournas *et al.* 2016), while fusion of the C-tail of Gap1 to Can1 is not sufficient to exclude the Arg permease from MCCs (Bianchi et al., 2018). Consistently, Mup1 without the N- and C-tails is still able to normally segregate in MCCs (Busto *et al.* 2018). The same also applies to C-tail less Can1 (Bianchi et al., 2018), all the mutants of the Ala-scanning mutagenesis of Can1's N-tail (Gournas *et al.* 2017), and the N-tail less Fur4 (Moharir *et al.* 2018).

MCCs as sites for protection from endocytosis - Substrate-induced exit of Can1, Mup1 and Fur4 from the MCC occurs independently of ubiquitin and the ubiquitylation machinery. More precisely, the loss of MCC partitioning occurs even in the absence of the Ub-acceptor Lys residues, the α-arrestins involved in their ubiquitylation, or in a hypomorph mutant of the Rsp5 Ub-ligase (Busto *et al.* 2018; Gournas *et al.* 2018; Moharir *et al.* 2018). This strongly indicates that ubiquitylation of transporters occurs outside MCCs. This was shown for Can1 and Mup1 after trapping them to MCC, by fusing the GFP-binder protein (Rothbauer *et al.* 2008) to Sur7 or Nce102 (Busto *et al.* 2018; Gournas *et al.* 2018). Using this approach, the MCC-trapped Can1-GFP was shown to have unaltered kinetic characteristics (K_m and V_{max}), by measuring the uptake rate of radiolabeled Arg, while under these conditions, fully functional Can1 was shown to be resistant to both ubiquitylation and endocytosis (Gournas *et al.* 2018). Similarly, Mup1-GFP trapped in MCCs was active and protected from degradation (Busto *et al.* 2018). Importantly, this protection of Can1 and Mup1 required intact eisosomes, as it was not present in *pil1*Δ cells. This was also the case for the C-tail-less Mup1 translationally fused to Ub, showing that eisosomes can even protect mono-ubiquitylated cargos from degradation. A protective role of eisosomes on the substrate-induced downregulation of Fur4 has also been shown; in the absence of MCCs and the presence of uracil the amount of Fur4 at the plasma membrane was reduced (Moharir *et al.* 2018). Interestingly, it was suggested that Fur4 is not active when in MCCs, based on a slightly lower accumulation of uracil, as determined by HPLC measurements of the uracil content of cells after 10 min accumulation. This, however, is not the case for Can1 and Mup1, for which radiolabeled amino acid uptake measurements showed that MCCs do not affect the activity or kinetic characteristics of the transporters, but only their abundance at the plasma membrane (Busto *et al.* 2018; Gournas *et al.* 2018). Nevertheless, it cannot be ruled out that not all MCC-resident transporters are active when in MCCs. Overall, recent studies converge to indicate that MCCs act as reservoir domains for transporters, protecting them from downregulation. This protection could occur either at the level of ubiquitylation

or at the level of endocytosis. Nowadays, it is well established that eisosomes do not colocalize with markers of clathrin-dependent endocytosis (see Paragraph 2.7.1). Thus, a plausible explanation for the protection of transporters in MCCs could be that formation of endocytic vesicles is not feasible there. An alternative scenario is that the ubiquitylation machinery (the Rsp5-α-arrestin complexes) does not have access to MCC-localized transporters, or cannot be recruited to eisosomes. In support of the latter, it is known that Art1 translocates from the Golgi to cortical foci of the PM only when it is activated by TORC1 in the presence of cycloheximide (increasing the pool of internal amino acids) or medium rich in nitrogen (MacGurn *et al.* 2011; Baile *et al.* 2019). Although the identity of these cortical PM foci is not known, it is possible that these are sites of endocytosis, as Rsp5 has similarly been reported to transiently localize to cortical, Abp1-labelled, sites of endocytosis (Kaminska *et al.* 2011). Consistently, Busto et al. (2018) have shown that the network-like domain that Mup1 occupies once outside MCCs, transiently co-localizes with endocytic sites, while ubiquitylated Mup1 seems to actively re-direct the endocytic machinery to this region.

MCCs as starvation-protective transporter reservoir domains - Despite showing that MCCs are sites where transporters are protected from Ub-dependent endocytosis, the fact that the transporters exit MCCs in the presence of their substrates questions their endocytosis-protective role. Exception to this is the case for Fur4, whose uracil-triggered endocytosis is enhanced in *pil1*Δ and *nce102*Δ cells. Moharir et al. (2018) suggested that a lower activity of Fur4 in MCCs could potentially protect Fur4 from endocytosis when in MCCs. This explanation was also supported by mathematical modeling, showing that a potentially lower activity of Fur4 in MCCs would result in slower endocytosis of the transporter in the presence of MCCs (Moharir *et al.* 2018). However, at least for Can1, we and others have reported that Arg-induced endocytosis is not accelerated in the absence of eisosomes (Brach, Specht and Kaksonen 2011; Gournas *et al.* 2018). Thus, we speculated eisosomes should protect transporters from other endocytosis-promoting conditions, in the absence of their substrate. We found such a condition to be endocytosis provoked as the cells grow and the cultures reach the stationary phase of growth (SP) (Gournas *et al.* 2018). This bulk endocytosis of many membrane proteins is known to occur in parallel with autophagy and is believed to help cells retrieve free amino acids (Jones *et al.* 2012; Crapeau, Merhi and André 2014). Under these conditions, cells keep a part of intact Can1 at the plasma membrane and are able to efficiently resume growth when nutrient conditions improve (Gournas *et al.* 2018). This is not the case, however, for *pil1*Δ cells that have remained in SP for 12 h. In these cells, nearly all Can1 is degraded, while cells show reduced efficiency to recover from SP in media with Arg as the sole nitrogen source, suggesting that MCCs act as reservoir domains protecting transporters from downregulation in SP. Consistently, MCCs themselves are regulated by nutrient availability. Their number at the plasma membrane is low in nitrogen rich conditions and increases in nitrogen poor conditions or nitrogen starvation (Gournas *et al.* 2018), consistent with a 3-fold difference in protein levels (Villers *et al.* 2017). The more pronounced increase in MCCs, however is seen as cells reach the SP. In SP, a 4-5 fold increase in the number of MCCs is observed, that also correlates with increased intensity of Pil1 in single eisosomes (Gournas *et al.* 2018), suggesting that the size of the invagination also increases. Indeed, it has been reported from the 70's using electron microscopy that the number and size of invaginations at the plasma membrane of both *S.*

cerevisiae and *S. pombe* increases in SP (Takeo, Shigeta and Takagi 1976; Walther, Müller and Schweingruber 1984). Importantly, we could additionally observe a 3-fold slower diffusion of Can1 in MCCs from cells in SP, compared to cells from mid log phase (Gournas *et al.* 2018). This slower diffusion in MCCs helps protecting a significant fraction of transporter from molecules from endocytosis in SP, and allows cells to more efficiently resume growth once nutrients become available again. Importantly, Lsp1, the paralogue of Pil1 whose function remained unknown, was found to be important for the expansion of MCCs in SP, as in *lsp1*Δ cells only a minor increase in the number of MCCs could be observed in SP (Gournas *et al.* 2018). Interestingly, Lsp1 has been reported to be among the first hits in a phosphoproteomics screen for targets of the Npr1 kinase upon TORC1 inhibition by rapamycin (MacGurn *et al.* 2011). As Npr1 is a well-known effector of TORC1 in response to nitrogen availability (Schmidt *et al.* 1998), this suggests a potential mechanism for the regulation of the number and size of MCCs in response to nutrient availability (Gournas *et al.* 2018).

Consistent with the view of MCCs as starvation-protective membrane domains, several lines of evidence suggest that eisosomes are generally important in nutrient limitation and SP and not only for protecting transporters from degradation. First, preliminary evidence suggest that in *A. nidulans* eisosomes are similarly regulated by nutrient availability (Athanasopoulos A, Gaitanos S, Gournas C and Sophianopoulou V, unpublished results). Such an intra-species conservation indicates that eisosomes could have an evolutionarily conserved function in nutrient-limiting conditions. Second, in *S. cerevisiae*, the Sur7-like MCC protein Pun1 is essential for the nitrogen stress-induced filamentation (Xu *et al.* 2010). Moreover, two eisosome components, Pst2 and Fmp45, are required for the recovery of cells from SP (Martinez *et al.* 2004). In *B. bassiana*, deletion of Pil1A or Pil1B differentially affected the expression of autophagy-related genes (Zhang *et al.* 2017). Importantly, Xrn1 exoribonuclease, the main mRNA decay enzyme, has been reported to accumulate in MCCs in stationary phase (Grousl *et al.* 2015). This accumulation occurs after glucose exhaustion, leads to inactivation of mRNA degradation activity that requires intact MCCs (Vaškovičová *et al.* 2017) and is specific for Xrn1, as other components of the mRNA decay machinery do not follow Xrn1 in MCCs (Grousl *et al.* 2015). MCC partitioning of Xrn1 is reversible, as glucose re-addition induces the release of Xrn1 from eisosomes (Grousl *et al.* 2015). Thus, spatial segregation of Xrn1 is a specific mechanism to regulate the degradation of mRNAs. Especially in SP, protecting specific mRNAs from degradation seems to be an energy-saving mechanism for the glucose-depleted cells, which could help them more efficiently recover when a fermentable carbon source becomes available again. Xrn1 was recently shown to be a post-transcriptional negative regulator of autophagy, both in *S. cerevisiae* and in mammalian cells (Delorme-Axford *et al.* 2018), while its activity is known to be regulated via phosphorylation by the glucose-sensing Snf1/AMP kinase (Braun *et al.* 2014). Consistently, a role of Snf1 in the organization of eisosomes has recently been reported by Moharir et al. 2018, who showed that disruption of the membrane potential by alkalization of the medium not only affected the MCC partitioning of Fur4, but also that of Nce102. A partial loss of Nce102 MCC partitioning causes a re-modelling of eisosomes, as supported by the Snf1/AMPK-dependent changes in the distribution of the eisosomal protein Slm1 in response to alkalization of the medium (Moharir *et al.* 2018). The above collectively show

that eisosomes respond to the nutrient status of the cell and have an important, and yet not fully understood starvation-protective role.

2.2. Membrane Compartment of Pma1 (MCP)

2.2.1 Assembly and Organization

The Membrane Compartment of Pma1 (MCP) is enriched in Pma1, the fungal PM H^+-ATPase. This enzyme, considered as the most abundant PM protein in fungi, pumps H^+ out of the cell thus establishing an electrochemical gradient across the PM, which drives active uptake of nutrients and inorganic ions via secondary transporters (Serrano, Kielland-Brandt and Fink 1986; Wolf *et al.* 2012). Pma1 also maintains the intracellular pH, ion homeostasis and membrane potential of fungal cells in the physiological ranges (Kane 2016). Pma1 belongs to the group of P-type cation pumps containing 10 spanning α-helices with both termini exposed to the cytoplasm (Rao *et al.* 1992). Pma1 homologues have been studied in several fungal species, such as *A. nidulans* (Reoyo *et al.* 1998), *A. fumigatus* (Burghoorn *et al.* 2002), *C. albicans* (Monk *et al.* 1991), *S. pombe* (Ito *et al.* 2010) and *N. crassa* (Bowman, O'Neill and Bowman 1997; Rhee, Scarborough and Henderson 2002). Early structural and functional information of Pma1 were obtained in *N. crassa* (Auer, Scarborough and Kühlbrandt 1998; Kühlbrandt, Zeelen and Dietrich 2002; Rhee, Scarborough and Henderson 2002), and it is also in this species that the existence of a H^+ pump has been initially predicted by electrophysiological measurements (Slayman and Slayman 1962; Slayman 2004).

The MCP in *S. cerevisiae* was first described as a network-like compartment containing Pma1 that is distinct from the MCC in which Can1 segregates (Malínská *et al.* 2003). The formation and/or stabilization of MCP was found to be independent of microtubules and cytoskeletal components (Malinska *et al.* 2004). The MCP network is also known to be stable over time (Malinska *et al.* 2004) and Pma1 molecules show lateral diffusion within it (Malínská *et al.* 2003). However, these early observations did not solve the question of whether the particular distribution of Pma1 in the PM is due to its exclusion from MCC or to active segregation into a domain displaying specific features. Spira et al. (2012) reported that other PM proteins are excluded from the MCP, providing evidence against a single global network-like domain occupied by all non-MCC proteins. On the other hand, network-like domains occupied by other PM proteins significantly overlapped with the MCP (Spira *et al.* 2012). In a later study exploiting super-resolution microscopy (stimulated emission depletion - STED, resolution of 70 nm), the distribution pattern of Pma1 appeared as isolated plasma membrane foci, potentially corresponding to the hexagonal honeycomb-like areas of the PM of *S. cerevisiae* observed by electron microscopy (Malinsky and Opekarová 2016).

Whether the MCP has a specific lipidic composition remains poorly known. From the observation that the sterol-binding florescent compound Filipin is excluded from the MCP, it was proposed that this compartment contains relatively low levels of ergosterol (Grossmann *et al.* 2007). Evidence also suggests links between Pma1 and sphingolipids. For instance, newly synthesized Pma1 partitions in DRMs already from the ER, where it forms large

oligomeric complexes (Lee, Hamamoto and Schekman 2002; Bagnat, Chang and Simons 2013). Furthermore, shortening the C26 fatty acid of sphingolipids to a C22 fatty acid induces rapid degradation of Pma1 (Gaigg, Toulmay and Schneiter 2006). The importance of surrounding lipid organization in Pma1 function is also illustrated by the influence of edelfosine, a compound that has high affinity for sterols and disorganizes liquid-ordered membranes (Ausili *et al.* 2018). Incubation of *S. cerevisiae* with edelfosine causes displacement of Pma1 from DRMs and its ubiquitin-dependent downregulation, thus provoking intracellular acidification and cell death (Zaremberg *et al.* 2005; Zhang, Muend and Rao 2012; Cuesta-Marbán *et al.* 2013; Czyz *et al.* 2013). In *Cryptococcus neoformans*, Pma1 also fractionates in DRMs (Farnoud *et al.* 2014), and lack of ceramide synthase interferes with Pma1 function and cell growth (Munshi *et al.* 2018). A recent study reported that the inability of *C. neoformans* to degrade complex sphingolipids into phytoceramide inhibits Pma1 function, an effect proposed to correlate with reduced oligomerization of the pump (Farnoud *et al.* 2014). Several studies support the view that fungal H^+-ATPase oligomerize (Auer, Scarborough and Kühlbrandt 1998; Lee, Hamamoto and Schekman 2002; Bagnat, Chang and Simons 2013), and a recent study indicates that this oligomerization correlates with the activated state of the H+-ATPase (Ruiz-Granados, De La Cruz-Torres and Sampedro 2019). Plant H^+-ATPases, highly similar in sequence to fungal Pma1, are also known to form hexamers (Kanczewska *et al.* 2005; Ottmann *et al.* 2007). In this case also, it is illustrated that this complex arrangement occurs upon stimulation of the H^+-ATPase activity, via phosphorylation of the C-terminal tail (Kanczewska *et al.* 2005). Further work is thus needed to clarify the possible interlinks between the oligomerization of fungal H^+-ATPases, the influence of surrounding lipids, the control by phosphorylation of their activity, and their lateral segregation in MCP.

2.2.2 Physiological roles of MCP

Why an essential PM enzyme like Pma1 segregates into a particular domain instead of being uniformly distributed remains unknown. From the observation that Can1 and Mup1 are less active when artificially tethered to the MCP (Spira *et al.* 2012; Busto *et al.* 2018), it has been proposed that proton symporters need to occupy a domain distinct from the MCP for being fully functional, although the reasons remain elusive. It has been suggested that this separation of the proton-consuming transporters and the proton-gradient forming proton-pump could be important for maintaining the proton gradient and membrane potential (Grossmann *et al.* 2007; Moharir *et al.* 2018). As mentioned before, the main roles of Pma1 is to energize the PM and control the intracellular pH (Kane 2016). The latter function is also influenced by the Vacuolar-type H^+-ATPase (V-ATPase), a multi-subunit enzyme pumping protons from the cytosol into the lumen of acidic organelles such as vacuoles/lysosomes, endosomes and Golgi compartments in all eukaryotic cells [Reviewed in (Deprez *et al.* 2018)]. It is therefore not surprising that the V-ATPase and Pma1 are highly interdependent pumps that act in close harmony. For instance, loss of V-ATPase activity leads to a partial sorting of Pma1 to the vacuole, a process that requires the α-arrestin family protein Rim8

and the Rsp5 ubiquitin ligase (Smardon and Kane 2014). Further support for the crosstalk between the vacuolar and plasma membrane proton ATPases was provided by studies in the field of biological aging (Thayer *et al.* 2014). Pma1 is an extremely long-lived protein that progressively accumulates at the PM of aging mother cells and is largely absent from nascent daughter cells (Malínská *et al.* 2003; Henderson, Hughes and Gottschling 2014). The cytosolic pH progressively increases in mother cells due to asymmetric distribution of Pma1 between mother and daughter cells. Consistently, vacuoles become less acidic as cells age, and inhibition of this process by reducing Pma1 activity leads to increased replicative lifespan (Hughes and Gottschling 2012; Henderson, Hughes and Gottschling 2014). A recent study also suggests that Pma1 might have signaling capabilities (Saliba *et al.* 2018). Specifically, it was observed that the influx of H^+ coupled to active nutrient uptake via H^+ symporters provides a signal for reactivation of TORC1. Interestingly, this TORC1 activation proved dependent on Pma1 as it was not observed in cells where Pma1 has been replaced with a functional plant H^+-ATPase. As Pma1 is known to be stimulated under acidic conditions (Eraso and Gancedo 1987), it has been proposed that this activated state of Pma1 signals to TORC1 (Saliba *et al.* 2018).

As a whole, these recent studies illustrate the complexity of the roles potentially undertaken by Pma1 in fungi. Furthermore, these roles are likely interlinked with the mechanisms controlling the intrinsic activity and endocytosis of Pma1. A promising direction for future works is to enlighten possible functional links between the multiple functions of Pma1 and its segregation in MCP. Such studies could also be extended to pathogenic fungi, where Pma1 is known to be crucial. For instance, mutants of *C. neoformans* altered in the H^+-ATPase display attenuated virulence, possibly due to their inability to survive inside acidic macrophage lysosomes (Farnoud *et al.* 2014). Moreover, in *C. albicans,* Pma1 activity was found to be upregulated during filamentation, and mutants unable to alkalinize their cytosol were found to be non-virulent (Mahanty *et al.* 1990; Stewart, Gow and Bowen 2009).

2.3. Membrane Compartment of TORC2 (MCT)

More than 25 years ago, Heitman and coworkers identified in *S. cerevisiae* the Tor (Target of rapamycin) kinase as the target of the naturally occurring immunosuppressant and potent inhibitor of proliferation, rapamycin (Heitman, Movva and Hall 1991). Rapamycin binds FKBP12, a proline isomerase, to form a prodrug complex that specifically inhibits TOR in both *S. cerevisiae* and mammals (Heitman, Movva and Hall 1991; Blenis 2017). In both budding (Heitman, Movva and Hall 1991) and fission yeasts (Hayashi *et al.* 2007; Ikai *et al.* 2011), two Tor paralogs exist and populate two structurally and physiologically distinct protein complexes known as the Target Of Rapamycin Complex 1 (TORC1) and 2 (TORC2) (Loewith *et al.* 2002; Wedaman *et al.* 2003). In other fungal species (such as *N. crassa* and *A. nidulans*), but also in mammals, a single Tor protein exists in both complexes (Fitzgibbon *et al.* 2005; De Souza *et al.* 2013; Ratnayake *et al.* 2018; Stuttfeld *et al.* 2018). The TORC1 is highly conserved in eukaryotes (Soulard, Cohen and Hall 2009; Tatebe and Shiozaki 2017), is sensitive to rapamycin and controls translation, transcription, cell cycle regulation, ribosome biogenesis, and autophagy in response to nutrient availability [Reviewed in (Saxton and

Sabatini 2017; Tatebe and Shiozaki 2017). TORC2 is absent in plants and is insensitive to rapamycin (Jacinto *et al.* 2004; Tatebe and Shiozaki 2017). TORC1 localizes at the vacuolar membrane (Urban *et al.* 2007; Sturgill *et al.* 2008) while TORC2 localizes at the plasma membrane and in *S. cerevisiae* forms highly dynamic punctate structures known as MCT (Membrane Compartment occupied by TORC2) (Sturgill *et al.* 2008; Berchtold and Walther 2009).

2.3.1. MCT assembly and organization

In *S. cerevisiae* TORC2 is composed of two copies of six subunits, namely Tor2, Avo1, Avo2, Avo3, Lst8 and Bit61 or its paralog Bit2 [More details on TORC2 assembly are reviewed in (Eltschinger and Loewith 2016; Gaubitz *et al.* 2016; Blenis 2017)]. These subunits form a 1.4 MDa rhombohedral structure, similar to, but slightly larger than mammalian TORC1 (Karuppasamy *et al.* 2017). Tor2 is a member of the phosphatidylinositol-3 kinase-related kinase (PIKK) family and the core component of TORC2 (Keith and Schreiber 1995). Tor2 possesses a combination of HEAT (Huntington, EF3A, ATM, TOR) repeats, a helical region known as FAT (FRAP, ATM, and TRRAP) domain, the PI3K kinase domain which includes the FRB domain and the FAT C-terminal (FATC) domain (Knutson 2010). The C-terminus of Avo1 contains a Pleckstrin homology (PH) domain that binds $PI(4,5)P_2$ and mediates the plasma membrane anchoring of TORC2 (Berchtold and Walther 2009). Avo2 is a relatively small subunit essential for cell survival during stress conditions (Leskoske *et al.* 2018). Bit61 also binds to TORC2 but its role remains unknown (Loewith *et al.* 2002). Avo3 binds close to the FRB domain of Tor2 kinase and occludes the FKBP12-rapamycin-binding site, rendering TORC2 insensitive to rapamycin (Gaubitz *et al.* 2015; Karuppasamy *et al.* 2017). Lst8 is a WD40 repeat-containing protein that exhibits a β-propeller architecture known to interact with the catalytic kinase domain (Karuppasamy *et al.* 2017). In *S. cerevisiae* MCTs localize exclusively at the cell periphery in highly dynamic foci that do not colocalize with the sites of actin patch formation, MCCs, MCP, MCW or MCL (see also below) (Berchtold and Walther 2009; Kock *et al.* 2016; Murley *et al.* 2017).

In *S. pombe* Tor1 (similar to the budding yeast Tor2) is not essential for cell growth, is found mainly in TORC2 and associates with Wat1/Lst8, Sin1/Avo1, Ste20/Avo3 and Bit61 (Hayashi *et al.* 2007; Matsuo *et al.* 2007; Soulard, Cohen and Hall 2009). The TORC2 of fission yeast contains three additional proteins, Tel2 (highly conserved essential protein and a key regulator of multiple cellular signaling pathways) (Inoue *et al.* 2017), Tti1 (Tel-two-interacting protein 1) and Orb5 (a catalytic subunit of CK2) (Hayashi *et al.* 2007). The *S. pombe* TORC2 forms PM foci throughout the cell surface and to the Cytokinetic Actomyosin Ring (CAR), where it possibly regulates the timing and fidelity of cytokinesis (Tatebe *et al.* 2010; Baker *et al.* 2016).

2.3.2. Physiological roles of MCT

Fungal TORC2 has been found to regulate a variety of functions ranging from cytokinesis and cell-cycle progression to maintenance of heterochromatin and aging, as detailed in previous reviews (Gaubitz *et al.* 2015; Baker *et al.* 2016; Cohen *et al.* 2018). Here we mainly focus on the roles of TORC2 that are related to plasma membrane organization.

Sphingolipid biosynthesis - Sphingolipids and ceramides are important PM structural components and signaling molecules in eukaryotes, and their homeostasis is regulated by multiple signaling pathways (Hannun and Obeid 2008; Luo *et al.* 2008; Berchtold *et al.* 2012), including MCC/eisosomes and TORC2 (for the relations of MCC-resident proteins in the regulation of TORC2, see chapter 2.6). Briefly, eisosomes disassemble in response to depletion of SLs and/or changes in membrane tension, thereby releasing the Slm1/2 proteins and the Pkh1/2 kinases. Slm1/2 is then recruited to MCT and this promotes TORC2-dependent phosphorylation of Ypk1 (Berchtold *et al.* 2012; Niles *et al.* 2012). Full activation of Ypk1 requires not only phosphorylation by TORC2, but also by the Pkh1/2 (Francoise M. Roelants, Pamela D. Torrance, Natalie Bezman and Thorner 2002; Leskoske *et al.* 2017), also released from eisosomes. Fully activated Ypk1 phosphorylates and inactivates the endoplasmic reticulum (ER) localized Orm1 and Orm2 proteins, negative regulators of sphingolipid biosynthesis (Roelants *et al.* 2011; Sun *et al.* 2012). Orm1/2 forms the SPOTS complex together with the serine palmitoyl transferase (SPT**,** comprises the subunits Lcb1, Lcb2 and Tsc3), which catalyzes the first enzymatic step in sphingolipid synthesis, and the phosphatidylinositol phosphatase Sac1 [(Breslow *et al.* 2010) reviewed in (Davis, Kannan and Wattenberg 2018)]. Once phosphorylated by Ypk1, Orm1/2 alleviate the inhibition of SPT, the rate limiting step of SL biosynthesis, thereby inducing the biosynthesis of complex sphingolipids [Reviewed in (Eltschinger and Loewith 2016; Olson *et al.* 2016; Davis, Kannan and Wattenberg 2018; Zahumensky *et al.* 2019)]. A recent study additionally shows that upon TORC2-mediated phosphorylation, Orm2 moves out of the ER to post-ER compartments and there it is selectively degraded by a mechanism called Endosome and Golgi-associated degradation (EGAD) (Schmidt *et al.* 2019) (Figure 2). In addition to the phosphorylation of Orm1/2 by Ypk1, Npr1 (a TORC1-regulated protein kinase) phosphorylates the Orms upon TORC1 inhibition, targeting residues different from the ones utilized by Ypk1, and this also contributes to increased *de novo* synthesis of complex sphingolipids (Shimobayashi *et al.* 2013). A role of Sch9 and TORC1 in SL biosynthesis has also been reported. Inactivation of Sch9 promotes SL biosynthesis via activation of the ceramidases (Liu *et al.* 2005; Swinnen *et al.* 2014). Furthermore, Ypk1 phosphorylates and stimulates the catalytic subunits (Lac1 and Lag1) of the ceramide synthase complex (CerS), by increasing the rate of the formation of ceramides and preventing hyper-accumulation of LCBs/LCBPs, thus blocking inadvertent induction of autophagy under nutrient-rich conditions (Muir *et al.* 2014). Likewise, CerS activity is additionally regulated through elongase Elo2, the rate-limiting enzyme in the elongation of fatty acids to very long chain fatty acids (VLCFAs) which combined with LCBs form ceramides, in a signaling pathway controlled by the CWI pathway (Olson *et al.* 2015). Olson *et al* suggested that during sphingolipid limitation the

activity of Wsc1 diminishes, provoking the down-regulation of Rom2 activity toward Rho1 and the downstream signaling cascade (Olson *et al.* 2015).

Response to membrane tension and regulation of endocytosis – Sensing of membrane tension is proposed to involve MCCs (discussed in chapter 2.6), as well as an MCC-independent mechanism (Riggi *et al.* 2018). The latter is based on the observation that hypo- and hyper-osmotic shocks do not affect Slm1 localization and eisosome assembly, suggesting that PM tension is regulated by TORC2 independently of MCCs. More specifically, PM tension was shown to trigger energy-independent $PI(4,5)P_2$ phase separation into discrete invaginated membrane domains, previously observed upon hyper-osmotic shock (Dupont *et al.* 2010). These domains cluster and inactivate TORC2 signalling, this way increasing PM tension (Riggi *et al.* 2018). TORC2 signaling affects endocytosis at various levels: inhibition of TORC2 blocks endocytosis by affecting the actin cytoskeleton (Riggi *et al.* 2018), and by a phosphorylation cascade via the Fpk1/2 kinases (deHart 2003; Rispal *et al.* 2015; Roelants *et al.* 2017; Bourgoint *et al.* 2018), that is additionally involved in the regulation of membrane asymmetry (Kamada *et al.* 2005; Roelants *et al.* 2009, 2017; Muir *et al.* 2014; Niles and Powers 2014; Rispal *et al.* 2015). Upon activation of TORC2 (e.g. by sphingolipid depletion, hypotonic stress, heat shock or elevated exogenous acetic acid) (Roelants *et al.* 2011; Berchtold *et al.* 2012; Niles and Powers 2012; Sun *et al.* 2012; Guerreiro *et al.* 2016), stimulated Ypk1 phosphorylates and negatively regulates Fpk1 (Roelants *et al.* 2009, 2011). Among the substrates of Fpk1 are the 4P-type ATPases Dnf1/2 that establish and repair bilayer asymmetry by translocating aminoglycerophospholipids from the outer to the inner leaflet of the PM (Chen *et al.* 2007; Roelants *et al.* 2009; Sebastian *et al.* 2012), helping relieve membrane tension.

An additional target of Fpk1 is the kinase Akl1, which modulates the dynamics of actin patch-mediated endocytosis (Roelants *et al.* 2017). Active Fpk1 hyperphosphorylates and negatively regulates Akl1. Substrates of Akl1 are multiple endocytic factors and coat proteins, like Sla1, Ent2, and Pan1 (Smythe and Ayscough 2003; Bourgoint *et al.* 2018). Therefore, when Fpk1 is active, multiple Akt1-substrates coat proteins are hypophosphorylated, thereby altering the dynamics of endocytic events (Roelants *et al.* 2017; Bourgoint *et al.* 2018).

2.4. MCL / ER-PM contact sites

The endoplasmic reticulum (ER) network undergoes constant remodeling in response to different growth conditions and can be divided into morphologically distinct subdomains including the nuclear envelope, sheet-like cisternae, tubules and the cortical ER (West *et al.* 2011; Stradalova *et al.* 2012). In order to accomplish its role in protein synthesis, modification and quality control, lipid and carbohydrate metabolism as well as calcium signaling [Reviewed in (Schwarz and Blower 2016)], ER contacts and crosstalks with several other cellular organelles, such as the trans-Golgi network (TGN), mitochondria, endosomes, the vacuole and the PM (Wu, Carvalho and Voeltz

2018). These ER/organelle Membrane Contact Sites (MCSs) are highly conserved and were observed by classical electron microscopy more than sixty years ago (Porter and Palade 1957), though frequently neglected due to the lack of knowledge on their functional significance. MCSs are closely juxtaposed and tethered membranes, are devoid of ribosomes and are spaced at 10 to 60 nm from one another (West *et al*. 2011).

An important physiological role for inter-organelle communication is membrane lipid exchange (Wong and Levine 2016). Lipid transfer between organelles is mediated by multiple mechanisms including vesicular transport and nonvesicular transport by lipid transfer proteins (LTPs) [Reviewed in (Prinz 2010; Tong, Manik and Im 2018)]. The highly conserved eukaryotic family of LTPs, known as lipid transfer proteins anchored at a membrane contact sites (Lam) or lipid transfer at contact site (Ltc) play key roles in transferring lipids between cellular compartments (Gatta *et al.* 2015; Horenkamp *et al.* 2018; Tong, Manik and Im 2018). Members of the Lam/Ltc protein family in *S. cerevisiae* were recently shown to localize at junctions between ER and PM (known also as PM-associated ER or ER-PM MCSs) and form the MCL (Membrane Compartment of Ltc3/4) (Murley *et al.* 2017).

MCL **assembly and organization -** In *S. cerevisiae* cells, about half of the PM remains closely associated with the cortical ER (West *et al.* 2011; Manford *et al.* 2012). This close association is stabilized by the MCSs, which are only a part of the cER. MCSs are enriched in members of the Lam/Ltc family of LTPs, which function in exchanging lipids between the cytosolic face of the ER and that of the PM and actively maintain the unique lipid composition of the PM (Horenkamp *et al.* 2018). This Ltc/Lam family of LTPs was discovered using structural bioinformatics on the basis of distant sequence homology with proteins containing a START-like domain (steroidogenic acute regulatory protein-related lipid transfer domain) (Gatta *et al.* 2015), also called VASt domain (Khafif *et al.* 2014). This domain is a truncated version of the StART domain, which is capable of forming a cuplike structure with a lid, able to selectively transports sterols between membranes *in vitro* (Gatta *et al.* 2015; Horenkamp *et al.* 2018; Tong, Manik and Im 2018). Proteins in the Lam/Ltc family all feature an unstructured N-terminus, followed by a structure similar to a pleckstrin homology (PH) domain, known as a GRAM domain, a transmembrane segment anchored to the ER and one or two StART-like domains (Gatta *et al.* 2015; Hanada 2018; Tong, Manik and Im 2018). Recently, Lam2/Ltc4/Ysp2 -contrary to prior observations that is a mitochondrial protein (Sokolov *et al.* 2006)- together with Lam1/Ysp1, Lam3/Sip3, and Lam4/Ltc3 members of the Lam/Ltc protein family, and the Ymr102c/Dgr2 paralogs involved in multidrug resistance (González Montoro and Ungermann 2015; Murley *et al.* 2017), were shown to localize to ER-PM MCSs and to form the unique MCL (Murley *et al.* 2017). Functional Ltc4 was observed by

confocal microscopy to form relatively stable, distinct domains that do not overlap with the MCT, MCP, MCW and MCC (Murley *et al.* 2017).

Physiological role of MCL - *de novo* ER synthesized sterols are, at least in part, delivered to the membranes of other cellular organelles by non-vesicular mechanisms requiring LTPs (Lev 2010; Johansen, Ramanathan and Beh 2012). In particular, Ltc/Lam proteins are involved in transport of sterols between PM and ER (Gatta *et al.* 2015; Murley *et al.* 2017). Although the MCL was only very recently described, evidence suggests that it is involved not only in the transport of sterols from the ER to the PM, but also to the sensing of PM sterol levels. This sensing seems to be mediated via TORC2 (more details in chapter 2.6), as the Slm1/2 regulators of TORC2 show partial localization at the MCL .

2.5. Membrane Compartment of Wsc1 (MCW)

Integrity of fungal cells requires the presence of the cell wall, a structure that provides mechanical support and protection against different environmental stresses (Gow, Latge and Munro 2017; Sanz *et al.* 2017). The cell wall integrity signaling pathway (CWI), highly conserved in fungi (Nikolaou *et al.* 2009), is involved in the sensing and the remodeling of the cell wall structure (Rodicio and Heinisch 2010; Jendretzki *et al.* 2011; Futagami and Goto 2012), using five sensors (Wsc1, Wsc2, Wsc3, Mid2 and Mtl1) [Reviewed in (Rodicio and Heinisch 2010; Jendretzki *et al.* 2011)]. One of the most studied is Wsc1, a cell wall stress mechanosensor (Dupres *et al.* 2009; Alsteens *et al.* 2012) that has recently been shown to form the MCW (Membrane Compartment containing Wsc1) (Dupres *et al.* 2009; Kock *et al.* 2016). MCW is distinct from most of the known membrane domains (MCC, MCP, MCT and MCL) (Kock *et al.* 2016; Murley *et al.* 2017). In the Aspergilli, homologues of Wsc1 localize at the PM and septa and are related to CWI signaling (Futagami *et al.* 2011).

Wsc1 contains a signal peptide, an extracellular region that is highly O-mannosylated, a cysteine-rich domain (CRD or WSC domain), a serine/threonine-rich region (STR domain), a single transmembrane domain and a relatively short highly charged cytoplasmic tail (Philip and Levin 2002). Wsc1 anchors at the cell wall via the CRD domain, which contains up to eight conserved cysteine residues that are believed to noncovalently bind to glycans of the cell wall (Levin 2005; Heinisch *et al.* 2010). Wsc1 forms patches at the PM of both S. cerevisiae and Kluyveromyces lactis (Straede and Heinisch 2007; Rodicio *et al.* 2008). These patches are highly dynamic, approximately 200 nm in size and concentrate at the tip and the neck of the bud prior to cytokinesis (Straede and Heinisch

2007; Heinisch *et al.* 2010; Kock *et al.* 2016). The polarized distribution of Wsc1 is determined by the cytoplasmic domain (Straede and Heinisch 2007) and maintained by clathrin-mediated endocytosis and recycling (Piao, Machado and Payne 2006; Dupres *et al.* 2009; Wilk *et al.* 2010).

The PM clustering of Wsc1 is enhanced under stress conditions (Heinisch *et al.* 2010; Kock *et al.* 2016). It is proposed that upon stretching of either the PM or the cell wall, a mechanical force is generated on the extracellular part of the Wsc1, which could be transmitted via conformational change to the cytoplasmic tail (Heinisch *et al.* 2010). This conformational change would trigger the activation of the small GTPase Rho1, probably via the major GDP/GTP exchange factor Rom2. GTP-bound Rho1 then activates various effectors, including the 1,3-glucan synthase (GS) (Mazur and Baginsky 1996) and the Pkc1-MAP kinase cascade (Schmitz, Lorberg and Heinisch 2002). Activated Pkc1 triggers the MAP Kinase cascade composed of a series of consecutive protein kinases, transmitting finally the signal to two known transcription factors, Rlm1 (Watanabe *et al.* 2015) and SBF (Swi4/6) (Baetz *et al.* 2001), and thus triggering new cell wall synthesis at sites of polar growth or surface lesions [reviewed in (Levin 2011; Dichtl, Samantaray and Wagener 2016; Gow, Latge and Munro 2017; Sanz *et al.* 2017; Heinisch and Rodicio 2018)].

2.6 Inter-domain relations

In this chapter we briefly refer to mechanisms and functions involving more than one membrane domains. Although the information provided here shows some overlap with previous chapters, we believe that it can contribute to a better understanding of the emerging interactions of the different PM domains. It should be noted that in this chapter we do not discuss the relations of MCCs with sites of endocytosis, which are discussed in the corresponding chapters (2.1.3 and 2.7.1) and illustrated in Figure 3, for the sake of clarity.

MCC-MCT – One of the first-described inter-domain relations was that of MCC and MCT in the control of sphingolipid biosynthesis and membrane stress (Berchtold *et al.* 2012; Riggi *et al.* 2018) (summarized in Figure 2). TORC2 is known to control the homeostasis of both sphingolipids (SLs) and phosphoinositides, and to induce the biogenesis of these lipids in response to depletion, heat (Bultynck *et al.* 2006), plasma membrane stress (Berchtold *et al.* 2012) or increased membrane tension (Riggi *et al.* 2018). MCC assembly has been reported to control SL biosynthesis via a TORC2-mediated negative feedback loop (Berchtold *et al.* 2012). More specifically, when eisosomes disassemble, the MCC/eisosome-resident proteins Nce102 (Fröhlich *et al.* 2009), Slm1/2 (Berchtold *et al.* 2012) and the Pkh1/2 (Walther *et al.* 2007) kinases, are released and relocate to other cellular compartments. Nce102 becomes homogeneous at the plasma membrane (Fröhlich *et al.* 2009), Pkh1/2 diffuse at the cytoplasm (Walther *et al.* 2007), while the Slm1/2 relocate to the MCT (Berchtold *et al.* 2012). Since Nce102 is the only transmembrane protein required for eisosome assembly (Grossmann *et al.* 2008; Fröhlich *et al.* 2009), it is an excellent candidate for sensing the levels of SLs (potentially enriched in the outer leaflet of the PM), and transmitting the signal

to the cytoplasmic side of the PM. This agreed with the observation that the exit of Nce102 from MCCs upon SL depletion occurs prior to and independently of eisosome dissasembly (Fröhlich *et al.* 2009). However, such an SL-sensing role of Nce102 is under debate (Malinsky and Opekarová 2016; Zahumensky *et al.* 2019), as subsequent evidence in *S. cerevisiae* identified the Slm1/2 proteins as better candidates for transmitting the signal from MCCs to the MCT. Briefly, Slm1/2 not only relocate from MCC to MCT upon SL depletion (Berchtold *et al.* 2012) and are required for the recruitment of Ypk1 there (Niles *et al.* 2012), but also move out of eisosomes much faster that Nce102 does upon inhibition of SL biosynthesis (Berchtold *et al.* 2012; Busto *et al.* 2018). The same relocation of Slm1/2 is also observed in response to plasma membrane stress and to an increase in plasma membrane tension (Riggi *et al.* 2018), suggesting that eisosomes act as sensors of membrane stress. However, the phylogenetic conservation of such a mechanism is not clear, since the homologues of the Slm proteins in *S. pombe* do not localize in eisosomes (Kabeche *et al.* 2011). Similarly, SlmA of *A. nidulans* is only found at the eisosomes of the hyphae (Pinar and Peñalva 2017) and not at those of the head of the germlings, which respond to SL depletion (Athanasopoulos *et al.* 2015). Differences in growth conditions and the existence of different populations of eisosomes with specialized roles (see paragraph 2.1.1) could potentially explain the apparent inconsistences.

The above described mechanism has been proposed to additionally contribute to the regulation of PM tension. According to the proposed model, an increase in membrane tension would be sensed by a flattening of eisosomes (Kabeche, Howard and Moseley 2015a) and would lead to the activation of TORC2. The latter would result in stimulation of the biosynthesis of sphingolipids, that would asymmetrically insert into the PM to diminish this tension [details in (Berchtold *et al.* 2012)].

MCT-MCW - In both fission and budding yeast, cell wall stress results in activation of CWI pathway that negatively regulates the growth-promoting functions of MCT (Cohen, Kupiec and Weisman 2014; Leskoske *et al.* 2018). Recently, it was shown that activation of either CWI via Pkc1 or the HOG pathway resulted in the phosphorylation of the Avo2 subunit of TORC2 by the mitogen-activated protein kinase Slt2, leading to the down-regulation of TORC2 activity (Leskoske *et al.* 2018).

MCT-MCL – Recent evidence suggests the existence of a feedback circuit between the MCL and the MCT, which would control sterol abundance at the PM, similarly to the above-described pathway controlling sphingolipid biosynthesis via TORC2 signaling. Ltc3/4 proteins, the main MCL components transfering sterols between the ER and the PM, seem to additionally regulate and be regulated by sterol availability. Ypk1, the effector of TORC2, phosphorylates Ltc3/Lam4 and Ltc4/Lam2, and this phosphorylation inhibits the retrograde transport of sterols from the PM to the ER (Roelants *et al.* 2018). Moreover, it was proposed that the increased sensitivity of *S. cerevisiae* cells to amphotericin B (AmB), a polyene antifungal antibiotic, in the presence of myriocin, an inhibitor of the *de novo* sphingolipid biosynthesis, was due to the TORC2 - Ypk1 inhibition of Lam2, resulting in PM ergosterol to be more accessible to the antibiotic (Roelants *et al.* 2018). In parallel, Murley and coworkers provided evidence that the activity of Ltc3/4 contributes to PM sterol homeostasis by negatively regulating Ypk1 phosphorylation. They additionally suggested that Slm1/2

proteins, which partially localize at the MCL, participate in the transmission of the sterol-sensing signal from the MCL to the MCT (Murley *et al.* 2017). Additional work is required in order to fully understand how MCT-MCL interactions regulate sterol levels.

2.7. Sites of endocytosis

Endocytosis in *S. cerevisiae* has been extensively studied, and excellent recent reviews regarding the mechanisms of formation / organization of clathrin-dependent endocytic vesicles (CCVs) are available (Boettner, Chi and Lemmon 2011; Merrifield and Kaksonen 2014; Lu, Drubin and Sun 2016; Kaksonen and Roux 2018). Cortical foci where endocytosis is initiated have long been observed in *S. cerevisiae* (Kaksonen, Toret and Drubin 2005; Newpher *et al.* 2005) and intensive work focused on the composition and ordered recruitment of proteins at these sites [reviewed in (Boettner, Chi and Lemmon 2011; Merrifield and Kaksonen 2014; Lu, Drubin and Sun 2016; Kaksonen and Roux 2018)]. Endocytosis has also gained much attention in filamentous fungi, since it is essential for polarized hyphal growth (see chapter 2.9). Endocytic cortical foci have also been observed *in Ustilago maydis* (Wedlich-Soldner *et al.* 2000)*, S. pombe* (Gachet 2005), *C. albicans* (Martin *et al.* 2007), *A. nidulans* (Taheri-Talesh *et al.* 2008), and *N. crassa* (Berepiki *et al.* 2010; Delgado-Álvarez *et al.* 2010). In this section, rather than providing an extensive review on the mechanisms of CCV formation and cargo recruitment, we focus on studies showing that endocytic patches are formed at PM sites that are distinct from the other known membrane compartments, and discuss on how these sites are defined.

2.7.1. Evidence for a unique domain – MCCs have initially been proposed to be sites of endocytosis (Walther *et al.* 2006). However, several lines of evidence suggest that this is not the case. First, sites of endocytosis in several fungi preferentially localize close to apically growing membranes, like the newly-forming bud of *S. cerevisiae* (Valdez-Taubas and Pelham 2003) and the sub-apical collar in the hyphae of filamentous fungi (Peñalva 2010). On the contrary, MCCs are excluded from these regions (see chapter 2.1). Second, sites of endocytosis are known to only be transiently formed, while eisosomes are static. Third and most important, eisosomes do not colocalize with several markers of clathrin-dependent endocytosis in *S. cerevisiae* [Rvs161, Ede1 (Grossmann *et al.* 2008), Abp1 and Sla1 (Brach, Specht and Kaksonen 2011)], *C. albicans* [cortical actin patches and Abp1 (Reijnst, Walther and Wendland 2011)] and *A. nidulans* [FM4-64 and AbpA (Vangelatos *et al.* 2010; Athanasopoulos *et al.* 2013)], while deletion of PilA or AoPil1 does not affect the endocytosis of FM4-64 or amino acid transporters in the *Aspergilli* (Higuchi *et al.* 2009; Vangelatos *et al.* 2010). Stradalova et al. (2012) subsequently reported that endocytic vesicles are formed in PM regions devoid of cortical ER (cER), and that these regions are defined by the positioning of the nearby MCC invaginations (the regions between MCC-invaginations are devoid of cER and it is proposed that sites of endocytosis are formed there) (Figure 1). Importantly, it was shown that even when artificially anchoring early endocytic proteins to eisosomes, endocytic

events occurred only next to eisosomes and not within eisosomes, suggesting that the dense protein coat beneath MCCs would prevent the assembly of the vesicle-budding machinery (Brach *et al.* 2014). Moreover, Abp1-defined endocytic sites have recently been shown to be formed at foci that are distinct not only from MCCs, but also from MCL, MCT, and MCW patches, as well as from the network-like MCP (Busto *et al.* 2018). Busto et al., (2018) proposed that sites specifically occur within the network-like domain where the active Mup1 permease relocalizes before undergoing endocytosis.

2**.7.2. Selection of the endocytic site formation** - The formation of endocytic vesicles is a complex and intensively studied process including the following steps: site initiation, cargo capture, coat assembly, membrane bending, invagination, scission, and uncoating of the vesicle [reviewed in (Merrifield and Kaksonen 2014; Lu, Drubin and Sun 2016)]. It can be simplified to two temporal phases, an "early phase" and a "late phase" (Lu, Drubin and Sun 2016). During these phases, more than 60 proteins are spatially recruited to endocytic sites (Merrifield and Kaksonen 2014). These proteins can be organized into five different modules: (i) early proteins; (ii) early, middle and late coat proteins; (iii) WASp/Las17 and myosin-related proteins, (iv) scission-related proteins and (v) actin and actin-associated proteins. Despite intensive research on the subject, how the exact position of endocytic sites is defined still remains elusive. Current evidence suggests that there is an interdependence on the presence of specific lipids, early endocytic factors and cargo proteins, both for defining the exact location of endocytic patch formation, but also for vesicle budding.

Regarding lipids, phosphatidylserine (PS) and $PI(4,5)P_2$ are specifically enriched at the inner leaflet of the membrane at endocytic sites (Antonescu, Danuser and Schmid 2010). PS has a role in the bud site selection and initiation, whereas $PI(4,5)P_2$ is required for efficient invagination of the vesicle (Sun and Drubin 2012). The asymmetry of lipids between the two leaflets and the presence of lipid-binding proteins have been proposed to be important for membrane bending (Kirchhausen 2012; Shen, Pirruccello and De Camilli 2012) and for the final scission of the CCV (Merrifield and Kaksonen 2014). Sphingolipids are also crucial for endocytosis, as mutants in SL biosynthesis pathway show defective internalization of α-factor pheromone, the classic clathrin-mediated endocytosis (CME) cargo in *S. cerevisiae* (Munn and Riezman 1994; Zanolari 2002). Moreover, genes related to SL biosynthesis have been initially isolated as Suppressors of an Rvs161 (SUR genes) salt-sensitive allele (Desfarges *et al.* 1993; Young *et al.* 2002), although the mechanism behind this suppression still remains unknown.

Regarding the role of proteins in defining the location of endocytic patch formation and vesicle budding, during the initiation phase of endocytic vesicle formation early proteins are recruited at the PM. It has been proposed that rather than controlling the recruitment of late proteins or the budding of the vesicle, early proteins, like Ede1, are important to define the spatial location of the assembly of the endocytic machinery (Brach *et al.* 2014; Lu and Drubin 2017). Importantly, cargo molecules have been found to arrive after the early phase, but before the late phase proteins (Toshima *et al.* 2006), suggesting the existence of a cargo-based checkpoint controlling the full assembly of the vesicle (Carroll *et al.* 2011). Consistent

with this view, Busto et al. 2018 showed that Mup1, once outside the endocytosis-protective MCCs, as it occurs upon substrate transport, partially localizes with endocytic sites. Importantly, Mup1, only when ubiquitylated, was able to redirect endocytic events from the bud to endocytic sites in mother cells (Busto *et al.* 2018), suggesting that it is ubiquitylated cargos that should stabilize the assembly of the endocytic machinery. This stabilization should occur in regions of the PM devoid of any other known membrane compartment. Due to the architecture of the endocytic patch (Merrifield and Kaksonen 2014; Lu, Drubin and Sun 2016), it is likely that the exclusion of sites of endocytosis from other membrane domains occurs via steric hindrance between the multi-protein coat, and proteins or membranes bound at the inner surface of certain PM compartments (e.g. cER, MCCs) (Brach, Specht and Kaksonen 2011; Stradalova *et al.* 2012; Brach *et al.* 2014; Gournas *et al.* 2018).

2.8. PAL/RIM foci

The RIM/Pal pathway in fungi, extensively studied in *A. nidulans* and *S. cerevisiae* [reviewed in detail in (Maeda 2012; Peñalva, Lucena-Agell and Arst 2014)], is responsible for sensing external pH alkalization and plasma membrane lipid asymmetry (Peñalva, Lucena-Agell and Arst 2014; Rockenfeller and Gourlay 2018). The master regulator of pH responses is the transcription factor PacC/Rim101, whose activity and nuclear localization is controlled by regulated two-step proteolysis from an inactive to an active form (Díez *et al.* 2002). PacC, in its active form, promotes the expression of genes related to alkaline pH and negatively regulates those related to acidic pH. The first step of PacC/Rim101 proteolytic activation is mediated by PalB/Rim13, a protease of the calpain family (Díez *et al.* 2002), while the second step occurs by the proteasome (Hervás-Aguilar *et al.* 2007). It is now established that PalB/Rim13-mediated proteolysis of PacC occurs under alkaline conditions by recruitment of PacC and PalB to distinct foci at the plasma membrane (details below). Importantly, this recruitment is mediated by an atypical, non-budding function of the ESCRT machinery at the plasma membrane (Obara and Kihara 2014; Peñalva, Lucena-Agell and Arst 2014).

Although whether these RIM/Pal foci form a distinct PM domain has not yet been thoroughly studied, several lines of evidence suggest so. Firstly, these foci seem to not correspond to MCCs, as only a minority of Pil1-mcherry spots colocalizes with RIM/Pal foci at alkaline pH (Obara, Yamamoto and Kihara 2012). Secondly, the RIM/Pal foci are found at PM regions devoid of cortical ER (Obara and Kihara 2017) and can thus be considered distinct from the MCL too. Thirdly, the RIM/Pal foci have been localized by epifluorescence microscopy at PM regions with reduced Pma1 intensity (Obara and Kihara 2014). Although this has to be systematically studied using confocal microscopy, it suggests that the RIM/Pal foci form at PM sites distinct from the MCP. Finally, the characteristics of the foci and their organization (see below) imply that they should occupy a unique region at the PM.

Assembly and Organization of PAL/RIM foci - Results suggest that the region of these PM foci is defined by PalH/Rim21, the sensor of pH and membrane lipid asymmetry (Herranz *et al.* 2005; Obara, Yamamoto and Kihara 2012; Nishino, Obara and Kihara 2015), which

eventually is able to promote a cascade of events leading to the recruitment of the ESCRT machinery at the PM. PM recruitment of components of the RIM/Pal pathway has been reported as early as 2007 (Galindo *et al.* 2007). Importantly, the foci were shown to be static (Galindo *et al.* 2012), while the recruitment of downstream components at these cortical structures is transient, upon exposure to alkaline pH. PalH/Rim21 is a 7-TMS protein, similar in sequence to GPCRs, whose cytosolic C-tail is in contact with the PM, but detaches from the PM and becomes exposed to the cytosol under external alkalization or loss of lipid asymmetry at the PM (Herranz *et al.* 2005; Obara, Yamamoto and Kihara 2012; Nishino, Obara and Kihara 2015; Lucena-Agell *et al.* 2016). Another membrane protein, PalI/Rim9 is involved in pH sensing, most probably by stabilizing PalH/Rim21 at the PM (Calcagno-Pizarelli *et al.* 2007). In *S. cerevisiae* yet another membrane protein, Dfg16, has been identified as part of the sensing complex and is also believed to stabilize Rim8 at the PM (Obara, Yamamoto and Kihara 2012). Interestingly, PalI/Rim9, part of the Sur7 family, shows partial MCC localization and labels only a few MCCs, both in *S. cerevisiae* (Obara, Yamamoto and Kihara 2012) and *A. nidulans* (Athanasopoulos A., Vangelatos I. and Sophianopoulou V., unpublished observations), but the physiological significance of this remains elusive. The third component of the sensor is PalF/Rim8, an arrestin-like protein (Herranz *et al.* 2005). Once the C-tail of PalH/Rim21 gets detached from the inner membrane surface (Nishino, Obara and Kihara 2015), it allows the ubiquitylation of PalF/Rim8 by HulA/Rsp5 Ub-ligase (Obara and Kihara 2014). This ubiquitylation is sufficient for activation of the signaling pathway (Hervás-Aguilar, Galindo and Peñalva 2010). The ubiquitylated complex is believed to be recognized by Vps23, which recruits ESCRT-I and ESCRT-II machinery at the PM, allowing further recruitment of Snf7 (Herrador *et al.* 2010; Maeda 2012). Snf7 then recruits the Bro1-domain-containing PalA/Rim20 and PalC/Rim23 (Galindo *et al.* 2007, 2012), eventually allowing recruitment of the protease, PalB/Rim13, and the full-length transcription factor, PacC/Rim101. Consequently, the first proteolytic processing of PacC/Rim101 occurs at the Pal/RIM foci.

Physiological role of PAL/RIM foci - Optimal response to differences in external pH is crucial in various important physiological responses (reviewed in (Peñalva *et al.* 2008; Davis 2009; Du and Huang 2016; Brown *et al.* 2018), including the biosynthesis of penicillin (Brakhage *et al.* 2012), the uptake of siderophores (Eisendle *et al.* 2004), and the virulence of *A. fumigatus* (Loss *et al.* 2017), *Candida albicans* (Sherrington *et al.* 2017) and *Cryptococcus neoformans* (Ost *et al.* 2017; Hommel *et al.* 2018). The response is mediated by the regulation of the expression of genes involved in synthesis and secretion of extracellular enzymes and metabolites, as well as some transporters (Peñalva *et al.* 2008). Additionally, the synthesis of specific cell wall components of *C. albicans* is also under the control of the Pal/RIM pathway and is critical for masking the fungus from the immune response of the host (Sherrington *et al.* 2017). Evidence accumulates that the Pal/RIM pathway additionally senses lipid asymmetry of the PM, and is involved in lipotoxicity [reviewed in (Rockenfeller and Gourlay 2018)]. More specifically, activation of the RIM pathway was shown to occur in the absence of lipid flippases or floppases and their regulators (Ikeda *et al.* 2008; Obara and Kihara 2014; Brown *et al.* 2018), by expression of phospholipase A2 (Mattiazzi *et al.* 2010), or in response to externally supplied excess of palmitoleic acid (Richard *et al.* 2014) or

diacylglycerol (Rockenfeller *et al.* 2018). The sensing mechanism again relies on Rim21 (see above), as both lipid stress and alkalization of the medium can result in loss of the proton gradient across the PM, and thus affect the conformation of PalH/Rim21 (Nishino, Obara and Kihara 2015; Rockenfeller and Gourlay 2018).

2.9. Sterol-Rich Domains (SRDs)

Polarized cell growth is a universal phenomenon in fungi, ranging from unicellular yeasts to multicellular filamentous fungi. Fungal cells grow exclusively via specific regions, the bud in *S. cerevisiae* or the tip in apically-grown unicellular and multicellular fungi. Apical growth requires two processes, polarity establishment and polarity maintenance. Both are believed to be defined by polarity markers that are constantly restricted to the tip region. Several lines of evidence suggest that the restriction of the apical marker to the tip region is maintained by constant directed exocytic flow of vesicles, coupled to endocytic recycling (Steinberg *et al.* 2017; Riquelme *et al.* 2018), and assisted by the extremely slow diffusion of proteins at the fungal plasma membrane (Valdez-Taubas and Pelham 2003). The above suggests that the fungal tip region can be considered as a specific membrane domain, having a distinct composition of proteins and lipids. Indeed, in several fungal species it has been reported that sites of active membrane growth are enriched in sterols (Sterol-Rich Domains, SRDs), as seen using the fluorescent and 3'-β-sterol-binding filipin (Alvarez, Douglas and Konopka 2007; Malinsky *et al.* 2013). Filipin-labelled SRDs have been observed at the bud tips and mating projections in *S. cerevisiae* (Bagnat and Simons 2002), the cell ends in *S. pombe* (Wachtler 2003), the hyphal tips of *A. nidulans* (Pearson *et al.* 2004) and *C. albicans* (Martin and Konopka 2004), the places of septum formation in *S. pombe* (Wachtler 2003) and *Ustilago maydis* (Woraratanadharm, Kmosek and Banuett 2018) and the germling tips in *N. crassa* (Weichert *et al.* 2016). In filamentous fungi in particular, the study of hyphal growth via the tip is a very active field of research and excellent recent reviews adequately cover the subject (Peñalva 2010; Steinberg *et al.* 2017; Riquelme *et al.* 2018). Here, we discuss the particular protein and lipid composition of the PM at the fungal apical regions of growth and briefly refer to the mechanisms related to their formation and maintenance.

Organization of apical regions

Composition - Growing membranes are enriched in sterols, which are required for the apical localization of proteins (see below). SLs, functional partners of sterols, could potentially also localize at the tip. Although direct visualization of SLs is missing, intact SL biosynthesis in *A. nidulans* is required for polarity establishment and maintenance, as well as for the apical localization of sterols (Cheng *et al.* 2002; Pearson *et al.* 2004; Li *et al.* 2006; Martzoukou *et al.* 2017). Among the proteins enriched in the hyphal tip in several fungi is the V-SNARE protein Snc1/SynA (Valdez-Taubas and Pelham 2003; Taheri-Talesh *et al.* 2008), used as a polarity marker. Additional proteins localizing at the tip are those involved in polarity

establishment and/or maintenance, but also proteins essential for the extension of the tip, like cell wall-building and remodeling enzymes (see below).

Mechanism of formation - Several studies have identified proteins that are enriched in SRDs and are required for proper organization of SRDs. The enrichment of these constituents at SRDs occurs by localized exocytosis coupled to endocytic recycling (Figure 4).

The region of apical growth is initially defined in uniformly-grown cells by the process of polarity establishment, which has been studied in detail in *S. cerevisiae* and *S. pombe* (Chiou, Balasubramanian and Lew 2017). In budding yeast, the Bud cortical landmark proteins are utilized for defining the new polarity axes. Fission yeast, on the contrary, utilizes the Tea proteins and a microtubule-based delivery system in order to position the new sites of growth (Chang and Martin 2009). Recent work in *S. pombe* suggests that it is SRDs (as evidenced by both filipin staining and the newly-identified SRD marker Tna1p) that define prospective growth sites after exit from starvation, while the role of Tea1 is to polarize the SRDs (Makushok *et al.* 2016). More specifically, shortly after exit from starvation, SRDs are initially formed intracellularly and are exocytosed at random positions of the plasma membrane (Wachtler 2003; Makushok *et al.* 2016), where they recruit polarity factors and the growth machinery. Subsequently, the Tea proteins along with microtubules polarize the SRDs, and are essential for the bipolar growth of fission yeast. Orthologues of the Bud and Tea proteins are conserved in filamentous fungi but seem to not participate in polarity establishment. In these organisms instead, the Bud proteins are required for the formation of septa (Justa-Schuch *et al.* 2010; Si *et al.* 2010), while the Tea proteins control the directionality of hyphal extension (Fischer, Zekert and Takeshita 2008). Despite controlling different polarization phenomena, the Tea proteins in *S. pombe* and *A. nidulans* seem to form similar 50-120 nm cortical foci at the plasma membrane, as seen by PALM and STORM (Dodgson *et al.* 2013; Ishitsuka *et al.* 2015). Another set of proteins, the septins, have been shown to control the formation of branches in the hyphae of filamentous fungi, thus allowing the establishment of multiple sites of polarity and the formation of mycelia (Harris 2008; Khan, McQuilken and Gladfelter 2015). Interestingly, septins in *A. gossypi* were shown to be able to sense membrane curvature, a function important for defining the region of hyphal branch formation (Bridges *et al.* 2016).

Long-term directional growth requires the maintenance of the established polarity. This process is known to require both endocytosis and exocytosis, as well as an active cytoskeleton, presumably for the recycling of polarity marker(s). Localized exocytosis occurs by targeted directed flow of Golgi-derived RabE/Rab11-containing vesicles to the tip region (Pantazopoulou *et al.* 2014) using the cytoskeleton. In filamentous fungi, cytoskeletal components and the polarized distribution of the Golgi in the sub-apical region of the hyphal tip form a specialized structure named “Spitzenkörper” (SPK) (Löpez-Franco and Bracker 2005; Steinberg *et al.* 2017; Riquelme *et al.* 2018). SPK is essential for polarity maintenance and hyphal tip growth and acts as a vesicle supply center for the tip of the hyphae. SPK-derived vesicles contain not only proteins related to polarity establishment/maintenance, but also several membrane and extracellular components that are essential for the

extension of the tip, like cell wall-building and remodeling enzymes. Importantly, the lipid flippases DnfA and DnfB, essential for hyphal growth, localize at the SPK and contribute to the asymmetric distribution of phosphatidylserine (PS) to the cytoplasmic side of exocytic vesicles (Schultzhaus, Yan and Shaw 2015). The homologous Dnf1 and Dnf2 of *S. cerevisiae* additionally recognize glycosylceramide as a substrate (Roland *et al.* 2019). Given that SLs are also essential for polarity maintenance (Cheng *et al.* 2002), the above suggest that exocytic vesicles towards the tip are enriched in PS and SLs, and that these lipids contribute to the polarization of hyphae.

Polarity maintenance is known to also necessitate endocytosis, coupled to recycling of apical markers. Endocytosis in filamentous fungi seems to occur via at least two different pathways. The first occurs along the total length of the hyphae, depends on clathrin and several related proteins (e.g. SagA/End3), and is coupled to highly-motile Early Endosomes (EEs), also known as sorting endosomes (SEs), which represents the first vesicle for material internalized from the PM (Peñalva 2010; Steinberg 2014). Seminar work, initially in *U. maydis,* has shown that EEs move bi-directionally, based on kinesin-3 or dynein, along hyphae on microtubule tracks, with the plus end of the microtubule being towards the hyphal tip (Lenz *et al.* 2006; Zhang *et al.* 2010; Schuster *et al.* 2011; Abenza *et al.* 2012; Galindo *et al.* 2012; Steinberg 2014; Reck-Peterson *et al.* 2018). Using this route, which interestingly is also hitchhiked by other organelles (Higuchi *et al.* 2014; Guimaraes *et al.* 2015; Salogiannis, Egan and Reck-Peterson 2016; Salogiannis and Reck-Peterson 2017), membrane cargos recycle back to the tip region. The second endocytic pathway crucially contributes to both polarity maintenance and the apical localization of several proteins and lipids. This endocytosis occurs mainly at the sub-apical region of the hyphae (Araujo-Bazán, Peñalva and Espeso 2008; Upadhyay and Shaw 2008) and is coupled to exocytosis in order to recycle cargos at the tip (Lenz *et al.* 2006; Taheri-Talesh *et al.* 2008; Peñalva 2010). Several endocytic factors have been found to preferentially localize at this endocytic ring, like $AbpA^{Abp1}$ and $SlaB^{Sla2}$ (Taheri-Talesh *et al.* 2008; Hervás-Aguilar and Peñalva 2010), but not clathrin (Martzoukou *et al.* 2017). Similarly, in *S. cerevisiae*, endocytic events are spatially and temporally organized and cell-cycle regulated (Bi and Park 2012). They are homogeneously distributed in unbudded cells, but are preferentially localized in the growing daughter cells during cell division, while they concentrate to the cell division plane between the mother and daughter cells (Jose *et al.* 2013). Shmoos are similarly enriched in endocytic sites (Valdez-Taubas and Pelham 2003).

Endocytic events at the subapical region are coupled to polarized exocytosis and mediate the recycling of polar cargos to the hyphal tip. Among the cargos endocytosed at the sub-apical collar and recycled back to the tip are the SNARE protein SynA/Snc1, the DnfA and DnfB lipid flippases and the TeaR, TeaA and TeaC proteins (Takeshita *et al.* 2008; Higashitsuji *et al.* 2009; Hervás-Aguilar and Peñalva 2010; Schultzhaus, Yan and Shaw 2015). It was recently shown that recycling of DnfA and DnfB, but not SynA, at the subapical endocytic collar is mediated by the AP-2 adaptor complex (Martzoukou *et al.* 2017). Interestingly, this function of AP-2 is clathrin independent, while AP-2 is dispensable for transporter endocytosis along hyphae, showing that in fungi it has a specialized role in the recycling of apical cargos. Indeed, it was additionally shown that the polarized distribution of sterols also requires AP-2-dependent endocytosis (Martzoukou *et al.* 2017). After endocytosis, the

cargos recycle back to the tip via the Golgi and the SPK (Hernández-González *et al.* 2018; Casler *et al.* 2019), and results suggest, in both *S. cerevisiae* and *A. nidulans,* that this recycling is mediated by the Golgi-Associated Retrograde Protein (GARP) complex. In detail, it was recently shown that Dnf1 and Dnf2 (orthologues of DnfA and DnfB) are substrates of the GARP complex and recycle back to the PM, while their endocytosis requires the AP-2 complex (Eising, Thiele and Fröhlich 2019). Interestingly, the GARP complex also contributes to the recycling of SLs (Fröhlich *et al.* 2015), and consistently SLs are known to be required for polarity maintenance (Cheng *et al.* 2002; Pearson *et al.* 2004; Li *et al.* 2006), probably by promoting the localization of the AP-2 complex in the subapical collar of the hyphae (Fernandes *et al.* 2016; Martzoukou *et al.* 2017). Overall these results show an interplay of the GARP complex with sterol- and SL-rich lipid raft-like membranes, which mediates the recycling of proteins back to apical membranes. Indeed, it was recently shown using PALM that the essential chitin synthetase ChsB of *A. nidulans* preferentially localizes at the SPK and accumulates in vesicles before targeted at the tip (Zhou *et al.* 2018). SPK localization of ChsB is mediated by endocytosis and recycling via the Golgi by the GARP complex, before being re-targeted to the PM (Hernández-González *et al.* 2018) (Figure 4). Recycling of cargos back to the PM via the Golgi has been also reported in *S. cerevisiae* (Becuwe and Léon 2014; Gournas *et al.* 2017), where the TGN has recently been proposed to act as an Early Endosome (Day, Casler and Glick 2018).

2.10. SPFH-domain protein-containing domains

Proteins containing the Stomatin / Prohibitin / Flotillin / HflK/C (SPFH) domain are found in all domains of life, from prokaryotes to human (Browman, Hoegg and Robbins 2007). These proteins form oligomers via the SPFH domain and participate in Detergent Resistant Membranes (DRMs) and cortical foci of several cellular membranes. In eukaryotes, members of the family localize at the PM, early endosomes, TGN, ER, mitochondria and lipid droplets (reviewed in (Browman, Hoegg and Robbins 2007), and have been implicated in numerous cellular processes including mechanosensation, cell fusion, apoptosis, respiration, hyphal morphogenesis, endocytosis, cell signaling and the regulation of the cortical cytoskeleton (Glebov, Bright and Nichols 2006; Browman, Hoegg and Robbins 2007; Solis *et al.* 2007, 2013; Ludwig *et al.* 2010; Bitsikas *et al.* 2014). Homologues of Stomatin, the founding member of the family, are found in many eukaryotic, bacterial and archaeal genomes (Green and Young 2008). Flotillins are highly conserved in metazoa, but are also found in bacteria (Lopez and Koch 2017; Wagner, Kricks and Lopez 2017) and plants (Tapken and Murphy 2015). Very few fungal members of the family have been studied to date, including the prohibitins Phb1 and Phb2 of *S. cerevisiae*, and the Stomatin StoB of *A. nidulans*, which localize at the inner mitochondrial membrane (Osman *et al.* 2006; Takeshita, Diallinas and Fischer 2012). The only plasma membrane-bound fungal SPFH members studied are the Flotillin FloA and the Stomatin StoA of *A. nidulans* (Takeshita, Diallinas and Fischer 2012), as well as the Stomatin-like protein Slp3 of *C. albicans* (Conrad *et al.* 2018). FloA or StoA have no obvious orthologues in *S. cerevisiae*.

FloA was shown to preferentially localize at cortical foci of the PM of hyphae, with maximal intensity close to the conidial head and gradually diminishing towards the hyphal tip, while it was absent from the SRDs of the tip. Despite being absent from SRDs, FloA indirectly affects their formation, as deletion of *floA* resulted in the diffusion of the filipin-stained SRDs and the related TeaA polarity marker to the subapical region of growth. Consequently, *floA*Δ strains showed reduced colony diameter and irregular hyphal widths. Despite showing a localization pattern similar to the eisosomal proteins of *A. nidulans*, confocal microscopy shows that FloA cortical foci are mutually excluded with the PilA spots along the hyphae (Zoi I., Athanasopoulos A. and Sophianopoulou V., unpublished).

On the other hand, StoA, localized at the apical regions of growth in new hyphae and shifted to subapical regions in older hyphae, while it was detected in highly motile endosomes. StoA alone did not significantly affect the formation of SRDs, however the *floA*Δ *stoA*Δ double mutant showed severely abnormal hyphae due to defects in the formation of SRDs. Intriguingly, the sole stomatin studied in *C. albicans*, Slp3, is a single cell-phase specific protein that is not expressed in the hyphae of this yeast (Conrad *et al.* 2018). Its expression is altered in response to several stress conditions, while it is induced in the late stationary phase of growth. Slp3 forms puncta at the cell periphery, however it has not been studied whether these foci form a distinct membrane domain. Lack of Slp3 showed no defects in several growth conditions, but importantly, its overexpression reduced cell viability by promoting apoptotic cell death. Further studies are needed in order to understand the function and role of SPFH proteins in fungi.

3. Concluding remarks

The continuously expanding list of membrane domains raises significant challenges and questions. We only now begin to understand the complexity of lateral PM organisation, and we still have limited knowledge on the mechanisms of formation of several membrane domains. Given the patchwork organisation of membrane proteins in multiple co-existing patches and network like domains (Spira *et al.* 2012), several more membrane compartments are expected to be defined in more detail in the future. In addition, the full spectrum of biological roles of most domains is probably underestimated. This is well reflected in the multitude of roles and the complex organisation of the more intensively studied membrane domains, like the MCC/eisosome (chapter 2.1) or the sites of endocytosis (chapter 2.7). From this complexity one can anticipate that much remains to be discovered regarding the functions and organisation of the less studied and the yet unidentified membrane domains. To add to this level of the complexity anticipated, inter-domain interactions (chapter 2.6) are only now beginning to be described.

A newly emerging concept is that membrane domains, although distinct, might partially overlap. For example, a small population of Rim9/PalI colocalizes with a subpopulation of MCCs, both in *S. cerevisiae* (Obara, Yamamoto and Kihara 2012) and in *A. nidulans* (Athanasopoulos A., Vangelatos I. and Sophianopoulou V., unpublished results). Similarly,

evidence suggests that a substantial fraction of the Avo2 MCT subunit is found in association with the core eisosomal protein Pil1 (Bartlett *et al.* 2015; Leskoske *et al.* 2018). In the same direction, the network-like domain into-which Mup1 segregates in the presence of its substrate, partially colocalizes with sites of endocytosis (Busto *et al.* 2018). The above observations have several interpretations, one being that although the domains are well-defined and non-overlapping, some specific proteins are able to change localization in between different PM domains, depending on the conditions. Indeed, a well-defined example of this situation is Slm1, which has been shown to partition in several domains (MCC, MCT and MCL), and its localization is regulated by the availability of lipids (Berchtold *et al.* 2012; Murley *et al.* 2017; Riggi *et al.* 2018). An alternative interpretation is that the domains overlap and that domain intermediates exist. These intermediates could be only a subpopulation of each domain, having specialized functions. In support of this, it has been shown that Slm1 of *S. cerevisiae* and its orthologue SlmA in *A. nidulans* are not found in all eisosomes or all MCTs, or even all MCL cortical foci (Berchtold *et al.* 2012; Murley *et al.* 2017; Pinar and Peñalva 2017; Busto *et al.* 2018). Further work is needed in order to show whether PM domain intermediates or specialised subpopulations of PM domains exist, which could potentially serve specialized functions.

Another very interesting field of future research will be the quantitative regulation of PM domains in response to environmental and/or developmental stimuli. It was previously established that the qualitative composition of the plasma membrane changes in response to different nutrient or stress conditions, via endocytosis of several transmembrane proteins (Jones *et al.* 2012; Zhao *et al.* 2013; Crapeau, Merhi and André 2014). However, it has only recently started to become evident that PM domains are also regulated at the level of their dynamics. The number, size and the proportion at the PM of specific domains are subject to tight control, like the expansion of MCCs by nutrient depletion (Gournas *et al.* 2018), the change in the subcellular localization of endocytic events in response to methionine-triggered endocytosis of Mup1 (Busto *et al.* 2018), and the clustering of MCT foci in response to membrane tension (Riggi *et al.* 2018). The mechanisms controlling the above phenomena, their evolutionary conservation, as well as whether the levels of more PM domains are regulated remain to be shown.

Finally, accumulating knowledge on fungal PM domains can shed new light on the organization of the PM of animal cells. For example, although eisosomes have no homologues in animals, they show important analogies to the caveolae of mammalian cells (Moreira *et al.* 2012). Caveolae, similarly to eisosomes, are invaginations of 60–80 nm diameter, that are formed by polymerization of integral membrane proteins called caveolins. Caveolae are believed to serve as platforms for signalling molecules and enzymes, as tension- dependent membrane reservoirs for expansion of cell surface, while they are also involved in intracellular trafficking [Reviewed in (Parton and Del Pozo 2013; Kovtun *et al.* 2015; Lamaze *et al.* 2017)]. Caveolae are considered as a subset of lipid rafts, since proteins found there partition in DRMs (Carquin *et al.* 2016; Sezgin *et al.* 2017). Thus, eisosomes and caveolae share many physiological roles. Similar to the previous situation with eisosomes, it is up to now a matter of debate whether or how caveolae are involved in endocytosis. Early evidence suggested that caveolae are sites of endocytosis, but this has been challenged the last years [(Cheng and Nichols 2016) and references therein]. More precisely, it was recently

proposed that only clathrin-independent endocytosis could occur from caveolae (Chaudhary *et al.* 2014), and that caveolar endocytosis could be specialised for lipids, while bulk membrane proteins would be excluded from caveolae (Shvets *et al.* 2015). It is tempting to speculate that, like eisosomes, caveolae could be places where relatively small membrane proteins are protected from endocytosis under specific conditions, and that caveolae only indirectly affect endocytic dynamics by controlling the biosynthesis and trafficking of lipids.

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References:

Abe F, Hiraki T. Mechanistic role of ergosterol in membrane rigidity and cycloheximide resistance in Saccharomyces cerevisiae. *Biochim Biophys Acta - Biomembr* 2009;**1788**:743–52.

Abe F, Iida H. Pressure-induced differential regulation of the two tryptophan permeases Tat1 and Tat2 by ubiquitin ligase Rsp5 and its binding proteins, Bul1 and Bul2. *Mol Cell Biol* 2003;**23**:7566–84.

Abenza JF, Galindo A, Pinar M *et al.* Endosomal maturation by Rab conversion in Aspergillus nidulans is coupled to dynein-mediated basipetal movement. *Mol Biol Cell* 2012;**23**:1889–901.

Alsteens D, Dupres V, Yunus S *et al.* High-resolution imaging of chemical and biological sites on living cells using peak force tapping atomic force microscopy. *Langmuir* 2012;**28**:16738–44.

Alvarez FJ, Douglas LM, Konopka JB. Sterol-Rich Plasma Membrane Domains in Fungi. *Eukaryot Cell* 2007;**6**:755–63.

Alvarez FJ, Douglas LM, Konopka JB. The Sur7 protein resides in punctate membrane subdomains and mediates spatial regulation of cell wall synthesis in Candida albicans. *Commun Integr Biol* 2009, DOI: 10.4161/cib.7478.

Alvarez FJ, Douglas LM, Rosebrock A *et al.* The Sur7 Protein Regulates Plasma Membrane Organization and Prevents Intracellular Cell Wall Growth in Candida albicans. *Mol Biol Cell* 2008;**19**:5214–25.

Antonescu CN, Danuser G, Schmid SL. Phosphatidic Acid Plays a Regulatory Role in Clathrin-mediated Endocytosis. Lemmon S (ed.). *Mol Biol Cell* 2010;**21**:2944–52.

Araujo-Bazán L, Peñalva MA, Espeso EA. Preferential localization of the endocytic internalization machinery to hyphal tips underlies polarization of the actin cytoskeleton in Aspergillus nidulans. *Mol Microbiol* 2008, DOI: 10.1111/j.1365-2958.2007.06102.x.

Aresta-Branco F, Cordeiro AM, Marinho HS *et al.* Gel domains in the plasma membrane of Saccharomyces cerevisiae: Highly ordered, ergosterol-free, and sphingolipid-enriched lipid rafts. *J Biol Chem* 2011;**286**:5043–54.

Atanasova L, Gruber S, Lichius A *et al.* The Gpr1-regulated Sur7 family protein Sfp2 is required for hyphal growth and cell wall stability in the mycoparasite Trichoderma atroviride. *Sci Rep* 2018;**8**:12064.

Athanasopoulos A, Boleti H, Scazzocchio C *et al.* Eisosome distribution and localization in the meiotic progeny of Aspergillus nidulans. *Fungal Genet Biol* 2013;**53**:84–96.

Athanasopoulos A, Gournas C, Amillis S *et al.* Characterization of AnNce102 and its role in eisosome stability and sphingolipid biosynthesis. *Sci Rep* 2015;**5**:15200.

Auer M, Scarborough GA, Kühlbrandt W. Three-dimensional map of the plasma membrane H+-atpase in the open conformation. *Nature* 1998;**392**:840–3.

Ausili A, Martínez-Valera P, Torrecillas A *et al.* Anticancer Agent Edelfosine Exhibits a High Affinity for Cholesterol and Disorganizes Liquid-Ordered Membrane Structures. *Langmuir* 2018;**34**:8333–46.

Babst M. Eisosomes at the Intersection of TORC1 and TORC2 Regulation. *Traffic* 2019:tra.12651.

Baetz K, Moffat J, Haynes J *et al.* Transcriptional coregulation by the cell integrity mitogen-activated protein kinase Slt2 and the cell cycle regulator Swi4. *Mol Cell Biol* 2001;**21**:6515–28.

Bagnat M, Chang A, Simons K. Plasma Membrane Proton ATPase Pma1p Requires Raft Association for Surface Delivery in Yeast. *Mol Biol Cell* 2013;**12**:4129–38.

Bagnat M, Keranen S, Shevchenko A *et al.* Lipid rafts function in biosynthetic delivery of proteins to the cell surface in yeast. *Proc Natl Acad Sci* 2012;**97**:3254–9.

Bagnat M, Simons K. Cell surface polarization during yeast mating. *Proc Natl Acad Sci* 2002;**99**:14183–8.

Baile MG, Guiney EL, Sanford EJ *et al.* The yeast Art1 arrestin domain contains disordered insertions that regulate its localization and activity. *bioRxiv* 2019, DOI: 10.1101/553677.

Baker K, Kirkham S, Halova L *et al.* TOR complex 2 localises to the cytokinetic actomyosin ring and controls the fidelity of cytokinesis. *J Cell Sci* 2016;**129**:2613–24.

Bartlett K, Gadila SKG, Tenay B *et al.* TORC2 and eisosomes are spatially interdependent, requiring optimal level of phosphatidylinositol 4, 5-bisphosphate for their integrity. *J Biosci* 2015;**40**:299–311.

Becuwe M, Herrador A, Haguenauer-Tsapis R *et al.* Ubiquitin-Mediated Regulation of Endocytosis by Proteins of the Arrestin Family. *Biochem Res Int* 2012a;**2012**:1–12.

Becuwe M, Léon S. Integrated control of transporter endocytosis and recycling by the arrestin-related protein Rod1 and the ubiquitin ligase Rsp5. *Elife* 2014;**3**, DOI: 10.7554/elife.03307.

Becuwe M, Vieira N, Lara D *et al.* A molecular switch on an arrestin-like protein relays glucose signaling to transporter endocytosis. *J Cell Biol* 2012b;**196**:247–59.

Berchtold D, Piccolis M, Chiaruttini N *et al.* Plasma membrane stress induces relocalization of Slm proteins and activation of TORC2 to promote sphingolipid synthesis. *Nat Cell Biol* 2012;**14**:542–7.

Berchtold D, Walther TC. TORC2 Plasma Membrane Localization Is Essential for Cell Viability and Restricted to a Distinct Domain. *Mol Biol Cell* 2009;**20**:1565–75.

Berepiki A, Lichius A, Shoji J-Y *et al.* F-Actin Dynamics in Neurospora crassa. *Eukaryot Cell* 2010;**9**:547–57.

Bernardino de la Serna J, Schütz GJ, Eggeling C *et al.* There Is No Simple Model of the Plasma Membrane Organization. *Front Cell Dev Biol* 2016;**4**, DOI: 10.3389/fcell.2016.00106.

Bharat TAM, Hoffmann PC, Kukulski W. Correlative Microscopy of Vitreous Sections Provides Insights into BAR-Domain Organization In Situ. *Structure* 2018;**26**:879-886.e3.

Bi E, Park HO. Cell polarization and cytokinesis in budding yeast. *Genetics* 2012;**191**:347–87.

Bianchi F, Syga Ł, Moiset G *et al.* Steric exclusion and protein conformation determine the localization of plasma membrane transporters. *Nat Commun* 2018;**9**:501.

Bieberich E. Sphingolipids and lipid rafts: Novel concepts and methods of analysis. *Chem Phys Lipids* 2018;**216**:114–31.

Bitsikas V, Riento K, Howe JD *et al.* The role of flotillins in regulating ab production, investigated using Flotillin 1-/-, Flotillin 2-/- double knockout mice. Seaman M (ed.). *PLoS One* 2014;**9**:e85217.

Björkbom A, Ohvo-Rekilä H, Kankaanpää P *et al.* Characterization of membrane properties of inositol phosphorylceramide. *Biochim Biophys Acta - Biomembr* 2010;**1798**:453–60.

Blenis J. TOR, the Gateway to Cellular Metabolism, Cell Growth, and Disease. *Cell* 2017;**171**:10–3.

Boettner DR, Chi RJ, Lemmon SK. Lessons from yeast for clathrin-mediated endocytosis. *Nat Cell Biol* 2011;**14**:2–10.

Bourgoint C, Rispal D, Berti M *et al.* Target of rapamycin complex 2– dependent phosphorylation of the coat protein Pan1 by Akl1 controls endocytosis dynamics in Saccharomyces cerevisiae. *J Biol Chem* 2018;**293**:12043–53.

Bowman EJ, O'Neill FJ, Bowman BJ. Mutations of pma-1, the gene encoding the plasma membrane H+-ATPase of Neurospora crassa, suppress inhibition of growth by concanamycin A, a specific inhibitor of vacuolar ATPases. *J Biol Chem* 1997;**272**:14776–86.

Brach T, Godlee C, Moeller-Hansen I *et al.* The initiation of clathrin-mediated endocytosis is mechanistically highly flexible. *Curr Biol* 2014;**24**:548–54.

Brach T, Specht T, Kaksonen M. Reassessment of the role of plasma membrane domains in the regulation of vesicular traffic in yeast. *J Cell Sci* 2011;**124**:328–37.

Brakhage AA, Spröte P, Al-Abdallah Q *et al.* Regulation of Penicillin Biosynthesis in Filamentous Fungi. 2012, 45–90.

Braun KA, Vaga S, Dombek KM *et al.* Phosphoproteomic analysis identifies proteins involved in transcription-coupled mRNA decay as targets of Snf1 signaling. *Sci Signal* 2014;**7**:ra64.

Breslow DK, Collins SR, Bodenmiller B *et al.* Orm family proteins mediate sphingolipid homeostasis. *Nature* 2010;**463**:1048–53.

Bridges AA, Jentzsch MS, Oakes PW *et al.* Micron-scale plasma membrane curvature is recognized by the septin cytoskeleton. *J Cell Biol* 2016;**213**:23–32.

Browman DT, Hoegg MB, Robbins SM. The SPFH domain-containing proteins: more than lipid raft markers. *Trends Cell Biol* 2007;**17**:394–402.

Brown HE, Ost KS, Esher SK *et al.* Identifying a novel connection between the fungal plasma membrane and pH-sensing. *Mol Microbiol* 2018;**109**:474–93.

Bultynck G, Heath VL, Majeed AP *et al.* Slm1 and Slm2 Are Novel Substrates of the Calcineurin Phosphatase Required for Heat Stress-Induced Endocytosis of the Yeast Uracil Permease. *Mol Cell Biol* 2006;**26**:4729–45.

Burghoorn HP, Soteropoulos P, Paderu P *et al.* Molecular evaluation of the plasma membrane proton pump from Aspergillus fumigatus. *Antimicrob Agents Chemother* 2002;**46**:615–24.

Busto J V, Elting A, Haase D *et al.* Lateral plasma membrane compartmentalization links protein function and turnover. *EMBO J* 2018;**37**:e99473.

Calcagno-Pizarelli AM, Negrete-Urtasun S, Denison SH *et al.* Establishment of the ambient pH signaling complex in Aspergillus nidulans: PalI assists plasma membrane localization of PalH. *Eukaryot Cell* 2007;**6**:2365–75.

Carquin M, D'Auria L, Pollet H *et al.* Recent progress on lipid lateral heterogeneity in plasma membranes: From rafts to submicrometric domains. *Prog Lipid Res* 2016;**62**:1–24.

Carroll SY, Stimpson HEM, Weinberg J *et al.* Analysis of yeast endocytic site formation and maturation through a regulatory transition point. Schmid SL (ed.). *Mol Biol Cell* 2011;**23**:657–68.

Casamayor A, Torrance PD, Kobayashi T *et al.* Functional counterparts of mammalian protein kinases PDK1 and SGK in budding yeast. *Curr Biol* 1999;**9**:186–97.

Casler JC, Papanikou E, Barrero JJ *et al.* Maturation-driven transport and AP-1-dependent recycling of a secretory cargo in the Golgi. *J Cell Biol* 2019, DOI: 10.1083/jcb.201807195.

Chang F, Martin SG. Shaping fission yeast with microtubules. *Cold Spring Harb Perspect Biol* 2009, DOI: 10.1101/cshperspect.a001347.

Chaudhary N, Gomez GA, Howes MT *et al.* Endocytic Crosstalk: Cavins, Caveolins, and Caveolae Regulate Clathrin-Independent Endocytosis. Schmid SL (ed.). *PLoS Biol* 2014;**12**:e1001832.

Chen D, Wilkinson CRM, Watt S *et al.* Dynamic Regulation of the Large Exocytotic Fusion Pore in Pancreatic Acinar Cells. *Mol Biol Cell* 2007;**19**:308–17.

Cheng J, Park T-S, Fischl AS *et al.* Cell Cycle Progression and Cell Polarity Require Sphingolipid Biosynthesis in Aspergillus nidulans. *Mol Cell Biol* 2002;**21**:6198–209.

Cheng JPX, Nichols BJ. Caveolae: One Function or Many? *Trends Cell Biol* 2016;**26**:177–89.

Chiou J, Balasubramanian MK, Lew DJ. Cell Polarity in Yeast. *Annu Rev Cell Dev Biol* 2017;**33**:77–101.

Cohen A, Habib A, Laor D *et al.* TOR complex 2 in fission yeast is required for chromatin-mediated gene silencing and assembly of heterochromatic domains at subtelomeres. *J Biol Chem* 2018;**293**:8138–50.

Cohen A, Kupiec M, Weisman R. Glucose activates TORC2-Gad8 protein via positive regulation of the cAMP/cAMP-dependent Protein Kinase A (PKA) pathway and negative regulation of the Pmk1 protein-mitogen-activated protein kinase pathway. *J Biol Chem* 2014;**289**:21727–37.

Coleman JA, Yang D, Zhao Z *et al.* Serotonin transporter–ibogaine complexes illuminate mechanisms of inhibition and transport. *Nature* 2019:1.

Conrad KA, Rodriguez R, Salcedo EC *et al.* The Candida albicans stress response gene Stomatin-Like Protein 3 is implicated in ROS-induced apoptotic-like death of yeast phase cells. *PLoS One* 2018;**13**:e0192250.

Cournia Z, Ullmann GM, Smith JC. Differential effects of cholesterol, ergosterol and lanosterol on a dipalmitoyl phosphatidylcholine membrane: A molecular dynamics simulation study. *J Phys Chem B* 2007;**111**:1786–801.

Crapeau M, Merhi A, André B. Stress conditions promote yeast Gap1 permease ubiquitylation and down-regulation via the arrestin-like bul and aly proteins. *J Biol Chem* 2014;**289**:22103–16.

Cuesta-Marbán Á, Botet J, Czyz O *et al.* Drug uptake, lipid rafts, and vesicle trafficking modulate resistance to an anticancer lysophosphatidylcholine analogue in yeast. *J Biol Chem* 2013;**288**:8405–18.

Czyz O, Bitew T, Cuesta-Marbán A *et al.* Alteration of plasma membrane organization by an anticancer lysophosphatidylcholine analogue induces intracellular acidification and internalization of plasma membrane transporters in yeast. *J Biol Chem* 2013;**288**:8419–32.

Davis D, Kannan M, Wattenberg B. Orm/ORMDL proteins: Gate guardians and master regulators. *Adv Biol Regul* 2018;**70**:3–18.

Davis DA. How human pathogenic fungi sense and adapt to pH: the link to virulence. *Curr Opin Microbiol* 2009;**12**:365–70.

Day KJ, Casler JC, Glick BS. Budding Yeast Has a Minimal Endomembrane System. *Dev Cell* 2018;**44**:56-72.e4.

deHart AKA. Receptor Internalization in Yeast Requires the Tor2-Rho1 Signaling Pathway. *Mol Biol Cell* 2003;**14**:4676–84.

Delgado-Álvarez DL, Callejas-Negrete OA, Gómez N *et al.* Visualization of F-actin localization and dynamics with live cell markers in Neurospora crassa. *Fungal Genet Biol* 2010;**47**:573–86.

Delorme-Axford E, Abernathy E, Lennemann NJ *et al.* The exoribonuclease Xrn1 is a post-transcriptional negative regulator of autophagy. *Autophagy* 2018;**14**:898–912.

Deng C, Xiong X, Krutchinsky AN. Unifying Fluorescence Microscopy and Mass Spectrometry for Studying Protein Complexes in Cells. *Mol Cell Proteomics* 2009;**8**:1413–23.

Deprez M-A, Eskes E, Wilms T *et al.* pH homeostasis links the nutrient sensing PKA/TORC1/Sch9 ménage-à-trois to stress tolerance and longevity. *Microb Cell* 2018;**5**:119–36.

Desfarges L, Durrens P, Juguelin H *et al.* Yeast mutants affected in viability upon starvation have a modified phospholipid composition. *Yeast* 1993;**9**:267–77.

Dichtl K, Samantaray S, Wagener J. Cell wall integrity signalling in human pathogenic fungi. *Cell Microbiol* 2016;**18**:1228–38.

Díez E, Álvaro J, Espeso EA *et al.* Activation of the Aspergillus PacC zinc finger transcription factor requires two proteolytic steps. *EMBO J* 2002;**21**:1350–9.

Dodgson J, Chessel A, Yamamoto M *et al.* Spatial segregation of polarity factors into distinct cortical clusters is required for cell polarity control. *Nat Commun* 2013;**4**:1834.

Douglas LM, Konopka JB. Fungal Membrane Organization: The Eisosome Concept. *Annu Rev Microbiol* 2014;**68**:377–93.

Douglas LM, Konopka JB. Plasma membrane architecture protects Candida albicans from killing by copper. Culotta VC (ed.). *PLoS Genet* 2019;**15**:e1007911.

Douglas LM, Wang HX, Keppler-Ross S *et al.* Sur7 promotes plasma membrane organization and is needed for resistance to stressful conditions and to the invasive growth and virulence of Candida albicans. *MBio* 2012, DOI: 10.1128/mBio.00254-11.

Douglas LM, Wang HX, Konopka JB. The MARVEL domain protein Nce102 regulates actin organization and invasive growth of Candida albicans. *MBio* 2013;**4**, DOI: 10.1128/mBio.00723-13.

Du H, Huang G. Environmental pH adaption and morphological transitions in Candida albicans. *Curr Genet* 2016;**62**:283–6.

Dufourc EJ. Sterols and membrane dynamics. *J Chem Biol* 2008;**1**:63–77.

Dupont S, Beney L, Ritt JF *et al.* Lateral reorganization of plasma membrane is involved in the yeast resistance to severe dehydration. *Biochim Biophys Acta - Biomembr* 2010;**1798**:975–85.

Dupres V, Alsteens D, Wilk S *et al.* The yeast Wsc1 cell surface sensor behaves like a nanospring in vivo. *Nat Chem Biol* 2009;**5**:857–62.

Edidin M. Rotational and Translational Diffusion in Membranes. *Annu Rev Biophys Bioeng* 2003;**3**:179–201.

Eisendle M, Oberegger H, Buttinger R *et al.* Biosynthesis and uptake of siderophores is controlled by the PacC-mediated ambient-pH regulatory system in Aspergillus nidulans. *Eukaryot Cell* 2004;**3**:561–3.

Eising S, Thiele L, Fröhlich F. A systematic approach to identify recycling endocytic cargo depending on the GARP complex. *Elife* 2019;**8**, DOI: 10.7554/elife.42837.

Eltschinger S, Loewith R. TOR Complexes and the Maintenance of Cellular Homeostasis. *Trends Cell Biol* 2016;**26**:148–59.

Eraso P, Gancedo C. Activation of yeast plasma membrane ATPase by acid pH during growth. *FEBS Lett* 1987;**224**:187–92.

Farnoud AM, Mor V, Singh A *et al.* Inositol phosphosphingolipid phospholipase C1 regulates plasma membrane ATPase (Pma1) stability in Cryptococcus neoformans. *FEBS Lett* 2014;**588**:3932–8.

Farnoud AM, Toledo AM, Konopka JB *et al.* Raft-Like Membrane Domains in Pathogenic Microorganisms. *Curr Top Membr* 2015;**75**:233–68.

Fernandes CM, de Castro PA, Singh A *et al.* Functional characterization of the Aspergillus nidulans glucosylceramide pathway reveals that LCB Δ8-desaturation and C9-methylation are relevant to filamentous growth, lipid raft localization and Psd1 defensin activity. *Mol Microbiol* 2016;**102**:488–505.

Fischer R, Zekert N, Takeshita N. Polarized growth in fungi - Interplay between the cytoskeleton, positional markers and membrane domains. *Mol Microbiol* 2008;**68**:813–26.

Fitzgibbon GJ, Morozov IY, Jones MG *et al.* Genetic analysis of the TOR pathway in Aspergillus nidulans. *Eukaryot Cell* 2005, DOI: 10.1128/EC.4.9.1595-1598.2005.

Foderaro J, Douglas L, Konopka J. MCC/Eisosomes Regulate Cell Wall Synthesis and Stress Responses in Fungi. *J Fungi* 2017;**3**:61.

Francoise M. Roelants, Pamela D. Torrance, Natalie Bezman A, Thorner J. Pkh1 and Pkh2 Differentially Phosphorylate and Activate Ypk1 and Ykr2 and Define Protein Kinase Modules Required for Maintenance of Cell Wall Integrity. *Mol Biol Cell* 2002;**13**:3005–28.

Fröhlich F, Christiano R, Olson DK *et al.* A role for eisosomes in maintenance of plasma membrane phosphoinositide levels. *Mol Biol Cell* 2014;**25**:2797–806.

Fröhlich F, Moreira K, Aguilar PS *et al.* A genome-wide screen for genes affecting eisosomes reveals Nce102 function in sphingolipid signaling. *J Cell Biol* 2009;**185**:1227–42.

Fröhlich F, Petit C, Kory N *et al.* The GARP complex is required for cellular sphingolipid homeostasis. *Elife* 2015;**4**, DOI: 10.7554/elife.08712.

Futagami T, Goto M. Putative cell wall integrity sensor proteins in Aspergillus nidulans. *Commun Integr Biol* 2012;**5**:206–8.

Futagami T, Nakao S, Kido Y *et al.* Putative Stress Sensors WscA and WscB Are Involved in Hypo-Osmotic and Acidic pH Stress Tolerance in Aspergillus nidulans. *Eukaryot Cell* 2011;**10**:1504–15.

Gachet Y. Endocytosis in fission yeast is spatially associated with the actin cytoskeleton during polarised cell growth and cytokinesis. *J Cell Sci* 2005;**118**:4231–42.

Gaigg B, Toulmay A, Schneiter R. Very long-chain fatty acid-containing lipids rather than sphingolipids per se are required for raft association and stable surface transport of newly synthesized plasma membrane ATPase in yeast. *J Biol Chem* 2006;**281**:34135–45.

Galindo A, Calcagno-Pizarelli AM, Arst HN *et al.* An ordered pathway for the assembly of fungal ESCRT-containing ambient pH signalling complexes at the plasma membrane. *J Cell Sci* 2012;**125**:1784–95.

Galindo A, Hervás-Aguilar A, Rodríguez-Galán O *et al.* PalC, one of two Bro1 domain proteins in the fungal pH signalling pathway, localizes to cortical structures and binds Vps32. *Traffic* 2007;**8**:1346–64.

Gatta AT, Wong LH, Sere YY *et al.* A new family of StART domain proteins at membrane contact sites has a role in ER-PM sterol transport. *Elife* 2015;**4**:1–46.

Gaubitz C, Oliveira TM, Prouteau M *et al.* Molecular Basis of the Rapamycin Insensitivity of Target Of Rapamycin Complex 2. *Mol Cell* 2015;**58**:977–88.

Gaubitz C, Prouteau M, Kusmider B *et al.* TORC2 Structure and Function. *Trends Biochem Sci* 2016;**41**:532–45.

Ghaddar K, Krammer EM, Mihajlovic N *et al.* Converting the yeast arginine Can1 permease to a lysine permease. *J Biol Chem* 2014a;**289**:7232–46.

Ghaddar K, Merhi A, Saliba E *et al.* Substrate-Induced Ubiquitylation and Endocytosis of Yeast Amino Acid Permeases. *Mol Cell Biol* 2014b;**34**:4447–63.

Glebov OO, Bright NA, Nichols BJ. Flotillin-1 defines a clathrin-independent endocytic pathway in mammalian cells. *Nat Cell Biol* 2006;**8**:46–54.

González Montoro A, Ungermann C. StARTing to understand membrane contact sites. *Trends Cell Biol* 2015;**25**:497–8.

Gournas C, Amillis S, Vlanti A *et al.* Transport-dependent endocytosis and turnover of a uric acid-xanthine permease. *Mol Microbiol* 2010;**75**:246–60.

Gournas C, Athanasopoulos A, Sophianopoulou V. On the Evolution of Specificity in Members of the Yeast Amino Acid Transporter Family as Parts of Specific Metabolic Pathways. *Int J Mol Sci* 2018;**19**:1398.

Gournas C, Gkionis S, Carquin M *et al.* Conformation-dependent partitioning of yeast nutrient transporters into starvation-protective membrane domains. *Proc Natl Acad Sci* 2018;**115**:E3145–54.

Gournas C, Prévost M, Krammer E-MM *et al.* Function and regulation of fungal amino acid transporters: Insights from predicted structure. *Advances in Experimental Medicine and Biology*. Vol 892. 2016, 69–106.

Gournas C, Saliba E, Krammer E-M *et al.* Transition of yeast Can1 transporter to the inward-facing state unveils an α-arrestin target sequence promoting its ubiquitylation and endocytosis. *Mol Biol Cell* 2017;**28**:mbc.E17-02-0104.

Gow NAR, Latge J-P, Munro CA. The Fungal Cell Wall: Structure, Biosynthesis, and Function. *Microbiol Spectr* 2017;**5**:267–92.

Grassmé H, Jendrossek V, Riehle A *et al.* Host defense against Pseudomonas aeruginosa requires ceramide-rich membrane rafts. *Nat Med* 2003;**9**:322–30.

Green JB, Young JPW. Slipins: Ancient origin, duplication and diversification of the stomatin protein family. *BMC Evol Biol* 2008;**8**, DOI: 10.1186/1471-2148-8-44.

Grossmann G, Malinsky J, Stahlschmidt W *et al.* Plasma membrane microdomains regulate turnover of transport proteins in yeast. *J Cell Biol* 2008;**183**:1075–88.

Grossmann G, Opekarová M, Malinsky J *et al.* Membrane potential governs lateral segregation of plasma membrane proteins and lipids in yeast. *EMBO J* 2007;**26**:1–8.

Grousl T, Opekarová M, Stradalova V *et al.* Evolutionarily conserved 5'-3' exoribonuclease Xrn1 accumulates at plasma membrane-associated eisosomes in post-diauxic yeast. *PLoS One* 2015;**10**, DOI: 10.1371/journal.pone.0122770.

Guerreiro JF, Muir A, Ramachandran S *et al.* Sphingolipid biosynthesis upregulation by TOR complex 2-Ypk1 signaling during yeast adaptive response to acetic acid stress. *Biochem J* 2016;**473**:4311–25.

Guimaraes SC, Schuster M, Bielska E *et al.* Peroxisomes, lipid droplets, and endoplasmic reticulum "hitchhike" on motile early endosomes. *J Cell Biol* 2015;**211**:945–54.

Guiney EL, Klecker T, Emr SD. Identification of the endocytic sorting signal recognized by the Art1-Rsp5 ubiquitin ligase complex. *Mol Biol Cell* 2016;**27**:4043–54.

Hanada K. Lipid transfer proteins rectify inter-organelle flux and accurately deliver lipids at membrane contact sites. *J Lipid Res* 2018;**59**:1341–66.

Hannun YA, Obeid LM. Principles of bioactive lipid signalling: Lessons from sphingolipids. *Nat Rev Mol Cell Biol* 2008;**9**:139–50.

Harris SD. Branching of fungal hyphae: regulation, mechanisms and comparison with other branching systems. *Mycologia* 2008;**100**:823–32.

Hatakeyama R, Kamiya M, Takahara T *et al.* Endocytosis of the Aspartic Acid/Glutamic Acid Transporter Dip5 Is Triggered by Substrate-Dependent Recruitment of the Rsp5 Ubiquitin Ligase via the Arrestin-Like Protein Aly2. *Mol Cell Biol* 2010;**30**:5598–607.

Hayashi T, Hatanaka M, Nagao K *et al.* Rapamycin sensitivity of the Schizosaccharomyces pombe tor2 mutant and organization of two highly phosphorylated TOR complexes by specific and common subunits. *Genes to Cells* 2007;**12**:1357–70.

Hein C, Springael J -Y, Volland C *et al.* NPI1, an essential yeast gene involved in induced degradation of Gap1 and Fur4 permeases, encodes the Rsp5 ubiquitin—protein ligase. *Mol Microbiol* 1995;**18**:77–87.

Heinisch JJ, Dupres V, Wilk S *et al.* Single-molecule atomic force microscopy reveals clustering of the yeast plasma-membrane sensor Wsc1. *PLoS One* 2010;**5**:e11104.

Heinisch JJ, Rodicio R. Protein kinase C in fungi-more than just cell wall integrity. *FEMS Microbiol Rev* 2018;**42**:22–39.

Heitman J, Movva NR, Hall MN. Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science (80-)* 1991;**253**:905–9.

Henderson KA, Hughes AL, Gottschling DE. Mother-daughter asymmetry of pH underlies aging and rejuvenation in yeast. *Elife* 2014;**3**:e03504.

Herman P, Vecer J, Opekarova M *et al.* Depolarization affects the lateral microdomain structure of yeast plasma membrane. *FEBS J* 2015;**282**:419–34.

Hernández-González M, Bravo-Plaza I, Pinar M *et al.* Endocytic recycling via the TGN underlies the polarized hyphal mode of life. Gladfelter AS (ed.). *PLoS Genet* 2018;**14**:e1007291.

Herrador A, Herranz S, Lara D *et al.* Recruitment of the ESCRT Machinery to a Putative Seven-Transmembrane-Domain Receptor Is Mediated by an Arrestin-Related Protein. *Mol Cell Biol* 2010;**30**:897–907.

Herranz S, Rodriguez JM, Bussink H-J *et al.* Arrestin-related proteins mediate pH signaling in fungi. *Proc Natl Acad Sci* 2005;**102**:12141–6.

Hervás-Aguilar A, Galindo A, Peñalva MA. Receptor-independent ambient pH signaling by ubiquitin attachment to fungal arrestin-like PalF. *J Biol Chem* 2010;**285**:18095–102.

Hervás-Aguilar A, Peñalva MA. Endocytic machinery protein SlaB is dispensable for polarity establishment but necessary for polarity maintenance in hyphal tip cells of Aspergillus nidulans. *Eukaryot Cell* 2010, DOI: 10.1128/EC.00119-10.

Hervás-Aguilar A, Rodríguez JM, Tilburn J *et al.* Evidence for the direct involvement of the proteasome in the proteolytic processing of the Aspergillus nidulans zinc finger transcription factor PacC. *J Biol Chem* 2007;**282**:34735–47.

Higashitsuji Y, Herrero S, Takeshita N *et al.* The cell end marker protein teaC is involved in growth directionality and septation in aspergillus nidulans. *Eukaryot Cell* 2009, DOI: 10.1128/EC.00251-08.

Higuchi Y, Ashwin P, Roger Y *et al.* Early endosome motility spatially organizes polysome distribution. *J Cell Biol* 2014;**204**:343–57.

Higuchi Y, Shoji JY, Arioka M *et al.* Endocytosis is crucial for cell polarity and apical membrane recycling in the filamentous fungus Aspergillus oryzae. *Eukaryot Cell* 2009;**8**:37–46.

Hommel B, Mukaremera L, Cordero RJB *et al.* Titan cells formation in Cryptococcus neoformans is finely tuned by environmental conditions and modulated by positive and negative genetic regulators. *PLoS Pathog* 2018;**14**:e1006982.

Horenkamp FA, Valverde DP, Nunnari J *et al.* Molecular basis for sterol transport by StART-like lipid transfer domains. *EMBO J* 2018;**37**:e98002.

Hughes AL, Gottschling DE. An early age increase in vacuolar pH limits mitochondrial function and lifespan in yeast. *Nature* 2012;**492**:261–5.

Ikai N, Nakazawa N, Hayashi T *et al.* The reverse, but coordinated, roles of Tor2 (TORC1) and Tor1 (TORC2) kinases for growth, cell cycle and separase-mediated mitosis in Schizosaccharomyces pombe. *Open Biol* 2011;**1**:110007–110007.

Ikeda M, Kihara A, Depoh A *et al.* The Rim101 Pathway Is Involved in Rsb1 Expression Induced by Altered Lipid Asymmetry. *Mol Biol Cell* 2008;**19**:1922–31.

Inagaki M, Schmelzle T, Yamaguchi K *et al.* PDK1 Homologs Activate the Pkc1–Mitogen-Activated Protein Kinase Pathway in Yeast. *Mol Cell Biol* 2015;**19**:8344–52.

Inoue H, Sugimoto S, Takeshita Y *et al.* CK2 phospho-independent assembly of the Tel2-associated stress-signaling complexes in Schizosaccharomyces pombe. *Genes to Cells* 2017;**22**:59–70.

Inp P, Products INPG, Stolz LE *et al.* Identification and Characterization of an Essential Family of Inositol. *Genetics* 1998;**148**:1715–29.

Ishitsuka Y, Savage N, Li Y *et al.* Cell Biology: Superresolution microscopy reveals a dynamic picture of cell polarity maintenance during directional growth. *Sci Adv* 2015;**1**:e1500947.

Ito H, Oshiro T, Fujita Y *et al.* Pma1, a P-type proton ATPase, is a determinant of chronological life span in fission yeast. *J Biol Chem* 2010;**285**:34616–20.

Jacinto E, Loewith R, Schmidt A *et al.* Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat Cell Biol* 2004;**6**:1122–8.

Jendretzki A, Wittland J, Wilk S *et al.* How do I begin? Sensing extracellular stress to maintain yeast cell wall integrity. *Eur J Cell Biol* 2011;**90**:740–4.

Johansen J, Ramanathan V, Beh CT. Vesicle trafficking from a lipid perspective. *Cell Logist* 2012;**2**:151–60.

Jones CB, Ott EM, Keener JM *et al.* Regulation of membrane protein degradation by starvation-response pathways. *Traffic* 2012;**13**:468–82.

Jose M, Tollis S, Nair D *et al.* Robust polarity establishment occurs via an endocytosis-based cortical corralling mechanis. *J Cell Biol* 2013;**200**:407–18.

Justa-Schuch D, Heilig Y, Richthammer C *et al.* Septum formation is regulated by the RHO4-specific exchange factors BUD3 and RGF3 and by the landmark protein BUD4 in Neurospora crassa. *Mol Microbiol* 2010;**76**:220–35.

Kabeche R, Baldissard S, Hammond J *et al.* The filament-forming protein Pil1 assembles linear eisosomes in fission yeast. *Mol Biol Cell* 2011;**22**:4059–67.

Kabeche R, Howard L, Moseley JB. Eisosomes provide membrane reservoirs for rapid expansion of the yeast plasma membrane. *J Cell Sci* 2015a;**128**:4057–62.

Kabeche R, Howard L, Moseley JB. Pil1 cytoplasmic rods contain bundles of crosslinked tubules. *Commun Integr Biol* 2015b;**8**, DOI: 10.4161/19420889.2014.990848.

Kabeche R, Roguev A, Krogan NJ *et al.* A Pil1-Sle1-Syj1-Tax4 functional pathway links eisosomes with PI(4,5)P2 regulation. *J Cell Sci* 2014;**127**:1318–26.

Kaksonen M, Roux A. Mechanisms of clathrin-mediated endocytosis. *Nat Rev Mol Cell Biol* 2018;**19**:313–26.

Kaksonen M, Toret CP, Drubin DG. A modular design for the clathrin- and actin-mediated endocytosis machinery. *Cell* 2005;**123**:305–20.

Kamada Y, Fujioka Y, Suzuki NN *et al.* Tor2 Directly Phosphorylates the AGC Kinase Ypk2 To Regulate Actin Polarization. *Mol Cell Biol* 2005;**25**:7239–48.

Kaminska J, Spiess M, Stawiecka-Mirota M *et al.* Yeast Rsp5 ubiquitin ligase affects the actin cytoskeleton in vivo and in vitro. *Eur J Cell Biol* 2011;**90**:1016–28.

Kanczewska J, Marco S, Vandermeeren C *et al.* Activation of the plant plasma membrane H+-ATPase by phosphorylation and binding of 14-3-3 proteins converts a dimer into a hexamer. *Proc Natl Acad Sci* 2005;**102**:11675–80.

Kane PM. Proton Transport and pH Control in Fungi. *Yeast Membrane Transport. Advances in Experimental Medicine and Biology*. Vol 892. 2016, 33–68.

Karachaliou M, Amillis S, Evangelinos M *et al.* The arrestin-like protein ArtA is essential for ubiquitination and endocytosis of the UapA transporter in response to both broad-range and specific signals. *Mol Microbiol* 2013;**88**:301–17.

Karnovsky MJ, Kleinfeld AM, Hoover RL *et al.* The concept of lipid domains in membranes. *J Cell Biol* 1982;**94**:1–6.

Karotki L, Huiskonen JT, Stefan CJ *et al.* Eisosome proteins assemble into a membrane scaffold. *J Cell Biol* 2011;**195**:889–902.

Karuppasamy M, Kusmider B, Oliveira TM *et al.* Cryo-EM structure of Saccharomyces cerevisiae target of rapamycin complex 2. *Nat Commun* 2017;**8**, DOI: 10.1038/s41467-017-01862-0.

Keener JM, Babst M. Quality Control and Substrate-Dependent Downregulation of the Nutrient Transporter Fur4. *Traffic* 2013;**14**:412–27.

Keith CT, Schreiber SL. PIK-related kinases: DNA repair, recombination, and cell cycle checkpoints. *Science* 1995;**270**:50–1.

Khafif M, Cottret L, Balagué C *et al.* Identification and phylogenetic analyses of VASt, an uncharacterized protein domain associated with lipid-binding domains in Eukaryotes. *BMC Bioinformatics* 2014;**15**:222.

Khan A, McQuilken M, Gladfelter AS. Septins and Generation of Asymmetries in Fungal Cells. *Annu Rev Microbiol* 2015;**69**:487–503.

Kirchhausen T. Bending membranes. *Nat Cell Biol* 2012;**14**:906–8.

Knutson BA. Insights into the domain and repeat architecture of target of rapamycin. *J Struct Biol* 2010;**170**:354–63.

Kock C, Arlt H, Ungermann C *et al.* Yeast cell wall integrity sensors form specific plasma membrane microdomains important for signalling. *Cell Microbiol* 2016;**18**:1251–67.

Kovtun O, Tillu VA, Ariotti N *et al.* Cavin family proteins and the assembly of caveolae. *J Cell Sci* 2015;**128**:1269–78.

Kühlbrandt W, Zeelen J, Dietrich J. Structure, mechanism, and regulation of the Neurospora plasma membrane H+-ATpase. *Science (80-)* 2002;**297**:1692–6.

Kusumi A, Suzuki KGN, Kasai RS *et al.* Hierarchical mesoscale domain organization of the plasma membrane. *Trends Biochem Sci* 2011;**36**:604–15.

Lamaze C, Tardif N, Dewulf M *et al.* The caveolae dress code: structure and signaling. *Curr Opin Cell Biol* 2017;**47**:117–25.

Lauwers E, André B. Association of yeast transporters with detergent-resistant membranes correlates with their cell-surface location. *Traffic* 2006;**7**:1045–59.

Lauwers E, Erpapazoglou Z, Haguenauer-Tsapis R *et al.* The ubiquitin code of yeast permease trafficking. *Trends Cell Biol* 2010;**20**:196–204.

Lauwers E, Grossmann G, Andre B *et al.* Evidence for coupled biogenesis of yeast Gap1 permease and sphingolipids: essential role in transport activity and normal control by ubiquitination. Sommer T (ed.). *Mol Biol Cell* 2007;**18**:3068–80.

Lauwers E, Jacob C, Andre B. K63-linked ubiquitin chains as a specific signal for protein sorting into the multivesicular body pathway. *J Cell Biol* 2009;**185**:493–502.

Lee JH, Heuser JE, Roth R *et al.* Eisosome ultrastructure and evolution in fungi, microalgae, and lichens. *Eukaryot Cell* 2015;**14**:1017–42.

Lee MCS, Hamamoto S, Schekman R. Ceramide biosynthesis is required for the formation of the oligomeric H+-ATPase Pma1p in the yeast endoplasmic reticulum. *J Biol Chem* 2002;**277**:22395–401.

Lenz JH, Schuchardt I, Straube A *et al.* A dynein loading zone for retrograde endosome motility at microtubule plus-ends. *EMBO J* 2006;**25**:2275–86.

Leskoske KL, Roelants FM, Emmerstorfer-Augustin A *et al.* Phosphorylation by the stress-activated MAPK Slt2 down-regulates the yeast TOR complex 2. *Genes Dev* 2018;**32**:1576–90.

Leskoske KL, Roelants FM, Marshall MNM *et al.* The stress-sensing TORC2 complex activates yeast AGC-family protein kinase ypk1 at multiple novel sites. *Genetics* 2017;**207**:179–95.

Lev S. Non-vesicular lipid transport by lipid-transfer proteins and beyond. *Nat Rev Mol Cell Biol* 2010;**11**:739–50.

Levental I, Veatch SL. The Continuing Mystery of Lipid Rafts. *J Mol Biol* 2016;**428**:4749–64.

Levin DE. Cell Wall Integrity Signaling in Saccharomyces cerevisiae. *Microbiol Mol Biol Rev* 2005;**69**:262–91.

Levin DE. Regulation of cell wall biogenesis in Saccharomyces cerevisiae: The cell wall integrity signaling pathway. *Genetics* 2011;**189**:1145–75.

Li S, Du L, Yuen G *et al.* Distinct Ceramide Synthases Regulate Polarized Growth in the Filamentous Fungus Aspergillus nidulans. *Mol Biol Cell* 2006;**17**:1218–27.

Lichtenberg D, Goñi FM, Heerklotz H. Detergent-resistant membranes should not be identified with membrane rafts. *Trends Biochem Sci* 2005;**30**:430–6.

Lin CH, MacGurn JA, Chu T *et al.* Arrestin-Related Ubiquitin-Ligase Adaptors Regulate Endocytosis and Protein Turnover at the Cell Surface. *Cell* 2008;**135**:714–25.

Lingwood D, Simons K. Lipid Rafts As a Membrane-Organizing Principle. *Science (80-)* 2009;**327**:46 LP – 50.

Liu K, Zhang X, Lester RL *et al.* The sphingoid long chain base phytosphingosine activates AGC-type protein kinases in Saccharomyces cerevisiae including Ypk1, Ypk2, and Sch9. *J Biol Chem* 2005;**280**:22679–87.

Loewith R, Jacinto E, Wullschleger S *et al.* Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. *Mol Cell* 2002;**10**:457–68.

Loibl M, Grossmann G, Stradalova V *et al.* C terminus of Nce 102 determines the structure and function of microdomains in the Saccharomyces cerevisiae plasma membrane. *Eukaryot Cell* 2010;**9**:1184–92.

Löpez-Franco R, Bracker CE. Diversity and dynamics of the Spitzenkörper in growing hyphal tips of higher fungi. *Protoplasma* 2005;**195**:90–111.

Lopez D, Koch G. Exploring functional membrane microdomains in bacteria: an overview. *Curr Opin Microbiol* 2017;**36**:76–84.

Lorent JH, Diaz-Rohrer B, Lin X *et al.* Structural determinants and functional consequences of protein affinity for membrane rafts. *Nat Commun* 2017;**8**, DOI: 10.1038/s41467-017-01328-3.

Loss O, Bertuzzi M, Yan Y *et al.* Mutual independence of alkaline- and calcium-mediated signalling in Aspergillus fumigatus refutes the existence of a conserved druggable signalling nexus. *Mol Microbiol* 2017;**106**:861–75.

Lu R, Drubin DG. Selection and stabilization of endocytic sites by Ede1, a yeast functional homologue of human Eps15. *Mol Biol Cell* 2017;**28**:567–75.

Lu R, Drubin DG, Sun Y. Clathrin-mediated endocytosis in budding yeast at a glance. *J Cell Sci* 2016;**129**:1531–6.

Lucena-Agell D, Hervás-Aguilar A, Múnera-Huertas T *et al.* Mutational analysis of the Aspergillus ambient pH receptor PalH underscores its potential as a target for antifungal compounds. *Mol Microbiol* 2016;**101**:982–1002.

Ludwig A, Otto GP, Riento K *et al.* Flotillin microdomains interact with the cortical cytoskeleton to control uropod formation and neutrophil recruitment. *J Cell Biol* 2010;**191**:771–81.

Luo G, Gruhler A, Liu Y *et al.* The sphingolipid long-chain base-Pkh1/2-Ypk1/2 signaling pathway regulates eisosome assembly and turnover. *J Biol Chem* 2008;**283**:10433–44.

Lv QZ, Yan L, Jiang YY. The synthesis, regulation, and functions of sterols in Candida albicans: Well-known but still lots to learn. *Virulence* 2016;**7**:649–59.

MacGurn JAA, Hsu P-CC, Smolka MBB *et al.* TORC1 regulates endocytosis via npr1-mediated phosphoinhibition of a ubiquitin ligase adaptor. *Cell* 2011;**147**:1104–17.

Maeda T. The signaling mechanism of ambient pH sensing and adaptation in yeast and fungi. *FEBS J* 2012;**279**:1407–13.

Mahanty SK, Gupta P, Banerjee U *et al.* Defective plasma membrane H(+)-ATPase or orthovanadate resistant mutants from Candida albicans, a pathogenic yeast. *Biochem Int* 1990;**22**:11–20.

Makushok T, Alves P, Huisman SM *et al.* Sterol-Rich Membrane Domains Define Fission Yeast Cell Polarity. *Cell* 2016;**165**:1182–96.

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

Malínská K, Malínský J, Opekarová M *et al.* Visualization of Protein Compartmentation within the Plasma Membrane of Living Yeast Cells. *Mol Biol Cell* 2003;**14**:4427–36.

Malinsky J, Opekarová M. New Insight Into the Roles of Membrane Microdomains in Physiological Activities of Fungal Cells. *International Review of Cell and Molecular Biology*. Vol 325. Academic Press, 2016, 119–80.

Malinsky J, Opekarová M, Grossmann G *et al.* Membrane Microdomains, Rafts, and Detergent-Resistant Membranes in Plants and Fungi. *Annu Rev Plant Biol* 2013;**64**:501–29.

Manford AG, Stefan CJ, Yuan HL *et al.* ER-to-Plasma Membrane Tethering Proteins Regulate Cell Signaling and ER Morphology. *Dev Cell* 2012;**23**:1129–40.

Marchal C, Haguenauer-Tsapis R, Urban-Grimal D. Casein Kinase I-dependent Phosphorylation within a PEST Sequence and Ubiquitination at Nearby Lysines Signal Endocytosis of Yeast Uracil Permease. *J Biol Chem* 2000;**275**:23608–14.

Martin R, Hellwig D, Schaub Y *et al.* Functional analysis of Candida albicans genes whose Saccharomyces cerevisiae homologues are involved in endocytosis. *Yeast* 2007;**24**:511–22.

Martin SW, Konopka JB. Lipid Raft Polarization Contributes to Hyphal Growth in Candida albicans. *Eukaryot Cell* 2004;**3**:675–84.

Martinez MJ, Roy S, Archuletta AB *et al.* Genomic Analysis of Stationary-Phase and Exit in Saccharomyces cerevisiae: Gene Expression and Identification of Novel Essential Genes. *Mol Biol Cell* 2004;**15**:5295–305.

Martzoukou O, Amillis S, Zervakou A *et al.* The ap-2 complex has a specialized clathrin-independent role in apical endocytosis and polar growth in fungi. *Elife* 2017;**6**, DOI: 10.7554/eLife.20083.001.

Maryanovich M, Gross A. A ROS rheostat for cell fate regulation. *Trends Cell Biol* 2013;**23**:129–34.

Mascaraque V, Hernáez ML, Jiménez-Sánchez M *et al.* Phosphoproteomic Analysis of Protein Kinase C Signaling in Saccharomyces cerevisiae Reveals Slt2 Mitogen-activated Protein Kinase (MAPK)-dependent Phosphorylation of Eisosome Core Components. *Mol Cell Proteomics* 2012;**12**:557–74.

Matsuo T, Otsubo Y, Urano J *et al.* Loss of the TOR Kinase Tor2 Mimics Nitrogen Starvation and Activates the Sexual Development Pathway in Fission Yeast. *Mol Cell Biol* 2007, DOI: 10.3724/SP.J.1003.2008.08027.

Mattiazzi M, Jambhekar A, Kaferle P *et al.* Genetic interactions between a phospholipase A2 and the Rim101 pathway components in S. cerevisiae reveal a role for this pathway in response to changes in membrane composition and shape. *Mol Genet Genomics* 2010;**283**:519–30.

Mazur P, Baginsky W. In vitro activity of 1,3-β-D-glucan synthase requires the GTP-binding protein Rho1. *J Biol Chem* 1996;**271**:14604–9.

Merhi A, André B. Internal Amino Acids Promote Gap1 Permease Ubiquitylation via TORC1/Npr1/14-3-3-Dependent Control of the Bul Arrestin-Like Adaptors. *Mol Cell Biol* 2012;**32**:4510–22.

Merrifield CJ, Kaksonen M. Endocytic Accessory Factors and Regulation of Clathrin-Mediated Endocytosis. *Cold Spring Harb Perspect Biol* 2014;**6**:1–16.

Moharir A, Gay L, Appadurai D *et al.* Eisosomes are metabolically regulated storage compartments for APC-type nutrient transporters. Fox TD (ed.). *Mol Biol Cell* 2018;**29**:2113–27.

Monk BC, Kurtz MB, Marrinan JA *et al.* Cloning and characterization of the plasma membrane H+-ATPase from Candida albicans. *J Bacteriol* 1991;**173**:6826–36.

Moor H, Mühletttaler K. Fine structure in frozen-etched yeast cells. *J Cell Biol* 1963;**17**:609–28.

Moreira KE, Schuck S, Schrul B *et al.* Seg 1 controls eisosome assembly and shape. *J Cell Biol* 2012;**198**:405–20.

Moreira KE, Walther TC, Aguilar PS *et al.* Pil1 Controls Eisosome Biogenesis. *Mol Biol Cell* 2009;**20**:809–18.

Moseley JB. Eisosomes. *Curr Biol* 2018;**28**:R376–8.

Mueller NS, Wedlich-Söldner R, Spira F. From mosaic to patchwork: Matching lipids and proteins in membrane organization. *Mol Membr Biol* 2012;**29**:186–96.

Muir A, Ramachandran S, Roelants FM *et al.* TORC2-dependent protein kinase Ypk1 phosphorylates ceramide synthase to stimulate synthesis of complex sphingolipids. *Elife* 2014;**3**, DOI: 10.7554/elife.03779.

Munn AL, Riezman H. Endocytosis is required for the growth of vacuolar H(+)-ATPase-defective yeast: identification of six new END genes. *J Cell Biol* 1994;**127**:373–86.

Munshi MA, Gardin JM, Singh A *et al.* The Role of Ceramide Synthases in the Pathogenicity of Cryptococcus neoformans. *Cell Rep* 2018;**22**:1392–400.

Murley A, Yamada J, Niles BJ *et al.* Sterol transporters at membrane contact sites regulate TORC1 and TORC2 signaling. *J Cell Biol* 2017;**216**:2679 LP-- 2689.

Newpher TM, Smith RP, Lemmon V *et al.* In vivo dynamics of clathrin and its adaptor-dependent recruitment to the actin-based endocytic machinery in yeast. *Dev Cell* 2005;**9**:87–98.

Nikko E, Pelham HR. Arrestin-mediated endocytosis of yeast plasma membrane transporters. *Traffic* 2009;**10**:1856–67.

Nikolaou E, Agrafioti I, Stumpf M *et al.* Phylogenetic diversity of stress signalling pathways in fungi. *BMC Evol Biol* 2009;**9**:44.

Niles BJ, Joslin AC, Fresques T *et al.* TOR Complex 2-Ypk1 signaling maintains sphingolipid homeostasis by sensing and regulating ROS accumulation. *Cell Rep* 2014;**6**:541–52.

Niles BJ, Mogri H, Hill A *et al.* Plasma membrane recruitment and activation of the AGC kinase Ypk1 is mediated by target of rapamycin complex 2 (TORC2) and its effector proteins Slm1 and Slm2. *Proc Natl Acad Sci U S A* 2012;**109**:1536–41.

Niles BJ, Powers T. Plasma membrane proteins Slm1 and Slm2 mediate activation of the AGC kinase Ypk1 by TORC2 and sphingolipids in S. cerevisiae. *Cell Cycle* 2012;**11**:3745–9.

Niles BJ, Powers T. TOR complex 2-Ypk1 signaling regulates actin polarization via reactive oxygen species. *Mol Biol Cell* 2014;**25**:3962–72.

Nishino K, Obara K, Kihara A. The C-terminal cytosolic region of rim21 senses alterations in plasma membrane lipid composition: Insights into sensing mechanisms for plasma membrane lipid asymmetry. *J Biol Chem* 2015;**290**:30797–805.

O'Donnell AF, Huang L, Thorner J *et al.* A calcineurin-dependent switch controls the trafficking function of α-arrestin Aly1/Art6. *J Biol Chem* 2013;**288**:24063–80.

Obara K, Kihara A. Signaling Events of the Rim101 Pathway Occur at the Plasma Membrane in a Ubiquitination-Dependent Manner. *Mol Cell Biol* 2014;**34**:3525–34.

Obara K, Kihara A. The Rim101 pathway contributes to ER stress adaptation through sensing the state of plasma membrane. *Biochem J* 2017;**474**:51–63.

Obara K, Yamamoto H, Kihara A. Membrane protein Rim21 plays a central role in sensing ambient pH in Saccharomyces cerevisiae. *J Biol Chem* 2012;**287**:38473–81.

Odorizzi G, Babst M, Emr SD. Phosphoinositide signaling and the regulation of membrane trafficking in yeast. *Trends Biochem Sci* 2000;**25**:229–35.

Okeke E, Dingsdale H, Parker T *et al.* Endoplasmic reticulum-plasma membrane junctions: structure, function and dynamics. *J Physiol* 2016;**594**:2837–47.

Olivera-Couto A, Grana M, Harispe L *et al.* The eisosome core is composed of BAR domain proteins. *Mol Biol Cell* 2011;**22**:2360–72.

Olivera-Couto A, Salzman V, Mailhos M *et al.* Eisosomes are dynamic plasma membrane domains showing Pil1-Lsp1 heteroligomer binding equilibrium. *Biophys J* 2015;**108**:1633–44.

Olson DK, Fröhlich F, Christiano R *et al.* Rom2-dependent phosphorylation of Elo2 controls the abundance of very long-chain fatty acids. *J Biol Chem* 2015;**290**:4238–47.

Olson DK, Fröhlich F, Farese R V. *et al.* Taming the sphinx: Mechanisms of cellular sphingolipid homeostasis. *Biochim Biophys Acta - Mol Cell Biol Lipids* 2016;**1861**:784–92.

Osman C, Wilmes C, Tatsuta T *et al.* Prohibitins Interact Genetically with Atp23, a Novel Processing Peptidase and Chaperone for the F1FO-ATP Synthase. *Mol Biol Cell* 2006, DOI: 10.1091/mbc.e06-09-0839.

Ost KS, Esher SK, Wager CML *et al.* Rim pathway-mediated alterations in the fungal cell wall influence immune recognition and inflammation. *MBio* 2017;**8**, DOI: 10.1128/mBio.02290-16.

Ottmann C, Marco S, Jaspert N *et al.* Structure of a 14-3-3 Coordinated Hexamer of the Plant Plasma Membrane H+-ATPase by Combining X-Ray Crystallography and Electron Cryomicroscopy. *Mol Cell* 2007;**25**:427–40.

Pantazopoulou A, Pinar M, Xiang X *et al.* Maturation of late Golgi cisternae into RabERAB11 exocytic post-Golgi carriers visualized in vivo. *Mol Biol Cell* 2014;**25**:2428–43.

Parton RG, Del Pozo MA. Caveolae as plasma membrane sensors, protectors and organizers. *Nat Rev Mol Cell Biol* 2013;**14**:98–112.

Parton RG, Simons K. The multiple faces of caveolae. *Nat Rev Mol Cell Biol* 2007;**8**:185–94.

Pearson CL, Xu K, Sharpless KE *et al.* MesA, a Novel Fungal Protein Required for the Stabilization of Polarity Axes in *Aspergillus nidulans*. *Mol Biol Cell* 2004;**15**:3658–72.

Peñalva MÁ. Endocytosis in filamentous fungi: Cinderella gets her reward. *Curr Opin Microbiol* 2010;**13**:684–92.

Peñalva MA, Lucena-Agell D, Arst HN. Liaison alcaline: Pals entice non-endosomal ESCRTs to the plasma membrane for pH signaling. *Curr Opin Microbiol* 2014;**22**:49–59.

Peñalva MA, Tilburn J, Bignell E *et al.* Ambient pH gene regulation in fungi: making connections. *Trends Microbiol* 2008;**16**:291–300.

Philip B, Levin DE. Wsc1 and Mid2 Are Cell Surface Sensors for Cell Wall Integrity Signaling That Act through Rom2, a Guanine Nucleotide Exchange Factor for Rho1. *Mol Cell Biol* 2002;**21**:271–80.

Piao HL, Machado IMP, Payne GS. NPFXD-mediated Endocytosis Is Required for Polarity and Function of a Yeast Cell Wall Stress Sensor. *Mol Biol Cell* 2006;**18**:57–65.

Pinar M, Peñalva MA. Aspergillus nidulans BapH is a RAB11 effector that connects membranes in the Spitzenkörper with basal autophagy. *Mol Microbiol* 2017;**106**:452–68.

Porter KR, Palade GE. Studies on the endoplasmic reticulum. *J Biophys Biochem Cytol* 1957;**3**:269–300.

Prinz WA. Lipid trafficking sans vesicles: Where, why, how? *Cell* 2010;**143**:870–4.

Prinz WA. Bridging the gap: Membrane contact sites in signaling, metabolism, and organelle dynamics. *J Cell Biol* 2014;**205**:759–69.

Rao R, Nakamoto RK, Verjovksi-Almeida S *et al.* Structure and Function of the Yeast Plasma-Membrane H+-ATPase. *Ann N Y Acad Sci* 1992;**671**:195–203.

Ratnayake L, Adhvaryu KK, Kafes E *et al.* A component of the TOR (Target Of Rapamycin) nutrient-sensing pathway plays a role in circadian rhythmicity in Neurospora crassa. *PLoS Genet* 2018;**14**, DOI: 10.1371/journal.pgen.1007457.

Reck-Peterson SL, Redwine WB, Vale RD *et al.* The cytoplasmic dynein transport machinery and its many cargoes. *Nat Rev Mol Cell Biol* 2018;**19**:382–98.

Reijnst P, Walther A, Wendland J. Dual-colour fluorescence microscopy using yEmCherry-/GFP-tagging of eisosome components Pil1 and Lsp1 in Candida albicans. *Yeast* 2011;**28**:331–8.

Reoyo E, Espeso EA, Peñalva MA *et al.* The essential Aspergillus nidulans gene pmaA encodes an homologue of fungal plasma membrane H+-ATPases. *Fungal Genet Biol* 1998;**23**:288–99.

Rhee KH, Scarborough GA, Henderson R. Domain movements of plasma membrane H+-ATPase: 3D structures of two states by electron cryo-microscopy. *EMBO J* 2002;**21**:3582–9.

Richard VR, Beach A, Piano A *et al.* Mechanism of liponecrosis, a distinct mode of programmed cell death. *Cell Cycle* 2014;**13**:3707–26.

Riggi M, Niewola-Staszkowska K, Chiaruttini N *et al.* Decrease in plasma membrane tension triggers PtdIns(4,5)P2 phase separation to inactivate TORC2. *Nat Cell Biol* 2018;**20**:1043–51.

Riquelme M, Aguirre J, Bartnicki-García S *et al.* Fungal Morphogenesis, from the Polarized Growth of Hyphae to Complex Reproduction and Infection Structures. *Microbiol Mol Biol Rev* 2018;**82**:1–47.

Rispal D, Eltschinger S, Stahl M *et al.* Target of rapamycin complex 2 regulates actin polarization and endocytosis via multiple pathways. *J Biol Chem* 2015;**290**:14963–78.

Rockenfeller P, Gourlay CW. Lipotoxicty in yeast: A focus on plasma membrane signalling and membrane contact sites. *FEMS Yeast Res* 2018;**18**, DOI: 10.1093/femsyr/foy034.

Rockenfeller P, Smolnig M, Diessl J *et al.* Diacylglycerol triggers Rim101 pathway-dependent necrosis in yeast: A model for lipotoxicity. *Cell Death Differ* 2018;**25**:765–81.

Rodicio R, Buchwald U, Schmitz HP *et al.* Dissecting sensor functions in cell wall integrity signaling in Kluyveromyces lactis. *Fungal Genet Biol* 2008;**45**:422–35.

Rodicio R, Heinisch JJ. Together we are strong - Cell wall integrity sensors in yeasts. *Yeast* 2010;**27**:531–40.

Roelants FM, Baltz AG, Trott AE *et al.* A protein kinase network regulates the function of aminophospholipid flippases. *Proc Natl Acad Sci* 2009;**107**:34–9.

Roelants FM, Breslow DK, Muir A *et al.* Protein kinase Ypk1 phosphorylates regulatory proteins Orm1 and Orm2 to control sphingolipid homeostasis in Saccharomyces cerevisiae. *Proc Natl Acad Sci* 2011;**108**:19222–7.

Roelants FM, Chauhan N, Muir A *et al.* TOR complex 2–regulated protein kinase Ypk1 controls sterol distribution by inhibiting StARkin domain–containing proteins located at plasma membrane–endoplasmic reticulum contact sites. *Mol Biol Cell* 2018;**29**:2128–36.

Roelants FM, Leskoske KL, Pedersen RTA *et al.* TOR Complex 2-Regulated Protein Kinase Fpk1 Stimulates Endocytosis via Inhibition of Ark1/Prk1-Related Protein Kinase Akl1 in Saccharomyces cerevisiae. *Mol Cell Biol* 2017;**37**:e00627-16.

Roland BP, Naito T, Best JT *et al.* Yeast and human P4-ATPases transport glycosphingolipids using conserved structural motifs. *J Biol Chem* 2019;**294**:1794–806.

Rothbauer U, Zolghadr K, Muyldermans S *et al.* A Versatile Nanotrap for Biochemical and Functional Studies with Fluorescent Fusion Proteins. *Mol Cell Proteomics* 2008;**7**:282–9.

Ruiz-Granados YG, De La Cruz-Torres V, Sampedro JG. The oligomeric state of the plasma membrane H + -ATPase from Kluyveromyces lactis. *Molecules* 2019;**24**:958.

Sako Y, Kusumi A. Barriers for lateral diffusion of transferrin receptor in the plasma membrane as characterized by receptor dragging by laser tweezers: Fence versus tether. *J Cell Biol* 1995;**129**:1559–74.

Saliba E, Evangelinos M, Gournas C *et al.* The yeast H+-ATPase Pma1 promotes Rag/Gtr-dependent TORC1 activation in response to H+-coupled nutrient uptake. *Elife* 2018;**7**:e31981.

Salogiannis J, Egan MJ, Reck-Peterson SL. Peroxisomes move by hitchhiking on early endosomes using the novel linker protein PxdA. *J Cell Biol* 2016;**212**:289–96.

Salogiannis J, Reck-Peterson SL. Hitchhiking: A Non-Canonical Mode of Microtubule-Based Transport. *Trends Cell Biol* 2017;**27**:141–50.

Sanz AB, García R, Rodríguez-Peña JM *et al.* The CWI Pathway: Regulation of the Transcriptional Adaptive Response to Cell Wall Stress in Yeast. *J Fungi* 2017;**4**:1.

Saxton RA, Sabatini DM. mTOR Signaling in Growth, Metabolism, and Disease. *Cell* 2017;**168**:960–76.

Scazzocchio C, Vangelatos I, Sophianopoulou V. Eisosomes and membrane compartments in the ascomycetes: A view from Aspergillus nidulans. *Commun Integr Biol* 2011;**4**:64–8.

Schmidt A, Beck T, Koller A *et al.* The TOR nutrient signalling pathway phosphorylates NPR1 and inhibits turnover of the tryptophan permease. *EMBO J* 1998;**17**:6924–31.

Schmidt O, Weyer Y, Baumann V *et al.* Endosome and Golgi-associated degradation (EGAD) of membrane proteins regulates sphingolipid metabolism. *EMBO J* 2019:e101433.

Schmitz HP, Lorberg A, Heinisch JJ. Regulation of yeast protein kinase C activity by interaction with the small GTPase Rho1p through its amino-terminal HR1 domain. *Mol Microbiol* 2002;**44**:829–40.

Schuberth C, Wedlich-Söldner R. Building a patchwork - The yeast plasma membrane as model to study lateral domain formation. *Biochim Biophys Acta - Mol Cell Res* 2015;**1853**:767–74.

Schultzhaus Z, Yan H, Shaw BD. Aspergillus nidulans flippase DnfA is cargo of the endocytic collar and plays complementary roles in growth and phosphatidylserine asymmetry with another flippase, DnfB. *Mol Microbiol* 2015;**97**:18–32.

Schuster M, Lipowsky R, Assmann M-A *et al.* Transient binding of dynein controls bidirectional long-range motility of early endosomes. *Proc Natl Acad Sci* 2011;**108**:3618–23.

Schwarz DS, Blower MD. The endoplasmic reticulum: Structure, function and response to cellular signaling. *Cell Mol Life Sci* 2016;**73**:79–94.

Sebastian TT, Baldridge RD, Xu P *et al.* Phospholipid flippases: Building asymmetric membranes and transport vesicles. *Biochim Biophys Acta - Mol Cell Biol Lipids* 2012;**1821**:1068–77.

Seger S, Rischatsch R, Philippsen P. Formation and stability of eisosomes in the filamentous fungus Ashbya gossypii. *J Cell Sci* 2011;**124**:1629–34.

Séron K, Blondel MO, Haguenauer-Tsapis R *et al.* Uracil-induced down-regulation of the yeast uracil permease. *J Bacteriol* 1999;**181**:1793–800.

Serrano R, Kielland-Brandt MC, Fink GR. Yeast plasma membrane ATPase is essential for growth and has homology with (Na++ K+), K+- and Ca2+-ATPases. *Nature* 1986;**319**:689–93.

Sezgin E, Levental I, Mayor S *et al.* The mystery of membrane organization: Composition, regulation and roles of lipid rafts. *Nat Rev Mol Cell Biol* 2017;**18**:361–74.

Shen H, Pirruccello M, De Camilli P. SnapShot: Membrane Curvature Sensors and Generators. *Cell* 2012;**150**:1300-1300.e2.

Sherrington SL, Sorsby E, Mahtey N *et al.* Adaptation of Candida albicans to environmental pH induces cell wall remodelling and enhances innate immune recognition. *PLoS Pathog* 2017;**13**:e1006403.

Shimobayashi M, Oppliger W, Moes S *et al.* TORC1-regulated protein kinase Npr1 phosphorylates Orm to stimulate complex sphingolipid synthesis. *Mol Biol Cell* 2013;**24**:870–81.

Shvets E, Bitsikas V, Howard G *et al.* Dynamic caveolae exclude bulk membrane proteins and are required for sorting of excess glycosphingolipids. *Nat Commun* 2015;**6**:6867.

Si H, Justa-Schuch D, Seiler S *et al.* Regulation of septum formation by the Bud3-Rho4 GTPase module in Aspergillus nidulans. *Genetics* 2010;**185**:165–76.

Simons K, Ikonen E. Functional rafts in cell membranes. *Nature* 1997;**387**:569–72.

Simons K, Sampaio JL. Membrane organization and lipid rafts. *Cold Spring Harb Perspect Biol* 2011;**3**:1–17.

Slayman CL. Electrical Properties of Neurospora crassa: Respiration and the intracellular potential. *J Gen Physiol* 2004;**49**:93–116.

Slayman CL, Slayman CW. Measurement of membrane potentials in Neurospora. *Science (80-)* 1962;**136**:876–7.

Smardon AM, Kane PM. Loss of vacuolar H + -ATPase activity in organelles signals ubiquitination and endocytosis of the yeast plasma membrane proton pump Pma1p. *J Biol Chem* 2014;**289**:32316–26.

Smythe E, Ayscough KR. The Ark1/Prk1 family of protein kinases. *EMBO Rep* 2003;**4**:246–51.

Sokolov S, Knorre D, Smirnova E *et al.* Ysp2 mediates death of yeast induced by amiodarone or intracellular acidification. *Biochim Biophys Acta - Bioenerg* 2006;**1757**:1366–70.

Solis GP, Hoegg M, Munderloh C *et al.* Reggie/flotillin proteins are organized into stable tetramers in membrane microdomains. *Biochem J* 2007;**403**:313–22.

Solis GP, Hulsbusch N, Radon Y *et al.* Reggies/flotillins interact with Rab11a and SNX4 at the tubulovesicular recycling compartment and function in transferrin receptor and E-cadherin trafficking. *Mol Biol Cell* 2013;**24**:2689–702.

Sonnino S, Prinetti A. Membrane Domains and the “Lipid Raft” Concept. *Curr Med Chem* 2012;**20**:4–21.

Soulard A, Cohen A, Hall MN. TOR signaling in invertebrates. *Curr Opin Cell Biol* 2009;**21**:825–36.

De Souza CP, Hashmi SB, Osmani AH *et al.* Functional Analysis of the Aspergillus nidulans Kinome. *PLoS One* 2013;**8**:e58008.

Spira F, Mueller NS, Beck G *et al.* Patchwork organization of the yeast plasma membrane into numerous coexisting domains. *Nat Cell Biol* 2012;**14**:640–8.

Stancevic B, Kolesnick R. Ceramide-rich platforms in transmembrane signaling. *FEBS Lett* 2010;**584**:1728–40.

Steinberg G. *Endocytosis and Early Endosome Motility in Filamentous Fungi*. Elsevier Current Trends, 2014:10–8.

Steinberg G, Peñalva MA, Riquelme M *et al.* Cell Biology of Hyphal Growth. *Microbiol Spectr* 2017;**5**:1–34.

Stewart E, Gow NAR, Bowen D V. Cytoplasmic Alkalinization during Germ Tube Formation in Candida albicans. *Microbiology* 2009;**134**:1079–87.

Stradalova V, Blazikova M, Grossmann G *et al.* Distribution of cortical endoplasmic reticulum determines positioning of endocytic events in yeast plasma membrane. *PLoS One* 2012;**7**, DOI: 10.1371/journal.pone.0035132.

Stradalova V, Stahlschmidt W, Grossmann G *et al.* Furrow-like invaginations of the yeast plasma membrane correspond to membrane compartment of Can1. *J Cell Sci* 2009;**122**:2887–94.

Straede A, Heinisch JJ. Functional analyses of the extra- and intracellular domains of the yeast cell wall integrity sensors Mid2 and Wsc1. *FEBS Lett* 2007;**581**:4495–500.

Sturgill TW, Cohen A, Diefenbacher M *et al.* TOR1 and TOR2 Have Distinct Locations in Live Cells. *Eukaryot Cell* 2008;**7**:1819–30.

Stuttfeld E, Aylett CHS, Imseng S *et al.* Architecture of the human mTORC2 core complex. *Elife* 2018;**7**, DOI: 10.7554/elife.33101.

Sun Y, Drubin DG. Pik1-ing clathrin adaptors. *Nat Cell Biol* 2012;**14**:231–2.

Sun Y, Miao Y, Yamane Y *et al.* Orm protein phosphoregulation mediates transient sphingolipid biosynthesis response to heat stress via the Pkh-Ypk and Cdc55-PP2A pathways. *Mol Biol Cell* 2012;**23**:2388–98.

Swinnen E, Wilms T, Idkowiak-Baldys J *et al.* The protein kinase Sch9 is a key regulator of sphingolipid metabolism in Saccharomyces cerevisiae. *Mol Biol Cell* 2014;**25**:196–211.

Taheri-Talesh N, Horio T, Araujo-Bazan L *et al.* The Tip Growth Apparatus of Aspergillus nidulans. *Mol Biol Cell* 2008;**19**:1439–49.

Takeo K, Shigeta M, Takagi Y. Plasma membrane ultrastructural differences between the exponential and stationary phases of Saccharomyces cerevisiae as revealed by freeze-etching. *J Gen Microbiol* 1976;**97**:323–9.

Takeshita N, Diallinas G, Fischer R. The role of flotillin FloA and stomatin StoA in the maintenance of apical sterol-rich membrane domains and polarity in the filamentous fungus Aspergillus nidulans. *Mol Microbiol* 2012;**83**:1136–52.

Takeshita N, Higashitsuji Y, Konzack S *et al.* Apical Sterol-rich Membranes Are Essential for Localizing Cell End Markers That Determine Growth Directionality in the Filamentous Fungus Aspergillus nidulans. Drubin D (ed.). *Mol Biol Cell* 2008;**19**:339–51.

Tanaka S, Tani M. Mannosylinositol phosphorylceramides and ergosterol coodinately maintain cell wall integrity in the yeast Saccharomyces cerevisiae. *FEBS J* 2018;**285**:2405–27.

Tapken W, Murphy AS. Membrane nanodomains in plants: Capturing form, function, and movement. *J Exp Bot* 2015;**66**:1573–86.

Tatebe H, Morigasaki S, Murayama S *et al.* Rab-Family GTPase regulates TOR complex 2 signaling in fission yeast. *Curr Biol* 2010;**20**:1975–82.

Tatebe H, Shiozaki K. Evolutionary conservation of the components in the tor signaling pathways. *Biomolecules* 2017;**7**, DOI: 10.3390/biom7040077.

Thayer NH, Leverich CK, Fitzgibbon MP *et al.* Identification of long-lived proteins retained in cells undergoing repeated asymmetric divisions. *Proc Natl Acad Sci* 2014;**111**:14019–26.

Tong J, Manik MK, Im YJ. Structural basis of sterol recognition and nonvesicular transport by lipid transfer proteins anchored at membrane contact sites. *Proc Natl Acad Sci* 2018;**115**:E856–65.

Toshima JY, Toshima J, Kaksonen M *et al.* Spatial dynamics of receptor-mediated endocytic trafficking in budding yeast revealed by using fluorescent -factor derivatives. *Proc Natl Acad Sci* 2006;**103**:5793–8.

Upadhyay S, Shaw BD. The role of actin, fimbrin and endocytosis in growth of hyphae in Aspergillus nidulans. *Mol Microbiol* 2008;**68**:690–705.

Urban J, Soulard A, Huber A *et al.* Sch9 Is a Major Target of TORC1 in Saccharomyces cerevisiae. *Mol Cell* 2007;**26**:663–74.

Valdez-Taubas J, Pelham HRB. Slow diffusion of proteins in the yeast plasma membrane allows polarity to be maintained by endocytic cycling. *Curr Biol* 2003;**13**:1636–40.

Vangelatos I, Roumelioti K, Gournas C *et al.* Eisosome organization in the filamentous ascomycete Aspergillus nidulans. *Eukaryot Cell* 2010;**9**:1441–54.

Vaškovičová K, Awadová T, Veselá P *et al.* MRNA decay is regulated via sequestration of the conserved 5'-3' exoribonuclease Xrn1 at eisosome in yeast. *European Journal of Cell Biology*. http://www.sciencedirect.com/science/article/pii/S0171933517300250?via%3Dihub. Published September 1, 2017. Accessed October 5, 2017.

Vaskovicova K, Stradalova V, Efenberk A *et al.* Assembly of fission yeast eisosomes in the plasma membrane of budding yeast: Import of foreign membrane microdomains. *Eur J Cell Biol* 2015;**94**:1–11.

Vecer J, Vesela P, Malinsky J *et al.* Sphingolipid levels crucially modulate lateral microdomain organization of plasma membrane in living yeast. *FEBS Lett* 2014;**588**:443–9.

Verna J, Lodder A, Lee K *et al.* A family of genes required for maintenance of cell wall integrity and for the stress response in Saccharomyces cerevisiae. *Proc Natl Acad Sci U S A* 1997;**94**:13804–9.

Villers J, Savocco J, Szopinska A *et al.* Study of the Plasma Membrane Proteome Dynamics Reveals Novel Targets of the Nitrogen Regulation in Yeast. *Mol Cell Proteomics* 2017;**16**:1652–68.

Wachtler V. Sterol-rich plasma membrane domains in the fission yeast Schizosaccharomyces pombe. *J Cell Sci* 2003;**116**:867–74.

Wagner RM, Kricks L, Lopez D. Functional Membrane Microdomains Organize Signaling Networks in Bacteria. *J Membr Biol* 2017;**250**:367–78.

Walther P, Müller M, Schweingruber ME. The ultrastructure of the cell surface and plasma membrane of exponential and stationary phase cells of Schizosaccharomyces pombe, grown in different media. *Arch Microbiol* 1984;**137**:128–34.

Walther TC, Aguilar PS, Fröhlich F *et al.* Pkh-kinases control eisosome assembly and organization. *EMBO J* 2007;**26**:4946–55.

Walther TC, Brickner JH, Aguilar PS *et al.* Eisosomes mark static sites of endocytosis. *Nature* 2006;**439**:998–1003.

Wang HX, Douglas LM, Aimanianda V *et al.* The Candida albicans Sur7 protein is needed for proper synthesis of the fibrillar component of the cell wall that confers strength. *Eukaryot Cell* 2011;**10**:72–80.

Wang HX, Douglas LM, Vesela P *et al.* Eisosomes promote the ability of Sur7 to regulate plasma membrane organization in Candida albicans. *Mol Biol Cell* 2016;**27**:1663–75.

Wang Q, Chang A. Sphingoid base synthesis is required for oligomerization and cell surface stability of the yeast plasma membrane ATPase, Pma1. *Proc Natl Acad Sci* 2002;**99**:12853–8.

Watanabe Y, Takaesu G, Hagiwara M *et al.* Characterization of a serum response factor-like protein in Saccharomyces cerevisiae, Rlm1, which has transcriptional activity regulated by the Mpk1 (Slt2) mitogen-activated protein kinase pathway. *Mol Cell Biol* 2015;**17**:2615–23.

Wedaman KP, Reinke A, Anderson S *et al.* Tor Kinases Are in Distinct Membrane-associated Protein Complexes in Saccharomyces cerevisiae. *Mol Biol Cell* 2003;**14**:1204–20.

Wedlich-Soldner R, Bolker M, Kahmann R *et al.* A putative endosomal t-SNARE links exo- and endocytosis in the phytopathogenic fungus Ustilago maydis. *EMBO J* 2000;**19**:1974–86.

Weichert M, Lichius A, Priegnitz B-E *et al.* Accumulation of specific sterol precursors targets a MAP kinase cascade mediating cell–cell recognition and fusion. *Proc Natl Acad Sci* 2016;**113**:11877–82.

West M, Zurek N, Hoenger A *et al.* A 3D analysis of yeast ER structure reveals how ER domains are organized by membrane curvature. *J Cell Biol* 2011;**193**:333–46.

Wilk S, Wittland J, Thywissen A *et al.* A block of endocytosis of the yeast cell wall integrity sensors Wsc1 and Wsc2 results in reduced fitness in vivo. *Mol Genet Genomics* 2010;**284**:217–29.

Wolf W, Kilic A, Schrul B *et al.* Yeast Ist2 recruits the endoplasmic reticulum to the plasma membrane and creates a ribosome-free membrane microcompartment. *PLoS One* 2012;**7**, DOI: 10.1371/journal.pone.0039703.

Wong LH, Levine TP. Lipid transfer proteins do their thing anchored at membrane contact sites… but what is their thing? *Biochem Soc Trans* 2016;**44**:517–27.

Woraratanadharm T, Kmosek S, Banuett F. UmTea1, a Kelch and BAR domain-containing protein, acts at the cell cortex to regulate cell morphogenesis in the dimorphic fungus Ustilago maydis. *Fungal Genet Biol* 2018;**121**:10–28.

Wu H, Carvalho P, Voeltz GK. Here, there, and everywhere: The importance of ER membrane contact sites. *Science (80-)* 2018;**361**:eaan5835.

Xu T, Shively CA, Jin R *et al.* A Profile of Differentially Abundant Proteins at the Yeast Cell Periphery during Pseudohyphal Growth. *J Biol Chem* 2010;**285**:15476–88.

Xu X, Bittman R, Duportail G *et al.* Effect of the structure of natural sterols and sphingolipids on the formation of ordered sphingolipid/sterol domains (rafts). Comparison of cholesterol to plant, fungal, and disease-associated sterols and comparison of sphingomyelin, cerebrosides, and cer. *J Biol Chem* 2001;**276**:33540–6.

Yáñez-Mó M, Barreiro O, Gordon-Alonso M *et al.* Tetraspanin-enriched microdomains: a functional unit in cell plasma membranes. *Trends Cell Biol* 2009, DOI: 10.1080/13549839.2010.487525.

Young MEME, Karpova TSTS, Brugger B *et al.* The Sur7p Family Defines Novel Cortical Domains in Saccharomyces cerevisiae, Affects Sphingolipid Metabolism, and Is Involved in Sporulation. *Mol Cell Biol* 2002;**22**:927–34.

Zahumensky J, Malinsky J, Zahumensky J *et al.* Role of MCC/Eisosome in Fungal Lipid Homeostasis. *Biomol 2019, Vol 9, Page 305* 2019;**9**:305.

Zanolari B. Sphingoid base synthesis requirement for endocytosis in Saccharomyces cerevisiae. *EMBO J* 2002;**19**:2824–33.

Zaremberg V, Gajate C, Cacharro LM *et al.* Cytotoxicity of an anti-cancer lysophospholipid through selective modification of lipid raft composition. *J Biol Chem* 2005;**280**:38047–58.

Zhang J, Zhuang L, Lee Y *et al.* The microtubule plus-end localization of Aspergillus dynein is important for dynein-early-endosome interaction but not for dynein ATPase activation. *J Cell Sci* 2010;**123**:3596–604.

Zhang L Bin, Tang L, Ying SH *et al.* Two eisosome proteins play opposite roles in autophagic control and sustain cell integrity, function and pathogenicity in Beauveria bassiana. *Environ Microbiol* 2017;**19**:2037–52.

Zhang X, Lester RL, Dickson RC. Pil1p and Lsp1p negatively regulate the 3-phosphoinositide-dependent protein kinase-like kinase Pkh1p and downstream signaling pathways Pkc1p and Ypk1p. *J Biol Chem* 2004;**279**:22030–8.

Zhang Y, Muend S, Rao R. Dysregulation of ion homeostasis by antifungal agents. *Front Microbiol* 2012;**3**, DOI: 10.3389/fmicb.2012.00133.

Zhao Y, MacGurn JA, Liu M *et al.* The ART-Rsp5 ubiquitin ligase network comprises a plasma membrane quality control system that protects yeast cells from proteotoxic stress. *Elife* 2013;**2**, DOI: 10.7554/elife.00459.

Zhou L, Evangelinos M, Wernet V *et al.* Superresolution and pulse-chase imaging reveal the role of vesicle transport in polar growth of fungal cells. *Sci Adv* 2018;**4**:e1701798.

Ziółkowska NE, Karotki L, Rehman M *et al.* Eisosome-driven plasma membrane organization is mediated by BAR domains. *Nat Struct Mol Biol* 2011;**18**:854–6.

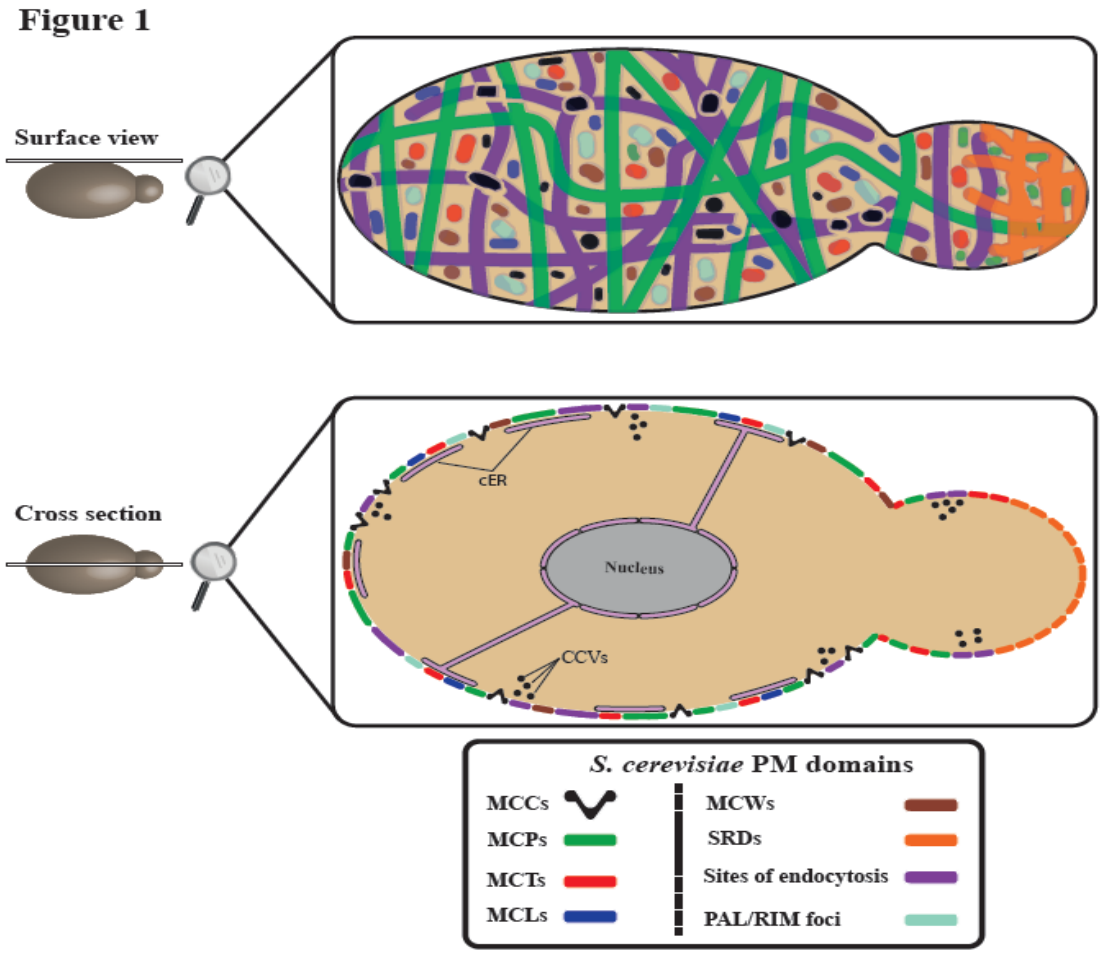

Figure 1. Schematic representation of the known plasma membrane domains in surface (upper) and cross (lower) sections of *Saccharomyces cerevisiae*. The representation is simplified and mostly qualitative, since quantitative data about the relative abundance of membrane domains is still scarce. An important percentage of the PM of *S. cerevisieae* (± 40 %) is in close contact with the cortical ER (cER). Contacts between the PM and the cER are mediated at specific positions by several tether proteins. A subset of these correspond to lipid transfer proteins of the Ltc/Lam family, which form the MCL patchy membrane domain. The network-like MCP hosts the highly abundant Pma1 and covers a significant part of the PM. MCCs/eisosomes are static patches that correspond to randomly distributed, furrow-like PM invaginations, where, among other proteins, certain nutrient transporters are localized (see also Figure 3). Outside MCCs, a network-like compartment was reported to exist, in which MCC-resident transporters additionally localize. This compartment overlaps with sites of endocytosis, where clathrin-coated vesicles (CCVs) form. Formation of CCVs most probably occurs in PM regions between MCCs/eisosomes that are devoid of cER. At the newly forming bud MCCs are not present and the sites of polar growth coincide with Sterol-Rich Domains (SRDs). Endocytosis is also polarized towards this region. Additional patchy-like domains

exist and are represented: the MCT, MCW and the Pal/Rim foci. For more details and references see text.

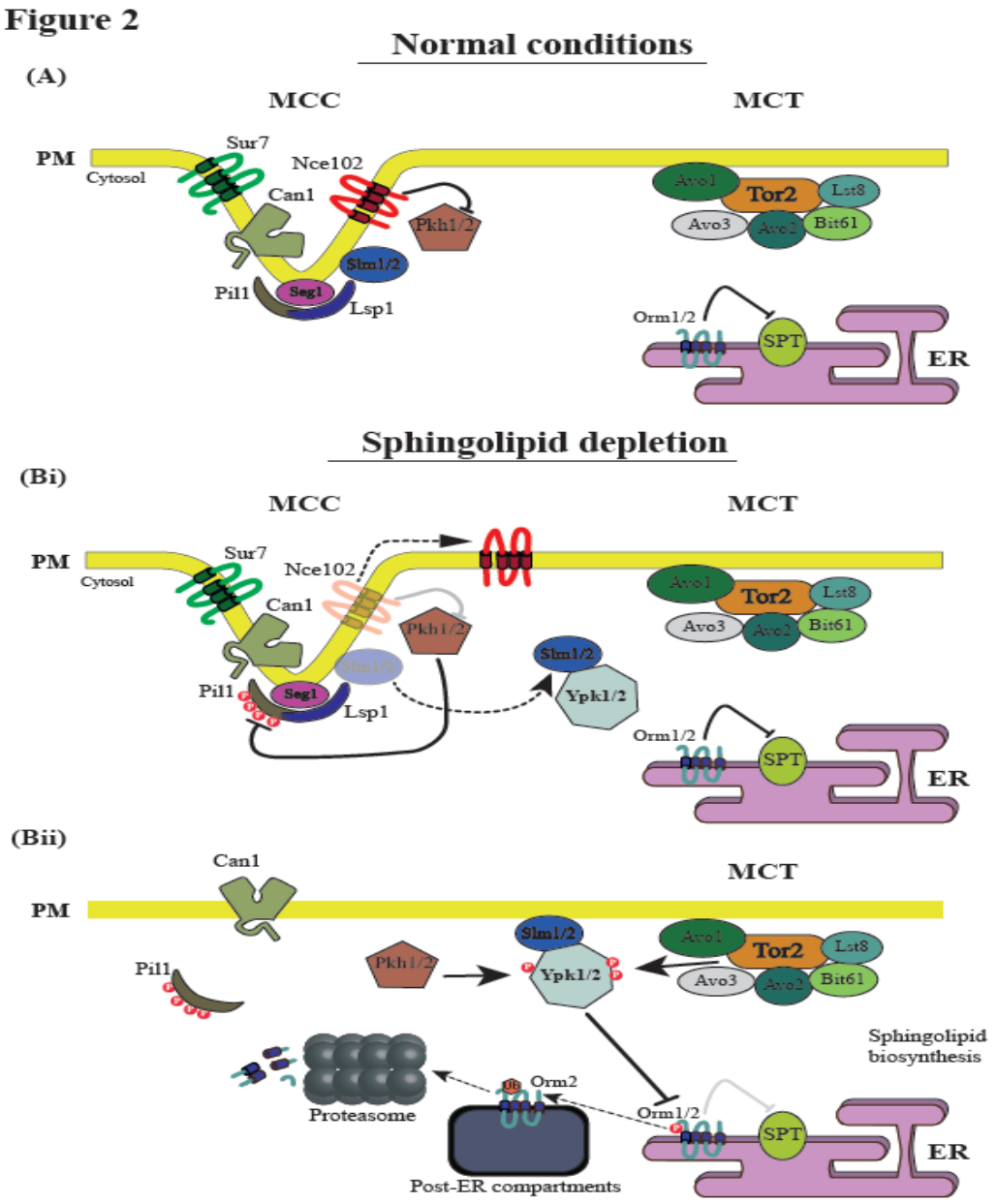

Figure 2. Organization of MCC/eisosomes and their relationships with MCTs, sphingolipid biosynthesis and membrane stress. (A) Schematic representation of the organization of MCCs. Under normal growth conditions, Nce102 in MCCs inhibits the Phk1/2 kinases, thereby promoting eisosome assembly. (Bi) Under SL depletion or mechanical membrane stretching, Nce102 moves out of MCC. The activated Pkh1/2 phosphorylate Pil1, inducing the disassembly of eisosomes. (Bii) At the same time, Slm1 relocates from MCC/eisosomes to MCT, and recruits Ypk1 to the PM for phosphorylation by TORC2. Ypk1 is additionally phosphorylated by Pkh1/2. Fully activated Ypk1 phosphorylates and inactivates the endoplasmic reticulum (ER) localized Orm1/2 proteins, negative regulators of sphingolipid

biosynthesis. Phosphorylated Orm2 is transported to post-ER compartments (such as Golgi and endosomes) where is degraded by a ubiquitin-dependent mechanism known as EGAD. For more details and references see text.

Figure 3

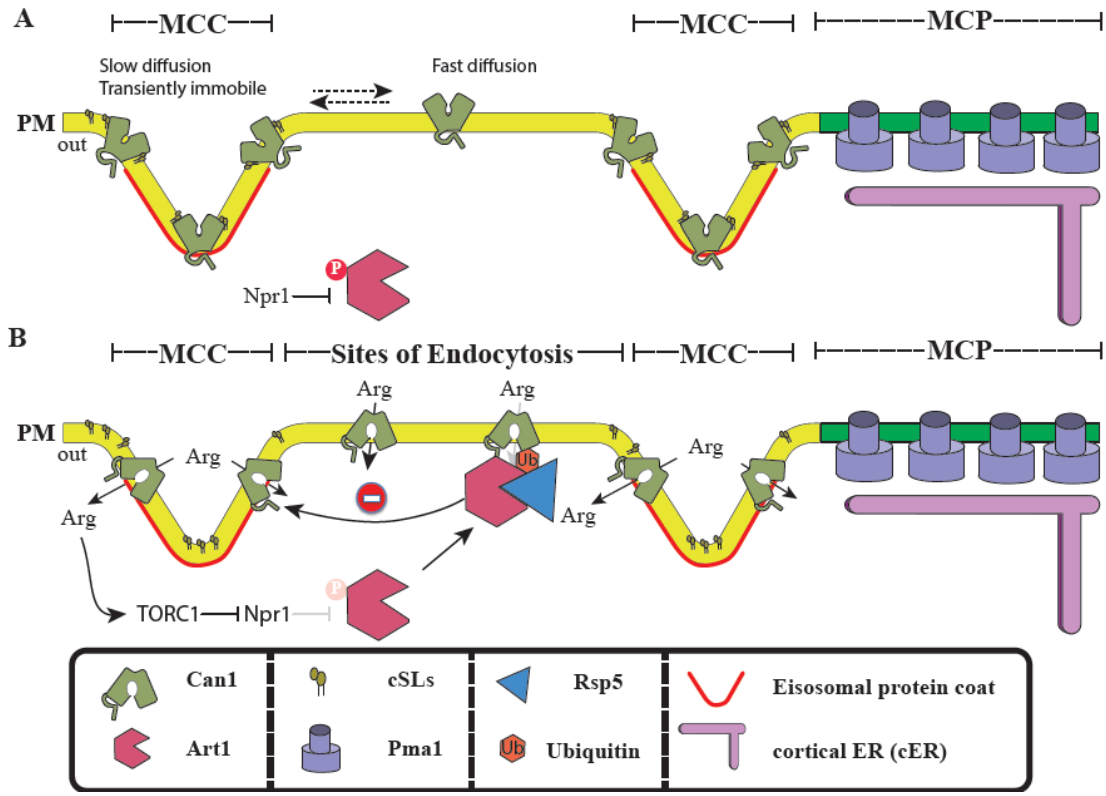

Figure 3. A model for the conformation-regulated partitioning of transporters in MCCs. The well-studied case of Can1 is shown as an example. (A). In the absence of substrate, some nutrient transporters (up to now known are Can1, Mup1, Fur4, Tat2 and Lyp1) preferentially localize in MCCs. This enrichment is mediated by complex sphingolipid (cSL)-dependent (direct or indirect) slower diffusion there, due to transient trapping of the transporters (via protein-protein and/or protein-lipid interactions) close to the centroid of MCCs. Transporters in MCCs are protected from the ubiquitylation machinery, potentially due to steric exclusion of the Rsp5-ART complex from the subcortical eisosomal protein scaffold. Similarly, transmembrane proteins with large cytosolic domains, like Pma1, are sterically excluded from MCCs. Pma1 participates in the network-like MCP (green). MCC resident transporters are able to diffuse outside MCCs and to exchange between different MCCs via another network-like compartment (see paragraph 2.1.2.3) (yellow). The cortical ER (cER) can partially cover the cytoplasmic side of these two network-like compartments. (B). In the presence of substrate (Arg for Can1), a shift of the transporter to an Inward-Facing conformation somehow abolishes the transient trapping and the slower diffusion of the permease in MCCs.

Transporter molecules are now equally distributed within MCCs and the network-like compartment outside MCCs. It is within this compartment, in regions devoid of cER on their cytoplasmic side, that the ubiquitylation machinery is able to recognize the transporter and mediate its ubiquitylation. The ubiquitylated transporter subsequently recruits/stabilizes components of the clathrin-mediated endocytic machinery, eventually leading to the formation of the clathrin coated vesicle and the endocytosis of transporter molecules. For more details and references see text.

Figure 4

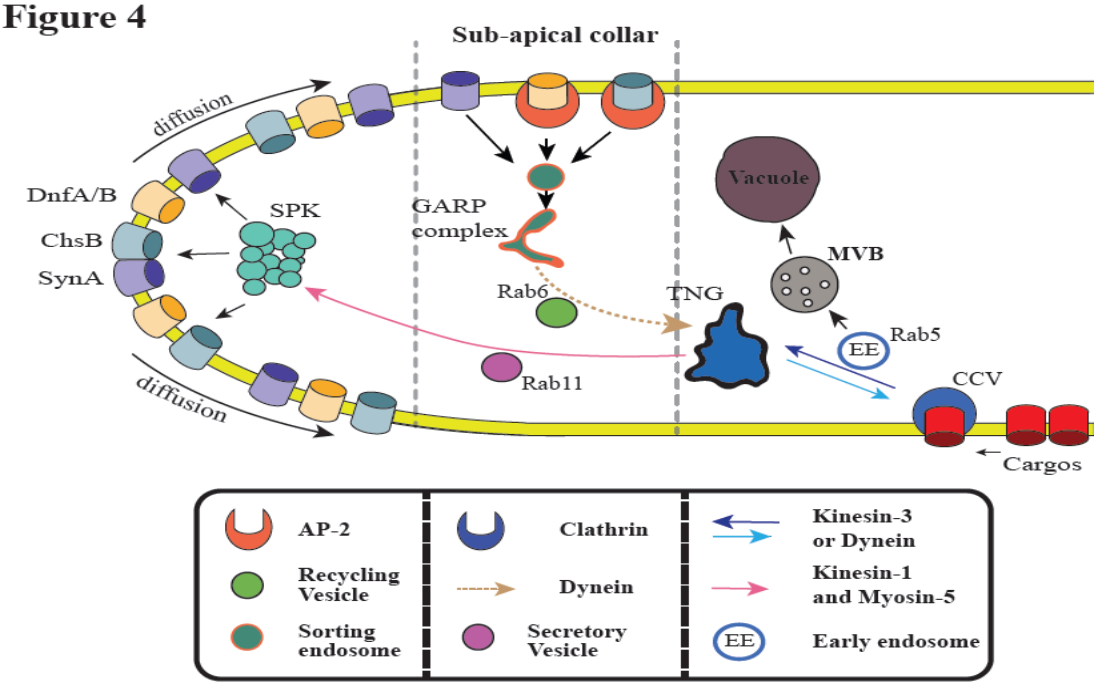

Figure 4. Schematic representation of the mechanisms contributing to the organization of the Sterol-Rich Domains (SRDs) in regions of apical fungal growth. The apical region is enriched in sterols (not shown) and several proteins, such as the polarity marker SynA/Snc1, the chitin synthetase ChsB and the lipid flippases DnfA,B/Dnf1,2. Proteins and lipids are preferentially secreted there from the Trans Golgi Network (TGN) via the Spitzenkörper structure (SPK). The secretory vesicles from the TGN to the SPK contain RabE/Rab11 and move on microtubules motorized by Kinesin-1. Proteins and lipids found in apical SRDs are restricted there by slow diffusion, and additionally via endocytosis-mediated recycling through at least two routes. The first endocytic route begins at the sub-apical collar, depends on AP-2, is clathrin independent (at least for ChsB, DnfA,B and sterols, but not SynA) and the vesicles formed are sorted to the GARP complex. From there, using recycling vesicles containing Rab6 and in a dynein-dependent way, the cargos recycle back to the TGN before being re-targeted at the PM. The second endocytic route occurs along the whole length of the hyphae and depends on clathrin. The cargoes are sorted in Early Endosomes, and if not targeted to the Vacuole for degradation via the Multi-Vesicular Bodies (MVB), they move along

microtubule tracks using kinesin-3, and are sorted at the TGN before eventually recycling back to the PM. For more details and references see text.

Table 1. The fungal plasma membrane domains.

Domain	Markers	Other components	Structure	Size	Abundance	Physiological roles	References
MCC	Sur7, Pil1, Lsp1, Seg1	Nce102, Can1, Mup1, Fur4, Tat2, Pkh1/2, Slm1/2, Fmp45, Pst2, Xrn1, Sterols (fillipin staining), Complex Sphingolipids*	Static patches, furrow like invaginations	200-300 nm long, 50-100 nm deep and 30-50 nm wide	1.5-2.5/μm^2, 20-50/ cell, increase in number and depth in stationary phase	Protection of transporters from endocytosis, regulation of lipid biosynthesis, cell wall assembly and stress responses	Malinsky et al. 2003; Walther et al. 2006; Stradalova et al. 2009; Berchtold et al. 2012; Spira et al., 2012; Gournas et al. 2018
MCP	Pma1, Pmp1	Sphingolipids*	Confocal and TIRF microscopy: Network-like domain, STED microscopy: isolated foci	n.r.	Probably the most abundant PM protein	Formation of the proton gradient, sensing of proton fluxes	Malinsky et al. 2003; Spira et al. 2012; Malinsky et al. 2016
MCT	Tor2, Avo3, Bit61	Avo1, Avo2, Lst8, Bit2, Slm1/2	Dynamic patches	2-6 protein complexes / foci	0.5-1.5/μm^2	TORC2 signaling	Berchtold et al.. 2009, 2012
MCL	Lam4/Ltc3, Lam2/Ltc4/Ysp2	Lam1/Ysp1, Lam3/Sip3, Slm1/2	Static patches, ER-PM Membrane Contact Sites	n.r.	n.r.	ER-PM crosstalk, transport of sterols	Gatta et al., 2015; Murley et al. 2017
MCW	Wsc1		Dynamic patches	200 nm	n.r.	Cell wall Stress sensing	Kock et al. 2016
Sites of Endocytosis	Abp1, Sla1, Sla2, Rvs161, Ede1	>60 proteins of the CCV	Dynamic patches	50-100 nm in diameter	0.6-1/μm^2 in unpolarized cells, more in buds	Endocytosis	Merrifield and Kaksonen, 2014 Stimpson et al., 2009
Pal/Rim foci	PalH/Rim21, PalI/Rim9	PalF/Rim8, Vps23, Snf7, PalB/Rim13, PacC/Rim101	Static patches	n.r.	*A. nidulans*: 0.1-0.3/μm at pH=5 0.6-1.2 at pH=7	External pH and PM lipid asymmetry sensing	Galindo et al., 2012 Penalva et al., 2014
SRDs	Sterols (fillipin staining), SpTna1p	Sphingolipids* Snc1/SynA, DnfA,B/Dnf1,2 ChsB	Dynamic and polarized, network-like domain	Up to several μm in diameter	Sites of polar growth	Establishment and maintenance of polar growth	Makushok et al. 2016; Riquelme et al 2018
SPFH-proteins	FloA, StoA/Slp3		n.r.	n.r.	n.r.	Affect the formation of SRDs	Takeshita et al., 2012; Conrad et al., 2018

n.r.: Not Reported; * Lack of direct evidence. Current evidence is indirect, showing that that SLs are essential for the organization of these domains or the function of proteins there.