Plasma Membrane MCC/Eisosome Domains Promote Stress Resistance in Fungi

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SUMMARY There is growing appreciation that the plasma membrane orchestrates a diverse array of functions by segregating different activities into specialized domains that vary in size, stability, and composition. Studies with the budding yeast

Saccharomyces cerevisiae have identified a novel type of plasma membrane domain known as the MCC (membrane compartment of Can1)/eisosomes that correspond to stable furrows in the plasma membrane. MCC/eisosomes maintain proteins at the cell surface, such as nutrient transporters like the Can1 arginine symporter, by protecting them from endocytosis and degradation. Recent studies from several fungal species are now revealing new functional roles for MCC/eisosomes that enable cells to respond to a wide range of stressors, including changes in membrane tension, nutrition, cell wall integrity, oxidation, and copper toxicity. The different MCC/eisosome functions are often intertwined through the roles of these domains in lipid homeostasis, which is important for proper plasma membrane architecture and cell signaling. Therefore, this review will emphasize the emerging models that explain how MCC/eisosomes act as hubs to coordinate cellular responses to stress. The importance of MCC/eisosomes is underscored by their roles in virulence for fungal pathogens of plants, animals, and humans, which also highlights the potential of these domains to act as novel therapeutic targets.

KEYWORDS Lsp1, MCC, Pil1, Slm1, Sur7, eisosome, furrow, membrane curvature, stress, yeast

INTRODUCTION

The role of the plasma membrane (PM) in acting as a protective barrier around the cell is complicated by the fact that it is also the site of a wide range of dynamic processes. A partial list of PM functions includes endocytosis, secretion, nutrient uptake, ion homeostasis, signal transduction, morphogenesis, and cell wall or extracellular matrix synthesis (1–3). Thus, an important question in cell biology is, how does the PM coordinate these diverse processes while maintaining its barrier function? In recent years, there has been significant progress in understanding how separating PM functions into different domains helps to orchestrate different activities. However, defining PM domains has been challenging for technical reasons (4). In addition to being hydrophobic and difficult to study *in vitro*, PM domains can be very small or very transient (5, 6). Therefore, studies on a relatively stable type of PM domain discovered in yeast, termed the membrane compartment of Can1 (MCC) (7–9), also known as an eisosome (10), have provided an important model for how compartmentalization of the PM facilitates its complex function (2, 11, 12).

There are typically about 50 MCC/eisosome domains distributed throughout the PM of each cell of the budding yeast *Saccharomyces cerevisiae*, resulting in a punctate appearance (Fig. 1). A major step forward in understanding the structure of these domains was the discovery that MCC domains correspond to inward furrows of the plasma membrane that had been reported previously but whose function was not known (13, 14). In *S. cerevisiae*, these domains are about 200 to 300 nm long, 50 nm wide, and 50 nm deep under standard conditions. Another major step forward was the discovery that the furrows are stabilized by a complex of cytosolic proteins termed the eisosome (10). The fact that these domains are protein-organized invaginations in the PM has led to comparisons with caveolae in mammalian cells (15). However, in the case of MCC/eisosomes, their formation in *S. cerevisiae* is promoted by two paralogous proteins, Pil1 and Lsp1 (16, 17). These proteins contain Bin-amphiphysin-Rvs (BAR) domains that bind the cytoplasmic surface of the PM. Pil1 and Lsp1 also interact to form long filaments that shape the PM into stable furrows (16–18). The MCC/eisosomes were found to contain a selected subset of proteins, suggesting that these domains were protected islands in the PM where proteins could avoid being endocytosed (7, 8). (Note that we will refer to the integral membrane proteins and PM lipids as part of the MCC, the peripheral membrane proteins at these sites as an eisosome, and the entire complex as an MCC/eisosome.)

More recent studies are now revealing that MCC/eisosomes are actually dynamic; they undergo changes in depth and length in response to stressful conditions. In some ways, these PM domains have been underappreciated because most previous studies were carried out on log-phase cells under ideal growth conditions. Therefore, this

FIG 1 Landmark research that converged to develop the current models for MCC/eisosome structure. PM furrows were discovered by freeze-etch EM (14). Sur7-GFP (9) and Can1-GFP (7, 8) were found to localize to stable PM patches termed MCC domains. Pil1-GFP was subsequently found to associate with the cytoplasmic side of the MCC in a complex termed the eisosome (10). Immuno-EM studies then revealed that MCC/eisosomes correspond to PM furrows with Sur7 found at the tops, whereas Pil1 was detected at the bottom (13). Determining the structure of Lsp1 revealed it to be a BAR domain protein (16), and demonstration of the ability of Pil1 and Lsp1 to bind membranes and form filaments that shape the furrows led to the “half-pipe” model for MCC/eisosome structure (18). New high-resolution microscopy methods are revealing dynamic aspects of MCC/eisosome furrow structure in response to stress (21, 22, 49). Space limitations prevent showing other key advances, which are described in the text. (The image of the PM furrows is reproduced from reference 14 with permission of Rockefeller University Press, the images of GFP-tagged Sur7 are reproduced from reference 9 with permission, the image of GFP-tagged Can1 is reproduced from reference 7 with permission, and the image of GFP-tagged Pil1 is reproduced from reference 10 with permission of Springer Nature.)

review will emphasize new results that document how MCC/eisosomes change their structure and function to promote critical stress responses. For example, several studies have converged to reveal that MCC/eisosomes act as sensors of altered membrane tension (2, 19, 20). Conditions such as hypoosmotic shock that increase membrane tension flatten the furrows at MCC/eisosomes (21–23) and release a subset of proteins that promote changes in other cellular processes (e.g., lipid homeostasis) (11). Other proteins can be selectively induced to exit, such as certain nutrient symporters whose substrates cause them to move out of MCC/eisosome so they can be endocytosed to help coordinate proper intracellular pools of amino acids (19, 24). In contrast, other proteins are recruited to MCC/eisosomes to prepare for starvation conditions (24–26). A third general area of MCC/eisosome function is to mediate proper morphogenesis and cell wall integrity, which are carried out in part by regulating phosphatidylinositol 4,5-bisphosphate [PI(4,5)P₂] levels and cell signaling pathways (12, 27). Other studies are also identifying roles for MCC/eisosomes in promoting resistance to environmental stress conditions, such as oxidation and copper (28, 29). As will be described, these MCC/eisosome functions are often intertwined through the homeostasis of different kinds of lipids that are important for PM architecture and cell signaling. Therefore, this review will emphasize the emerging models for how MCC/eisosomes act as hubs to coordinate cellular responses to a wide range of stress conditions.

MCC/EISOSOME STRUCTURE

Plasma Membrane Domains in Yeast

PM studies are in a golden age. Research on cellular membranes initially lagged

FIG 2 Diversity of PM domains in *S. cerevisiae*. Illustration of different types of PM domains. MCC/eisosomes are stable under steady-state conditions and do not move laterally in the PM. Other domains are transient, such as sites of endocytosis, secretion, and cER tethering, or they diffuse laterally in the PM. Sensor domains include the ambient pH sensor (pH), the membrane compartment of TORC2 (MCT), cell wall integrity sensors (CWI), and Mss4 (phosphatidylinositol 4-phosphate 5-kinase). Note that the MCP (membrane compartment of Pma1) domain is not specifically labeled in the figure, as it is thought to comprise all the areas of the PM that are not occupied by the domains mentioned above. Recent studies also indicate that additional domains can be identified by high-resolution fluorescence microscopy (2, 20, 44).

behind other areas because of the difficulties in studying hydrophobic complexes *in vitro*. It was not until the 1950s and 1960s that experiments were published demonstrating that the PM is a lipid bilayer (30). The fluid mosaic model, published in 1972, suggested that lipid membranes also contained integral membrane proteins that span across the bilayer, which provided an explanation for how polar molecules could cross membranes (31). However, it took years of challenging work, honored by recent Nobel Prizes, to define the structure and function of water channels (aquaporins) and ion channels that revealed the molecular mechanisms that enable polar molecules to cross the PM. In recent years, further insights into PM structure and function have been driven by the fact that the majority of pharmaceutical drugs target membrane proteins (32).

A major challenge is to understand how the PM coordinates diverse activities. Part of the answer is that the PM is compartmentalized into different subdomains (1, 2, 5). To provide a better context for understanding the special properties of MCC/eisosomes, some of the other types of PM domains found in yeast will be described (Fig. 2). In a general sense, the largest domain in *S. cerevisiae* is the bud, which is partitioned off by septin filaments that prevent diffusion of proteins back into the mother cell (33). Additional large-scale domains include sites of fusion during yeast conjugation and the leading edge of polarized growth in filamentous fungi (1). Other studies have proposed that a large portion of the PM is part of a network that is referred to as the MCP (membrane compartment of Pma1, the plasma membrane H⁺-ATPase) (7, 8, 34). The MCP was initially described as a dynamic zone in which proteins diffuse rapidly, sites of endocytosis and secretion form, and cell wall synthesis occurs. However, new super-resolution microscopy methods indicate that the MCP is segregated into different subdomains, such that sites where endocytosis occurs are distinct from Pma1 (35).

The MCP also includes, or envelops, a variety of other small, protein-organized domains (2). One domain that has functional relationships with MCC/eisosomes is termed the MCT (membrane compartment of TORC2) because it contains the target of rapamycin complex 2 (TORC2) kinase complex that regulates cell polarity, endocytosis, and sphingolipid synthesis (36, 37). Other domains include protein complexes involved in sensing pH (38), the phosphatidylinositol 4- and phosphatidylinositol 5-kinases (39, 40), cell wall integrity sensors (41, 42), and endoplasmic reticulum-PM contact sites (43). Interestingly, a high-throughput screen found that a large number of PM proteins formed punctate patches of various stability, suggesting that they may be regulated by

compartmentalization (44). Specialized lipid domains are also important, as they have been observed at MCC/eisosomes as well as sites of polarized growth and cytokinesis (45–47). Altogether, an emerging view in the field is that this type of nanoclustering plays a dominant role in coordinating membrane functions (3, 48).

MCC/Eisosome Components and Organization

This section will summarize the proteins and lipids that have been detected in MCC/eisosomes and, if it is known, describe their location within these structures. This information will be important for understanding the role of these components in promoting MCC/eisosome formation and in responding to different kinds of stress, as will be described in the subsequent sections.

A brief history of PM furrows and MCC/eisosomes. Several different avenues of research converged to identify MCC/eisosomes in *S. cerevisiae* as a novel type of membrane domain (Fig. 1). One line of work started with the discovery that yeast plasma membranes contain furrow-like invaginations, which was made possible by advances in freeze-etch electron microscopy (EM) (14). There was a long gap until another advance in technology, the use of green fluorescent protein (GFP) fusions to study protein localization, revealed that Sur7 and the arginine transporter Can1 localized to punctate patches in the PM (7–9). These domains were distinct from cortical actin patches, revealing a new PM domain that was termed the MCC. Another key discovery was that two paralogous proteins, Pil1 and Lsp1, bind to the PM and form a complex underneath the MCC that was termed an eisosome (10). These MCC/eisosomes were clearly novel structures, as they did not colocalize with cortical actin patches. A landmark study then tied everything together by showing that MCC/eisosomes correspond to the membrane furrows that had been described about 40 years earlier (13). The next major advances showed that Pil1 and Lsp1 contain BAR domains, which are known to promote membrane curvature, and that these proteins can link together to create filaments that shape the furrows in the PM (16, 18). New methods, including superresolution microscopy, are now being used to define novel properties of MCC/eisosomes, such as the dynamic regulation of furrow depth and the location of proteins within the furrows (21, 22, 35, 49).

Proteins in the MCC. Additional integral membrane proteins that localize to the MCC have been discovered, primarily by screening GFP-tagged proteins. They are listed in Table 1, and the proteins that will be discussed in this review are diagrammed in Fig. 3. These include members of two different families of tetraspan proteins, of which the prototypes are Sur7 and Nce102 (9, 34). Sur7 has been localized to the upper ends of the furrows, close to the rims, by immuno-EM and by high-resolution fluorescence microscopy (13, 49). The exact biochemical function of these tetraspan proteins is not known, but they are important in responding to stress (9, 50, 51). Another major class of MCC proteins consists of nutrient transporters in the APC family of proton symporters (APC stands for amino acid-polyamine-organocation) (2, 19, 20, 52). The arginine transporter Can1 was the first APC symporter to be localized to these domains (7, 8). Others now include the Tat2 transporter for tryptophan and tyrosine (53), Mup1 methionine transporter (35), Lyp1 lysine transporter (24), and the Fur4 uracil transporter (34, 54). Superresolution fluorescence microscopy indicates that Fur4, and likely other transporters, localize near the rim of the furrow, similar to Sur7 (21, 49). A common feature of the APC transporters is that they are proton symporters that utilize a cotransported proton to drive uptake of the substrate (2, 19, 20, 52). Although not all APC transporters localize to the MCC (e.g., Gap1 [24]), it has been proposed that many of the APC symporters localize to MCC domains as a way to coordinate their cell surface levels when an external proton gradient is not available, as will be described further below. If true, then the MCC could be home to more than 30 different proteins (Table 1).

Proteins in the eisosome. Pil1 and Lsp1 are the key players in the eisosome, both in terms of function and abundance. The data supporting a direct role for Pil1 and Lsp1 in forming the furrows will be described in the next section. Consistent with this, it is

TABLE 1 *S. cerevisiae* MCC/eisosome proteins

Protein	Location	Function	Localization reference no.	No. of copies/cells found through ^a :		
				Western blotting	Mass spectrometry	GFP tagging
Sur7	MCC	Sur7 family tetraspan	9	17,000	4,045	14,332
Fmp45	MCC	Sur7 family tetraspan	9	329	4,627	4,326
Pun1	MCC	Sur7 family tetraspan	34	1,660	2,735	5,167
Ynl194c	MCC	Sur7 family tetraspan	9	ND ^c	1,971	1,995
Nce102	MCC	Nce102 family tetraspan	34	ND	7,567	55,428
Fhn1	MCC	Nce102 family tetraspan	34	ND	ND	1,133
Can1	MCC	Arg/H ⁺ symporter	7	ND	1,812	8,324
Fur4	MCC	Ura/H ⁺ symporter	8	ND	756	ND
Tat2	MCC	Trp and Tyr/H ⁺ symporter	53	752	523	5,710
Lyp1	MCC	Lys/H ⁺ symporter	49	2,580	1,560	9,947
Mup1	MCC	Met/H ⁺ symporter	54	ND	1,916	14,268
Pil1	Eisosome	BAR domain	10	115,000	48,996	38,869
Lsp1	Eisosome	BAR domain	10	104,000	41,516	6,109
Pkh1	Eisosome	Ser/Thr protein kinase	63, 64	ND	1,786	1,262
Pkh2	Eisosome	Ser/Thr protein kinase	63, 64	ND	1,080	1,336
Eis1	Eisosome	Eisosome assembly and stability	34	5,570	12,142	7,173
Slm1	Eisosome	BAR domain and PH domain	34, 133	5,190	4,116	3,289
Slm2	Eisosome	BAR domain and PH domain	34, 133	2,610	980	ND
Seg1	Eisosome	Eisosome assembly and stability	59, 178	ND	4,834	3,890
Mdg1	Eisosome	Unknown	34	1,240	5,981	3,508
Ygr130c	Eisosome	Unknown	34, 59	10,300	10,608	6,932
Pst2	Eisosome	Flavodoxin-like protein/NAD(P)H quinone oxidoreductase	34	2,330	92,741 ^b	5,349
Rfs1	Eisosome	Flavodoxin-like protein	34	7,060	12,403	6,881
Ycp4	Eisosome	Flavodoxin-like protein/NAD(P)H quinone oxidoreductase	34	14,600	18,901	11,181
Msc3	Eisosome	Unknown	57, 76	131	3,915	2,707
Xrn1	Eisosome	5'–3' mRNA exoribonuclease	26	11,700	14,753	14,897

^aEstimates of protein levels from Western blotting data as reported by Ghaemmaghami et al. (179). Protein-level estimates from mass spectrometry and analysis of the fluorescence of GFP-tagged proteins represent the average of several independent data sets, as reported by Ho et al. (180).

^bThere was wide variation in values reported for Pst2 copies/cell as determined by mass spectrometry analysis.

^cND, not determined.

estimated that there are about 100,000 copies each of Pil1 and Lsp1 in the cell, which are predicted to be enough to cover each furrow (55). Immuno-EM studies localized Pil1 to the bottom of the furrows (13), and this has now been confirmed by new super-resolution fluorescence microscopy (35, 49) and electron cryo-tomography (22). At least 13 other peripheral membrane proteins were found to localize to eisosomes in *S. cerevisiae* (Fig. 3 and Table 1) (34, 56–60). One group of these proteins is noteworthy because they regulate the formation and stability of eisosomes. This includes Seg1 (61, 62), Eis1 (58), and the redundant paralogous protein kinases Pkh1 and Pkh2 that will be described further below (63, 64). The Pkh1/2 kinases are also significant because they have broad roles in regulating diverse cellular processes, including sphingolipid synthesis, endocytosis, and cell wall integrity (37). Some eisosome proteins, such as Pil1, are relatively stable in the eisosome (65, 66). Interestingly, other proteins, such as the paralogous BAR domain proteins Slm1 and Slm2, exit the eisosome under certain stress conditions that cause an increase in membrane tension (17, 67, 68). Conversely, the Xrn1 5' to 3' exonuclease that is a major component of cytoplasmic processing (P) bodies involved in mRNA decay only gets recruited to eisosomes when cells experience glucose limitation (26, 69).

Lipids in the MCC. Lipids are difficult to localize to specific sites in the PM, but several indirect lines of evidence indicate that MCC domains have a distinct lipid composition. For example, MCC domains stain preferentially with filipin (53), a naturally fluorescent sterol-binding antibiotic (46). However, it is unclear whether this represents an increase in ergosterol content or an increase in the accessibility of the ergosterol due to membrane curvature or changes in sphingolipid content (70). Consistent with this, analysis of the lipids immediately surrounding Sur7, a protein that is tightly localized to

FIG 3 Proteins associated with MCC/eisosomes. A subset of proteins that localize to MCC/eisosomes shown in Table 1 is illustrated. Proteins that can exit the furrows under stress are also shown outside the furrows on the right (i.e., Nce102, Slm1/2, and APC family symporters such as Can1). Sur7 and Can1 localize to the tops of the furrows (13, 35, 49). Pil1, Lsp1, Seg1, and Pkh1/2 appear to localize to the bottom of the furrows (18, 22, 37, 61). The other proteins were positioned where space was available and do not reflect experimentally determined locations.

the MCC, showed no enrichment in ergosterol, suggesting that there could be zones of distinct lipid composition in the MCC (47). The inner leaflet of the MCC is thought to be enriched in PI(4,5)P₂, in part because Pil1, Lsp1, and Slm1/2 bind this lipid (16, 18, 62). It is also likely that the MCC contains a distinct lipid composition because membrane curvature attracts specific types of lipids (3, 71).

MCC/Eisosome Formation and Stability

Regulation of MCC/eisosome assembly. (i) **Regulation of eisosome initiation and furrow length.** Pil1 was identified as a central component because it localized to these eisosomes and is essential for their formation (10). Subsequently, two key studies supported a direct role for Pil1 and Lsp1 and laid the groundwork for the current model of eisosome assembly. One line of research determined the 2.9-Å crystal structure of Lsp1, which revealed that Pil1 and Lsp1 contain banana-shaped BAR domains that are known to bind to membranes and promote curvature (16, 17, 72). Another key discovery was that purified Pil1 and Lsp1 assembled into long filaments that were able to tubulate membrane vesicles *in vitro* (18). These observations led to a “half-pipe” model for the formation of membrane furrows in which filaments of Pil1 and Lsp1 align their BAR domains along the plasma membrane to sculpt the membrane into an invagination (18). Interestingly, new electron cryo-tomography methods indicate that the PM is thicker at the base of deeper furrows, suggesting that the presence of increased copies of Pil1 and Lsp1 promotes deeper furrow formation (22).

Although *S. cerevisiae* Lsp1 is ~72% identical to Pil1, it cannot promote eisosome formation (10). This is thought to be due to a reduced ability of Lsp1 to bind the plasma membrane (10, 17). The impaired function of Lsp1 is not seen in all species, suggesting it may be restricted to fungi that are closely related to *S. cerevisiae* (73). It is possible that the unique properties of Lsp1 may have special physiological significance, as Lsp1 was needed to see an expansion of the size and number of MCC/eisosomes in stationary-phase *S. cerevisiae* cells (24).

Additional proteins aid in MCC/eisosome formation and in regulating the length of the furrow (13, 34, 58, 60, 74, 75). Seg1 is thought to facilitate the limiting steps in the initiation of eisosome assembly, which include the membrane association of Pil1/Lsp1 and the subsequent nucleation to polymerize these proteins into a membrane-bound filament that can form a furrow (61). Part of the evidence for this is that cells lacking Seg1 formed eisosomes with reduced efficiency, whereas overproduction of Seg1 led to

longer furrows (61, 74, 75). This model for Seg1 function also helps to explain the observation that decreased levels of Pil1 lead to fewer eisosomes that are the same size, rather than the same number of smaller eisosomes (61). A similar role is carried out by Sle1 in *Schizosaccharomyces pombe* (61, 62, 75). Eis1 also promotes eisosome assembly, possibly through a direct effect since it localizes to these domains (58). Nce102 and Slm1/2 are important for efficient MCC/eisosome formation, but determining their specific roles is complicated because they also regulate the synthesis of sphingolipids and can therefore indirectly affect eisosome formation via their role in proper lipid homeostasis (11, 13, 34, 58, 60). That proper PM lipids are needed for MCC/eisosome formation was also indicated by studies showing that genes involved in lipid synthesis can influence MCC/eisosome formation (34, 68). Another important factor is that the medium in which the cells are grown can impact eisosome length and number (24, 25, 54).

(ii) Spatial regulation of MCC/eisosome formation. MCC/eisosomes appear to be positioned randomly in the PM (Fig. 1), but, in fact, the position of the sites is regulated. In log-phase cells, new MCC/eisosome formation is usually restricted to the growing bud and is not seen in the mother cell (9, 76). New buds are initially devoid of MCC/eisosomes, and then there is a wave of MCC/eisosome formation progressing from the junction with the mother cell and spreading toward the tip of the bud (7, 9, 76). MCC/eisosomes do not typically form directly adjacent to each other, and the furrows do not overlap (76). This suggests that there is a mechanism that prevents the formation of a new MCC/eisosome close to an existing one. Although the curvature of the furrows likely prevents formation of overlapping furrows, the observation that some mutant cells can form end-to-end chains of furrows suggests that there is more to learn about the spatial regulation of MCC/eisosome formation (73).

(iii) Regulation of eisosome lateral stability and furrow depth. Additional factors must keep the eisosome in place, as they are immobile in the PM (7, 9, 10). The restricted lateral mobility of these domains does not appear to be due to a direct connection to the cell wall, actin filaments, or microtubules (8, 77). A contributing factor may be that Pil1 and Lsp1 bind PI(4,5)P₂, as other BAR domain-containing proteins form stable protein-lipid microdomains by inhibiting the lateral diffusion of phosphoinositides (78). However, studies in *S. pombe* indicate that cycles of PI(4,5)P₂ generation and hydrolysis are also important for proper eisosome assembly (62). The PI(4,5)P₂ hydrolysis may be related to the observation that the subunits at the ends of the Pil1/Lsp1 filaments undergo exchange, which would presumably allow for exposure of lipids at this zone of Pil1/Lsp1 filament assembly (65, 66). Consistent with MCC/eisosomes being stable domains, Sur7 is one of the longest-lived proteins in yeast (79). Although the lateral position of MCC/eisosomes in the PM is static, the depth of the furrows is not. As will be discussed below, furrow depth can vary in response to factors that influence tension (21, 23, 24, 35, 49). Nce102 is required for deep furrows to form (80), and its presence or absence from the MCC may be one mechanism used to control furrow depth. Increased furrow depth may be promoted by greater amounts of Pil1 and Lsp1 at the base of the furrow (22).

Regulation of MCC/eisosome disassembly. (i) **MCC/eisosome disassembly during increased membrane tension and initiation of budding.** Once established, MCC/eisosomes are thought to be very stable under ideal growth conditions. However, these domains disassemble under certain physiological conditions, such as inhibition of sphingolipid synthesis, as will be described further below (11). Interestingly, MCC/eisosomes have been observed to disassemble at the nascent bud site in *S. cerevisiae* (future site of septation) and at the site of septation in fission yeast (77, 81). An interesting future area for study will be to determine what selectively regulates eisosome disassembly in such a narrow subregion of the cell. Treating *S. cerevisiae* with mating pheromone has been reported to promote MCC/eisosome disassembly (59), but this was not obvious in a separate study (34).

(ii) **Phosphorylation of Pil1 and Lsp1 promotes eisosome disassembly.** Pil1 and Lsp1 are phosphorylated on multiple sites, and at least a subset of these modifications

is thought to promote eisosome disassembly. Mutation of four phosphorylation sites (Ser-45, Ser-59, Ser-230, and Thr-233) to the phosphomimetic aspartic acid led to decreased eisosomes, whereas substitution with nonphosphorylatable alanine led to greater eisosome stability (60, 64). Phosphorylating these sites likely promotes eisosome disassembly by interfering with membrane association since the phosphorylated residues lie within the membrane-binding surface of Pil1 (16, 60, 64). Although eisosomes are stable, the individual subunits at the ends of the filaments formed by Pil1 and Lsp1 have been shown to undergo exchange (65, 66). Thus, this exchange provides opportunities for phosphorylation to disassemble established Pil1/Lsp1 filaments.

Further studies have indicated that the role of phosphorylation is complex, as mutations affecting different groups of phosphorylation sites on Pil1 caused different phenotypes (59, 64, 82, 83). For example, mutating a set of six phosphorylation sites (Ser-6, Thr-27, Ser-59, Thr-233, Ser-273, and Ser-299) led to the opposite conclusion—that phosphorylation promotes eisosome assembly (82). Perhaps this difference was because some of these sites are predicted to face away from the PM, raising the possibility that phosphorylation of these residues might stabilize Pil1 filament formation. However, further research is needed in this area, as different interpretations have been made concerning the effects of mutating residues Ser-230 and Thr-233 (25, 59, 83). A comparison of these studies suggests that different growth conditions and media may contribute to the different phenotypes that were observed. Consistent with this, nutrition limitation stimulates an increase in the number and length of MCC/eisosomes that will be described further below (24, 25, 54).

Several protein kinases have been implicated in phosphorylating Pil1 and Lsp1, including the paralogous Pkh1 and Pkh2 that localize to eisosomes (59, 64, 82, 83). Pkh1/2 kinase activity was previously thought to be stimulated by sphingolipid long-chain bases, but subsequent studies have not found evidence to support this (37). The Pkh1/2 kinases are implicated because their mutation increased the assembly of Pil1 into filaments (63, 64). In addition, activation of the Pkc1 pathway leads to phosphorylation of Pil1 on Ser-230 and Thr-233, likely mediated by the Slt2 mitogen-activated protein (MAP) kinase (25, 83). An interesting area for future study will be to determine whether phosphorylation releases Pil1 and Lsp1 from the eisosomes to promote disassembly or if it acts to prevent reassociation of these proteins that is needed to maintain filament length. It will also be interesting to determine if other protein kinases regulate stress-induced changes in eisosome structure and function that will be described in the next section.

MCC/EISOSOME FUNCTION

Overview of MCC/Eisosome Functions in Responding to Stress

Recent studies are beginning to reveal the interrelated functional roles of MCC/eisosomes. Although the PM furrows were first reported in 1963 (14), understanding their function has lagged. Initial reports were controversial, as the first reports on the MCC concluded they were protected from endocytosis (34), whereas the first eisosome studies proposed that they were alternative sites of endocytosis (10). In fact, the term eisosome comes from the Greek “eis,” meaning into or portal, and “soma,” meaning body, that was meant to signify a role in endocytosis (10). Subsequent studies failed to detect endocytosis at eisosomes (9, 74, 77, 84, 85), although eisosomes can indirectly influence endocytosis through their roles in lipid homeostasis (37, 86). Newer studies now indicate that the major function of MCC/eisosomes is to protect cells from a wide range of stress conditions, which are outlined in Fig. 4 and will be summarized below in four subsections. The first section will describe how the MCC/eisosomes sense increased membrane tension (i.e., stretching). This will be presented first because membrane tension has broad effects on MCC/eisosome structure that impact the overall function of these domains. The next section on nutrient stress will highlight landmark new research on the regulation of nutrient symporters and how certain proteins are recruited to MCC/eisosomes to prepare for starvation conditions. The third section will review the roles of MCC/eisosomes in promoting proper cellular morpho-

FIG 4 MCC/eisosome functions. MCC/eisosome functions are displayed according to the different sections in which they are described in the text.

genesis and cell wall integrity, and the final section will describe the roles of MCC/eisosomes in promoting resistance to environmental stresses, such as oxidation and copper toxicity. It is important to note that these diverse functions are intertwined, often through the involvement of MCC/eisosomes in regulating PM lipids. Thus, a model is emerging that MCC/eisosomes are hubs that cross-regulate key functions in response to a range of different stressful conditions.

Membrane Tension

Overview. Membrane tension is the lateral stretching of the PM (87). All cells experience changes in membrane tension and need homeostatic mechanisms to counteract it. Decreased tension results in excess membrane, as might occur in response to a switch to hyperosmotic conditions that cause cells to shrink (88, 89). In yeast, decreased membrane tension promotes deepening of the MCC/eisosome furrows (21), but this is not sufficient to deal with all of the slack membrane. Other mechanisms include a recent discovery that cells experiencing decreased tension form inward protrusions of the PM that contain a lipid-ordered phase enriched in PI(4,5)P₂ (23). Conversely, increased membrane tension, as can be caused by hypoosmotic shock, stretches the PM. Therefore, it is not surprising that the first report of PM furrows in yeast recognized their potential to provide a reservoir of extra membrane (14). Recent studies found that MCC/eisosomes flatten under increased tension (21, 90), but other researchers concluded that flattening furrows would not contribute enough new membrane area to play a major role in responding to tension (21). Instead, it now appears that MCC/eisosome furrows primarily act as sensors of membrane tension. Cells are known to have several ways of sensing membrane tension, including mechanosensitive ion channels and proteins that differentially bind to membranes under tension due to changes in curvature or membrane fluidity (87). MCC/eisosomes may act similarly to mammalian cell caveolae in the way that these protein-organized domains disassemble in response to increased tension and release proteins that activate responses in other areas of the cell (15). This section will describe the effects on MCC/eisosome structure and function caused by changes in PM tension due to a blockade in sphingolipid synthesis, hypoosmotic stress, and a sudden change to alkaline pH.

Lipid stress. A screen for genes affecting MCC/eisosomes discovered that these domains are involved in sphingolipid signaling (60). MCC/eisosomes have also been linked to the regulation of phosphoinositide lipids (27), but this will be discussed below in the section “Cell wall stress and morphogenesis.” Studies from several labs led to a model that blocking sphingolipid synthesis would increase membrane tension, causing

Nce102 and Slm1/2 to exit the MCC/eisosome (35, 60, 68), which subsequently activates a pathway in which TORC2 stimulates Ypk1 to phosphorylate Orm1/2 and halt its repression of the sphingolipid synthesis pathway (37, 91). Similar events are also thought to occur in response to other types of stress that increase membrane tension, as will be described below for hypoosmotic shock or a shift to alkaline pH. This model for sphingolipid regulation has been reviewed in detail in other recent articles (2, 11, 19, 37). Therefore, this section will summarize the key findings and the emerging questions in this area.

(i) **Nce102.** The tetraspan integral membrane protein Nce102 was discovered to be a component of the MCC and was subsequently shown to regulate eisosome formation and sphingolipid signaling by acting upstream of Pkh1/2 (34, 60). Nce102 was first implicated as a regulator of the Pkh1/2 kinases because an *nce102*Δ mutant contained fewer MCC/eisosomes. Since phosphorylation of Pil1 by the Pkh1/2 kinases can promote eisosome disassembly, it was concluded that Nce102 acts as a negative regulator of Pkh1/2 in the eisosomes and that its absence leads to increased phosphorylation of Pil1 and eisosome disassembly (60). These conclusions were supported in part by data showing that a mutant form of Pil1, in which four phosphorylation sites were mutated to Ala, rescued the MCC/eisosome defect of an *nce102*Δ mutant. Nce102 was subsequently shown to also regulate Ypk1, which promotes sphingolipid synthesis (60, 82). Other studies showed that Nce102 is required for furrows to form at sites of MCC/eisosomes (80). Altogether, these results suggested that the presence of Nce102 in the MCC inhibits Pkh1/2 from activating Ypk1 and from phosphorylating Pil1 to promote eisosome disassembly and furrow flattening. Although Nce102 is clearly an important regulator, an important goal for future studies is to determine how it regulates Pkh1/2; it is not known if there is a direct or indirect relationship (92). It has been suggested that the transmembrane domains of Nce102 could recruit special lipids and proteins to the MCC, but Nce102 likely acts at least in part on cytoplasmic proteins since the last 6 amino acids of the cytoplasmic C-terminal domain are critical for its function (80). In addition, it will also be interesting to determine whether increased membrane tension flattens the eisosome furrow, causing Nce102 to exit, or does Nce102 directly sense increased tension, causing it to exit the MCC, leading to eisosome flattening?

(ii) **Paralogous Slm1 and Slm2.** Slm1/2 localize to eisosomes and contain a Fer-CIP4 homology (FCH)-BAR (F-BAR) domain that binds to membrane curvature as well as a specialized pleckstrin homology (PH) domain that coordinately binds to PI(4,5)P₂ and sphingolipids (17, 67, 93, 94). A current model suggests that Slm1/2 exit the eisosome in response to the inhibition of sphingolipid synthesis or other causes of increased membrane tension, and then they activate a signaling pathway resulting in increased sphingolipid synthesis. This pathway involves activation of TORC2 to phosphorylate the Ypk1 protein kinase, which then phosphorylates and inactivates Orm1/2 repressors of sphingolipid synthesis (23, 37, 68). There is general agreement that Slm1/2 are needed to stimulate TORC2 to phosphorylate Ypk1, but recent studies have raised questions about the underlying mechanisms (23, 95). An earlier study indicated that Slm1/2 exited the eisosomes and colocalized with TORC2, suggesting a direct role in TORC2 activation (68). Fusing the PH domain of Slm1 onto Ypk1 was sufficient to enable activation of Ypk1, indicating one key role of Slm1/2 is to recruit Ypk1 to the PM (96).

Recent studies have raised questions about the current model for Slm1/2 function. For example, one intriguing report indicated that Slm1 exits the eisosome and enters into a new PM domain that does not colocalize with TORC2 (21). Although part of this difference may be due to the different ways Slm1 was triggered to exit from the eisosome (sphingolipid stress versus alkaline pH stress), it was surprising that Slm1 was concluded to localize to patches in the PM that were distinct from both eisosomes and TORC2. A different study found that a fraction of Slm1 localized to special sites of contact between the cortical ER (cER) and the PM that contain the sterol transfer protein Ltc4, and this pool of Slm1 was implicated in activating TORC2 under conditions that disrupt the contact sites (95). Other investigators found that a small portion of TORC2 colocalized with MCC/eisosomes, suggesting there could be direct communi-

cation between these domains (97). It is also unclear what causes Slm1 to exit the eisosome. One study reported that reactive oxygen species generated as a consequence of inhibiting sphingolipid synthesis were primarily responsible for triggering the exit of Slm1 from the eisosome (98). It has also been proposed that increased membrane tension causes a change in membrane fluidity, which could be a key underlying factor for whether Slm1/2 and other proteins exit the MCC/eisosomes (21). Altogether, these results demonstrate that further work needs to be done to fully understand how Slm1/2 regulate TORC2. It will be important for future studies to determine which factors recruit Slm1/2 to the eisosomes, why they exit in response to stress, and how they lead to activation of TORC2.

(iii) Role of TORC2-Ypk1 in stress responses. The concept that a TORC2-mediated increase in sphingolipid synthesis could decrease membrane tension is supported by data showing that uptake of lipids relieves tension in mechanically stressed membranes (99). However, the model that TORC2 stimulates Ypk1 to phosphorylate Orm1/2 and derepress sphingolipid synthesis in order to decrease membrane tension has been questioned recently. In particular, questions have been raised about whether the timing of Orm1/2 phosphorylation would be rapid enough for new sphingolipid synthesis to help alleviate increased tension. For example, there was a 40-min delay before Orm2 phosphorylation was observed when membrane tension was increased by a shift to alkaline pH, which would presumably be too late to relieve a rapid change in membrane tension (21). In contrast, when membrane tension was rapidly increased by mechanical forces or osmotic shock, Slm1 exited eisosomes and Ypk1 became activated in a matter of seconds to minutes (23, 68) and returned to eisosomes within 2 min (23). Furthermore, myriocin treatment to inhibit sphingolipid synthesis did not stop membrane expansion in cells that were given a hypoosmotic shock, suggesting that a mechanism other than sphingolipid synthesis accounts for an initial increase in membrane area after hypoosmotic shock (21). In some cases, it is not clear that the kinetics of Ypk1 activation corresponds to Slm1/2 leaving eisosomes (23, 68). Perhaps Ypk1 works in other ways to counteract membrane tension in addition to Orm1/2 repression. Thus, further studies need to be done to determine how MCC/eisosomes relieve increased membrane tension caused by defects in lipid synthesis.

Osmotic stress. Rapid adaptation to changes in osmolarity is important for fungi, which may encounter wide swings in the environment. For example, *S. cerevisiae* can experience different levels of solute depending on whether the cells are living on ripe or decaying fruits, water, or insects. Since *S. cerevisiae* is nonmotile and does not have aerosolized spores, it must adapt to these changes to disseminate (100). Although the fungal cell wall offers some osmotic stress protection, it is not inflexible. Cell wall thickness changes rapidly when exposed to hyper- or hypoosmotic conditions independently of gene regulation (101, 102). Thus, cells maintain several mechanisms for dealing with osmotic stress, such as the high-osmolarity glycerol (HOG) pathway response (103, 104). Recent studies now indicate that MCC/eisosomes are involved in the response to changes in osmolarity by sensing the consequent changes in membrane tension.

The discovery of PM furrows first suggested that they might function as a reservoir of extra membrane to counteract stress caused by increased membrane tension (14). Studies on *S. pombe* spheroplasts showed that *pil1* Δ mutants were more sensitive to lysis under hypoosmotic conditions (90). However, because protoplasts were used, it was not clear if furrow flattening was an important factor in cells with intact walls, and experiments in *S. cerevisiae* did not find a difference in lysed cells when comparing WT to *pil1* Δ or *nce102* Δ cells (21). This group estimated that eisosomes only contain 2% of the plasma membrane surface area and are thus unable to account for the 12% increase in surface area observed after an increase in membrane tension (21). This contrasts with the reported ability of caveolae to act as membrane reservoirs; however, caveolae can form into very deeply invaginated rosette shapes that can provide more membrane (105). Rather than acting as membrane reservoirs, the evidence now indicates that MCC/eisosomes respond to changes in osmolarity by acting as sensors of membrane

tension, as described above for lipid stress. Riggi et al. used FLipTR (fluorescent lipid tension reporter) to demonstrate that membrane tension changes in *S. cerevisiae* after altering the osmotic environment (23). As expected, hypoosmotic shock increased membrane tension, Slm1 exited the MCC/eisosomes, and TORC2 was stimulated to phosphorylate Ypk1 (21, 23).

Hyperosmotic shock resulting in decreased membrane tension had the opposite effects in that Slm1 and Nce102 remained in eisosomes and there was a rapid and transient Ypk1 dephosphorylation, indicating inhibition of TORC2 (21, 23, 104). Inhibition of TORC2 is a logical response to membrane slack, as it would suppress new sphingolipid synthesis. An interesting effect of decreased tension due to hyperosmotic shock is that TORC2 clustered at abnormal membrane invaginations that were enriched in PI(4,5)P₂ (23). TORC2 was initially inactive at these PI(4,5)P₂ invagination sites, but its reactivation correlated with localization of Slm1 to these domains. Therefore, these data support the model that Slm1/2 activate TORC2 upon colocalization. Similar PI(4,5)P₂-rich invaginations were observed in *S. pombe* as a result of hyperosmotic stress (106). These abnormal invaginations required Pil1 for their formation, and they shared a boundary with eisosomes (106). It is significant that the membrane invaginations are enriched in PI(4,5)P₂, as this lipid can promote formation of curved membranes (107). However, more research needs to be done in this area, as, for example, changes in membrane tension also impact membrane fluidity, which can have broad effects on the organization of PM proteins and lipids.

Alkaline pH stress. MCC/eisosomes have recently been linked to how *S. cerevisiae* cells respond to a sudden change to alkaline pH in their environment (21, 54). This transition from acidic to neutral/alkaline ambient pH poses very big challenges. For example, mechanisms must be adjusted for nutrient uptake and acquisition of essential metals, such as iron. One well-studied pathway for responding to changes in ambient pH involves a protein sensor complex that localizes to punctate domains in the PM that are distinct from MCC/eisosomes (Fig. 2) (38). In *S. cerevisiae*, this pH sensor complex stimulates a pathway leading to proteolytic activation of the Rim101 transcription factor, which then induces the expression of genes needed to adapt to stress from an alkaline environment (38). Another major adjustment is that nutrient symporters, which localize to the MCC, are downregulated as proton import into the cell becomes an even bigger drain on the energy used by the Pma1 H⁺-ATPase. This section will focus on the effects of increased membrane tension caused by alkaline pH on MCC/eisosomes. The regulation of the nutrient symporters will then be described in more detail below.

Studies on the effects of ambient pH on the function of the Fur4 uracil symporter revealed that a sudden switch from pH 4 to pH 7.5 caused Fur4 to exit the MCC and become endocytosed (21, 54). This correlated with MCC/eisosome furrow flattening and exit of Slm1 and Nce102, leading to TORC2 activation. Analysis of the underlying mechanisms indicated that cells swelled by about half a micron, an estimated 12% greater surface area, indicating increased membrane tension. Cell swelling was thought to be caused in part by an imbalance in ion homeostasis (21, 54). The loss of the proton gradient during a shift to alkaline pH was predicted to impair the activity of the proton-driven Na⁺/K⁺ pump Nha1, and the increased demands for ATP by Pma1 were thought to divert ATP away from the Na⁺/K⁺ pump Ena1, causing water to flow into the cell and subsequent cell swelling (21). In addition, endocytosis of the large family of nutrient symporters that exit the MCC under these conditions also contributed to increased membrane tension, presumably by removing lipids from the PM at a time when increased membrane is needed (21).

Appadurai et al. predicted that eisosome furrow flattening might be due to a change in membrane lipid fluidity, rather than a mechanical pull on the membrane (21). To test this, they examined the effects of heat stress, which is known to increase fluidity. Interestingly, a rapid shift from 25°C to 35°C was associated with about an 80% loss of Nce102-containing MCC/eisosomes. This temperature effect could be counteracted by adding sorbitol to decrease membrane tension. This suggested that increased membrane fluidity, and consequent lipid mixing, could underlie the flattening of MCC/

eisosome furrows in response to a variety of different kinds of stress that cause increased tension and is an important area for future research.

Nutrient Stress

Overview. MCC/eisosomes play important roles in regulating nutrient uptake and the transitions that prepare cells to enter the later stages of growth and stationary phase. In particular, nutrient uptake is regulated by controlling the levels of the APC family of nutrient symporters, several of which have been shown to be enriched in MCC domains. This type of transporter relies on an external proton gradient to drive the cotransport of a proton and a substrate molecule across the PM (2, 19, 52). The function of the APC symporters therefore relies on the proton exporting Pma1 H⁺-ATPase, one of the largest consumers of ATP in the cell (19, 108). A mechanism for regulating the levels of a broad range of APC symporters occurs when increased membrane tension causes MCC/eisosomes to flatten, thereby enabling the transporters to exit to other regions in the PM where they can be endocytosed (21). This permits some of the energy used by Pma1 to be diverted to essential processes needed to counteract different kinds of stress. In addition to this type of global regulation, studies will be described that have shown how the levels of specific APC symporters can be fine-tuned to the appropriate level. This is important, as, for example, too much of an amino acid can be toxic, and the transporters can also allow leakage of substrates out of the cell (54, 109). Elegant recent studies that will be described in this section have shown how nutrient substrates promote a conformational change in their cognate transporters that causes them to exit the MCC, where they can then be ubiquitinated and then downregulated by endocytosis (21, 24, 54). In addition, the MCC/eisosomes have also been shown to undergo changes during late stages of growth that prepare cells for low-nutrient conditions and for the quick resumption of growth when nutrients return. This includes recruiting new proteins, such as a key regulator of mRNA turnover (Xrn1), the assembly of stress granules nearby, and an increase in furrow size and depth that has been suggested to stabilize additional nutrient transporters (24–26).

APC nutrient symporters. (i) Regulation of APC symporter levels in the absence of their nutrient substrate. The discovery that Can1 localized to MCC domains led to other types of transporters being tested, but it appears that localization to the MCC may be specific to a subset of the APC family of nutrient symporters. Transporters in the major facilitator superfamily (e.g., glucose transporters Hxt1 and Hxt3) and the proton exporter Pma1 H⁺-ATPase were not enriched in the MCC (7, 44). In contrast, localization to the MCC was detected for Tat2, Lyp1, Mup1, and Fur4, which are the proton symporters for tryptophan, lysine, methionine, and uracil, respectively (8, 49, 53, 54). It has therefore been proposed that many of the 26 APC symporters may localize to MCC/eisosomes (19, 52). The localization of Can1 to stable MCC domains and the increased turnover of Can1 in *pil1Δ* and *nce102Δ* *S. cerevisiae* mutants, which have impaired MCC/eisosomes, indicated that sequestration in these domains would protect against endocytosis (7, 34). A subsequent study challenged this because they observed that Can1 moves in and out of the MCC at a frequency they predicted would undercut any stabilizing role for MCC localization (110). However, other mathematical models support a role for the MCC in stabilizing APC symporters at the cell surface, and direct analysis of the stability of GFP-tagged symporters provided further support (21, 24, 35, 54).

Localization of APC symporters to the MCC is saturable, as evidenced in cells engineered to overproduce Can1 or Lyp1, indicating that one of the roles of MCC domains is to regulate the level of symporters at the cell surface (52). An interesting open question is whether all the APC symporters compete for a limited number of the same binding sites or if they have separate ways of being recruited to the MCC. Also, future studies should use prototrophic strains to be able to modulate the nutrient content of the medium, as it has been shown that the presence or absence of amino acids in the medium can have effects on symporter localization and on the morphology of the MCC/eisosomes (54).

FIG 5 Regulation of APC family nutrient symporters. Model of APC symporter regulation in MCC/eisosomes, which rely on a proton gradient across the PM to transport substrate into the cell. The symporters cluster to the upper region of the MCC/eisosome (in light blue) in an outward-facing (OF) conformation. The presence of substrate induces a transition to an inward-facing (IF) conformation, which enables the symporter to exit the MCC and exposes a site in the N-terminal tail that binds an arrestin-related trafficking adaptor (ART). The arrestin then recruits the Rps5 ubiquitin ligase that transfers ubiquitin onto lysine residues in the N-terminal tail of symporter, leading to endocytosis and degradation in the vacuole. This model was adapted from several studies, including the following: references 21, 24, 49, 54, 176, and 177.

A major advantage of localization of APC symporters to the MCC is that it provides a global way to coordinately regulate nutrient symport. The APC symporters are energy demanding in that they are one of the largest consumers of the proton gradient maintained by Pma1 (21, 35). Thus, stress conditions that increase membrane tension (described above) will stimulate APC symporters to exit the MCC and get endocytosed, thereby reducing the utilization of ATP by Pma1 for proton-pumping activity. The molecular mechanisms that promote exit from the MCC remain to be determined, but it likely occurs in a manner similar to that for triggering the exit of Nce102 and Slm1/2. Thus, the energy saved by downregulating APC symporters from the cell surface is advantageous in that it allows cells to redirect more energy to stress-response pathways (19, 21, 35).

(ii) Substrate-induced endocytosis of symporters. Levels of each individual symporter can also be regulated independently without globally affecting all members of the APC family. This occurs by a process in which the transport of the substrate also promotes increased endocytosis of the cognate transporter. An important advantage of this form of regulation is that it allows a rapid initial influx while preventing toxicity due to excessive nutrient uptake (109, 111–113). Recent studies on several different members of the APC symporter family are converging on a model that this selective endocytosis occurs because the symporters transition between two different conformations (Fig. 5). In the absence of substrate, symporters assume an outward-facing (OF) conformation, which allows proton and substrate binding in a pocket facing the extracellular space. This conformation is thought to have a higher affinity for localization to the MCC. The binding of substrate and a proton then induces a conformational switch to an inward-facing (IF) conformation, thereby transporting the cargo into the cytoplasm (19, 52). The change to the inward-facing conformation enables the symporter to exit the MCC. Substrate-induced exit from the MCC has been observed for several APC symporters, including Can1, Mup1, Lyp1, and Fur4 (21, 24, 35, 49). Studies with special mutant forms of Can1 have provided strong support for this model. For example, a mutated form of Can1 that is incapable of binding arginine does not leave the MCC/eisosome in the presence of this amino acid (24). An open question is, what causes the inward-facing conformation to exit the MCC? It has been suggested that the conformational change could disrupt interactions with binding partner(s), enabling diffusion from MCC/eisosomes (35, 49).

Further studies indicate that once outside the MCC, symporters in the inward-facing

conformation are modified by ubiquitination and then targeted for endocytosis (21, 24, 54). The transition to the inward conformation is thought to expose a region, typically in the cytoplasmic N terminus of the symporter, that contains a binding site for an α -arrestin, such as Art1 (19, 24, 52, 114). Arrestin binding then recruits the ubiquitin ligase Rsp5 that modifies lysine residues in the cytoplasmic tails of the symporters, leading to endocytosis (19, 24, 52, 114). Strong experimental support for this model comes in part from studies in which the arrestin binding site is mutated or there is a mutation of the ubiquitination sites, which leads to transporters that can exit the MCC in the presence of substrate but are not rapidly endocytosed. In addition, further support for this model comes from studies that leveraged sets of symporter mutants, such as special mutant forms of Can1 that are restricted to the outward-facing conformation that are not rapidly endocytosed even if they exit the MCC (24). Thus, the symporter must also undergo the conformational change to the inward-facing conformation that exposes new sites on the cytoplasmic side that lead to ubiquitination and endocytosis.

A novel PM compartment appears to be involved in symporter endocytosis. Recent studies indicate that the inward-facing conformation of Mup1 exits the MCC and then enters into a PM compartment that is distinct from the MCP (Pma1-containing domain), where it is ubiquitinated and subsequently stimulates the formation of a new endocytic site (35). Other studies have examined why symporters localized in the MCC are not ubiquitinated and targeted for endocytosis. Earlier studies suggested that the MCC/eisosome lipid composition may stabilize the symporters in an inactive state. However, Can1 and Fur4 are able to transport when restrained in the MCC, indicating that they can achieve the inward-facing conformation. Although there are conflicting data as to whether the different APC symporters transport more efficiently when they are in or out of the MCC, any differences appear to be minor compared to their overall ability to function efficiently in the MCC (21, 24, 35, 54). It was subsequently proposed that localization to MCC/eisosomes prevents Art1/Rsp5 from gaining access to symporters since Can1 was not ubiquitinated when restrained in the MCC (24). This does not simply appear to be due to the symporters being masked by the Pil1 eisosome protein complex at the base of the furrows, as superresolution microscopy assays have detected symporters closer to the surface of the MCC/eisosome (21, 49). However, additional mechanisms must also prevent endocytosis from occurring in the MCC, as a special Mup1-ubiquitin fusion protein, which is usually endocytosed rapidly, was stable in the MCC (35). This indicates that bypassing Art1/Rsp5 by fusing ubiquitin directly onto the symporter is still not sufficient, consistent with many previous failures to detect endocytosis at MCC/eisosomes (8, 9, 34, 59, 115).

Late stages of growth and stationary phase. Recent studies are beginning to reveal important roles for MCC/eisosomes in response to stress caused by nutrient limitation. This role of MCC/eisosomes in nutrient stress has largely been overlooked because most studies have focused on cells in the log phase of growth. One new role involves recruitment to the eisosome of Xrn1, a 5' to 3' exonuclease that is one of the main factors that promotes mRNA decay (26). Xrn1 is present in P bodies in log-phase cells and in eIF3a/Rpg1-containing stress granules under heat stress, which are both sites of mRNA degradation (26). In contrast, as glucose is depleted and cells approach the diauxic shift, Xrn1 relocates to the eisosome, which coincides with a reduced rate of mRNA degradation (69). Other components of the mRNA decay machinery were not detected in eisosomes in postdiauxic-phase cells. After restoration of fermentable nutrients, Xrn1 exited the eisosome. This provides a regulatory mechanism in which mRNA degradation components can be kept spatially separated to slow mRNA degradation, but they can be ready for immediate use when nutritional conditions are appropriate (26, 69).

MCC/eisosomes have also been linked to the assembly of stress granules, which are cytoplasmic complexes of proteins and untranslated mRNA that form under conditions of nutrient limitation (25). Glucose starvation induced a Pkc1-dependent clustering of MCC/eisosomes that was associated with the formation of stress granules adjacent to

the PM furrows. The authors of this study proposed a model in which Pkc1 induces a feed-forward loop that promotes MCC/eisosome clustering, assembly of stress granules, and then storage of Pkc1 in those stress granules (25). Stress granules enable a faster return to cell growth when nutrients are restored. MCC/eisosomes play an important role, as deletion of *PIL1* impaired stress granule formation and delayed resumption of growth after nutrients were restored (64). This novel clustering of MCC/eisosomes was associated with phosphorylation of Pil1 on Ser-163 and Ser-230, although it is not clear if Pkc1 plays a direct or indirect role. However, phosphorylation of Pil1 was concluded to be important, as a phosphomimetic Pil1-S230D mutant promoted clustering and stress granule formation, whereas a nonphosphorylated Pil1-S230A mutant was defective (25). These results are also interesting because phosphorylation of Ser-230 has been linked to a very different effect of promoting eisosome disassembly under log-phase growth conditions (64). Thus, these results are significant for identifying an important role for MCC/eisosomes in stress granule formation and raise interesting questions about new mechanisms for MCC/eisosome regulation under nutrient stress.

Nutrient limitation has also been shown to alter MCC/eisosome structure to provide a greater reserve of APC symporters (24). Cells in the stationary phase showed an increased number of MCC/eisosomes as well as an increase in their length and depth (24). Although more needs to be learned about the mechanisms underlying these alterations, it is very interesting that these changes were dependent on Lsp1, which is not critical for the MCC/eisosome structure during the log phase (24). The increased number of MCC/eisosomes coupled with longer and deeper furrows appears to play a role in protecting more APC symporters from endocytosis stimulated by TORC1 inhibition that occurs under conditions of nutrient depletion (21, 24, 116). Altogether, several emerging lines of study indicate that MCC/eisosomes play multiple roles in coordinating cellular responses to nutrient limitation.

PM Organization and Morphogenesis

Overview. PM organization underlies the ability of cells to properly synthesize the cell wall, which is important for protecting against stress and in shaping cell morphogenesis. MCC/eisosomes impact cell wall and PM organization in ways that are not just intrinsic to the membrane furrows; they also affect components outside the furrows. Although there are many ways in which MCC/eisosomes can influence different PM functions, such as through Pkh1/2 and TORC2, this section will focus on two recently discovered roles for MCC/eisosomes. The subsection "Cell wall stress and morphogenesis" will describe the role of MCC/eisosomes in regulating $PI(4,5)P_2$, which is under dynamic control by kinases and phosphatases that convert it to $PI4P$ and back again (117, 118). $PI(4,5)P_2$ is present at low levels in cells, but it plays a central role in recruiting to the PM the components that regulate cell wall synthesis and morphogenesis (119, 120). This section will also include studies on the human fungal pathogen *Candida albicans*, which have revealed roles of MCC/eisosomes in cell wall synthesis and morphogenesis that are needed for invasive growth and virulence (50, 51, 73). The next section will describe new results showing that direct contact sites form between the cER and eisosomes in *S. pombe* that mediate proper PM organization and are likely important for lipid homeostasis (121). MCC/eisosomes also contribute to PM organization and cell wall morphogenesis in several other fungi that will be described below in a section on the diversity of MCC/eisosomes in different fungi.

Cell wall stress and morphogenesis. Studies with *S. cerevisiae*, *S. pombe*, and *C. albicans* are converging on a model in which the regulation of $PI(4,5)P_2$ by MCC/eisosomes promotes proper morphogenesis and resistance to cell wall stress. In *S. cerevisiae*, *pil1* Δ mutants display abnormal inward growth of the cell wall, and morphological defects are detected for other MCC/eisosome mutants (9, 10, 61). The role of eisosomes in regulating $PI(4,5)P_2$ was analyzed because Pil1 binds to this lipid (18, 62) and because $PI(4,5)P_2$ recruits to the PM the proteins needed for a variety of functions, including cell wall morphogenesis, secretion, and endocytosis (117, 122, 123). It is also

interesting that other studies showed that mutating three 5' PI(4,5)P₂ phosphatases in *S. cerevisiae* (encoded by *INP51/SJL1*, *INP52/SJL2*, and *INP53/SJL3*), which raises the level of PI(4,5)P₂, mirrors the abnormal inward cell wall growth seen in some *S. cerevisiae* eisosome mutants (124). Genetic analysis of the three PI(4,5)P₂ phosphatases indicated that *INP51* has the closest functional relationship with *PIL1* (27). Further studies suggested that there was a direct relationship, as Inp51-GFP was detected at eisosomes and physically interacted with eisosome proteins (27, 86). A *pil1Δ* mutant was shown to have elevated levels of PI(4,5)P₂ in the PM, similar to an *inp51Δ* mutant (27). Pil1 and Lsp1 were concluded to play two roles in regulating PI(4,5)P₂, one in recruiting Inp51 to the PM and another role in acting as a buffer by binding thousands of copies of PI(4,5)P₂ (27).

Independent studies with *S. pombe* also identified a role for MCC/eisosomes in the regulation of PI(4,5)P₂. Genetic screens to identify the functional roles of eisosomes discovered that Pil1 acts in a pathway with the 5' PI(4,5)P₂ phosphatase Syj1 (*S. cerevisiae* Inp51) (62). Syj1 localized primarily in the cytoplasm, but some puncta were detected at the PM that might localize with eisosomes. Interestingly, further genetic studies revealed a Pil1-Sle1-Syj1-Tax4 pathway for the regulation of PI(4,5)P₂ that also intersected with the regulation of TORC2 (62).

The role of MCC/eisosomes in cell wall morphogenesis has been studied in more detail in the fungal pathogen *C. albicans* because these domains are needed for invasive growth, biofilm formation, and virulence (50, 51, 125). The stronger morphological phenotypes of *sur7Δ* and *pil1Δ lsp1Δ* mutants in *C. albicans* than in *S. cerevisiae* has helped to uncover novel aspects of MCC/eisosome function (50, 51, 73, 126–128). *C. albicans sur7Δ* and *pil1Δ lsp1Δ* mutants display similar morphological abnormalities during budding, such as formation of extremely large mother cells, and have defects in undergoing highly polarized growth to form hyphae (50, 51, 73, 127). They both form extensive invaginations of cell wall growth, including distinctive tubes of the cell wall (50, 73). Similar cell wall invaginations were seen in an *inp51Δ* mutant (129), which suggested that abnormal regulation of PI(4,5)P₂ underlies the altered cell wall growth in *C. albicans* MCC/eisosome mutants. Subsequent studies with a PH domain probe revealed that *sur7Δ* and *pil1Δ lsp1Δ* mutants contained abnormal patches of PI(4,5)P₂ at the sites of cell wall invaginations, as well as abnormal recruitment of septin proteins, which are organizing centers for cell wall synthesis (73). Interestingly, Sur7 overproduction rescued the defects of a *pil1Δ lsp1Δ* mutant, indicating that one key function of the eisosome furrows is to stabilize the tetraspan protein Sur7 in the PM so that it can regulate PI(4,5)P₂ (73). Thus, there appear to be conserved roles for MCC/eisosomes in regulating PI(4,5)P₂, but it will be important to determine whether the molecular mechanisms for PI(4,5)P₂ regulation are the same in *S. cerevisiae*, *S. pombe*, and *C. albicans* or if the MCC/eisosomes in these species have specialized in different ways to regulate PI(4,5)P₂.

C. albicans sur7Δ and *pil1Δ lsp1Δ* mutants are more sensitive to a variety of factors that cause cell wall stress (e.g., calcofluor white), antifungal drugs that impair PM lipids (e.g., fluconazole), the protein kinase C inhibitor cercosporamide, and elevated temperature (73, 127). These mutants are also more sensitive to other stress conditions that will be described below, such as oxidation and copper toxicity (29). Sur7-GFP was found to localize to sites of cell wall damage caused by neutrophils, which suggests MCC/eisosomes protect against cell wall stress *in vivo* (130). These results suggest that drugs targeting MCC/eisosome components may represent novel antifungal therapeutic strategies for blocking the virulence of fungal pathogens or enhancing the effectiveness of current antifungal drugs.

Although this section has focused on the roles of MCC/eisosomes in regulating PI(4,5)P₂, it is also important to note that MCC/eisosomes can impact morphogenesis in other ways that need to be investigated. For example, Pkh1/2, Slm1/2, and the TORC2 pathway influence actin organization, and Pkh1 is also known to regulate Pkc1, which plays a key role in responding to cell wall stress (37, 131–133). The convergence of

these signaling pathways on morphogenesis is not fully elucidated and offers many interesting areas for further research.

cER-PM contacts during stress. Another key regulator of PI(4,5)P₂ levels is the cortical ER (cER) (134–136), a specific subdomain of the ER that was recently shown to physically interact with MCC/eisosomes in *S. pombe* (121). The cER consists of a network of cisternae and tubules that is located 30 to 50 nm away from the PM (121, 137). This proximity allows for the formation of direct cER-PM contact sites that are protein organized and mediate lipid transfer between these domains, including PI4P and sterols (138–140). Two of the key proteins involved in these cER-PM contacts are the vesicle-associated membrane protein-associated-protein (VAP) homologs Scs2 and Scs22 (141, 142). The cER is also very dynamic and can cover ~65% of the PM at one time, so it is thought to have a major influence on PM function, as it can block the formation of domains needed for endocytosis, secretion, cell signaling, and other events (138, 143, 144). Interestingly, studies of *S. cerevisiae* showed that the cER appears to preferentially localize adjacent to or away from MCC/eisosomes but not underneath the furrows (13, 138). In *S. pombe*, a species in which the cER and MCC/eisosomes are reported to have a coupled distribution, only a small fraction of the cER was located beneath the furrows (121). These findings suggest that the eisosome furrows could provide more consistent access from the PM to the cytoplasm, enabling eisosome proteins, such as the Pkh1/2 kinases, to have an open avenue for regulating cytoplasmic target proteins.

MCC/eisosomes are important for normal cER structure and function in *S. cerevisiae*, as they regulate cER distribution and fragmentation (138). Interestingly, a recent study with *S. pombe* showed that there was a direct interaction between Pil1 and the cER-tethering protein Scs2 that stabilized cER-PM contacts and restricted cER remodeling (121). Consistent with a stabilizing interaction, the disassembly of eisosomes and furrow flattening due to increased PM tension in *S. pombe* was followed by remodeling of the cER and a decrease in cER-PM contacts (121). Disrupting these cER-eisosome contacts is predicted to alter lipid homeostasis and other cellular processes. For example, it would be interesting to know how this affects the ability of the cER to control PM lipid homeostasis during membrane stress, including the ability to regulate PI4P levels, as previous studies have shown that removal of the VAP proteins in *S. pombe* resulted in increased levels of PI4P in the PM (121, 144). Altering PI4P levels would likely influence PI(4,5)P₂ levels, which could contribute to some of the morphogenesis defects described in previous sections. In addition, since another study found that TORC2 could be activated by removal of the Ltc3/4 sterol transporters, which are located in cER-PM contact domains known as the MCL (membrane compartment of Ltc4), disrupting the cER-eisosome contacts could contribute to the signaling pathways activated by increased PM tension (95). These novel roles of cER-eisosome contact sites will be an interesting area of future study (121).

Environmental Stress

Overview. This section will focus on recent results demonstrating new ways that MCC/eisosomes promote resistance to oxidation and copper toxicity. The roles of MCC/eisosomes in resisting oxidation and copper have been studied primarily in *C. albicans*, as this human pathogen has to resist these and other types of stress to grow in the host and avoid attack by the immune system (12). In fact, these two types of stress can act synergistically at some sites in the body, as it has been shown that copper is pumped into macrophage phagosomes, where it can react with H₂O₂ formed by the oxidative burst to generate a broader range of toxic reactive oxygen species (ROS) to kill pathogens (145). These studies have provided some of the first insights into the physiological roles for a family of proteins known as flavodoxin-like proteins (FLPs) that localize to eisosomes and are widely conserved from bacteria to fungi (28). Studies on the increased sensitivity of *C. albicans* *sur7*Δ and *pil1*Δ *lsp1*Δ mutants to copper have identified a novel role for MCC/eisosomes in maintaining lipid asymmetry in the PM. Phosphatidylserine that was abnormally exposed in the outer leaflet of the PM of these

MCC/eisosome mutants was concluded to react with copper to damage PM integrity, which represents a novel mechanism for cell killing by copper (29).

Oxidative stress. The ability to resist oxidative stress is important to prevent lethal damage to DNA, proteins, and lipids (146). Interestingly, MCC/eisosomes have been implicated in resistance to oxidative stress in several different fungi (29, 147–149). In particular, a family of three proteins known as flavodoxin-like proteins (FLPs) (Rfs1, Pst2, and Ycp4) are of particular interest because they localize to *S. cerevisiae* eisosomes and have been implicated in the oxidative stress response (34, 150–152). Functional analysis of Pst2 and the determination of its crystal structure support the conclusion that it, like other FLPs, acts as a NAD(P)H quinone oxidoreductase (153). These oxidoreductases play important roles in detoxifying quinones by avoiding the formation of toxic semiquinone intermediates, which are themselves ROS (154–158).

Resisting oxidative stress is particularly important for human fungal pathogens like *C. albicans* to counteract the oxidative burst generated by innate immune cells (i.e., macrophages and neutrophils). Studies in *C. albicans* demonstrated that four FLPs (Pst1, Pst2, Pst3, and Ycp4) that localize to eisosomes are needed to combat oxidative stress, as a mutant lacking all four proteins showed increased sensitivity to small-molecule quinones such as benzoquinone and menadione (28). Interestingly, the *pst1Δ pst2Δ pst3Δ ycp4Δ* quadruple mutant was also more susceptible to killing by H₂O₂ and other types of oxidants. Of note, this mutant was more sensitive to lipid peroxidation, which draws attention to the fact that *C. albicans* contains roughly 30% polyunsaturated fatty acids, while *S. cerevisiae* has little to none (159, 160). Polyunsaturated fatty acids are more sensitive to lipid peroxidation than mono- or unsaturated lipids and are a serious threat to cells, as an initial lipid peroxidation event can start a chain reaction that propagates the damage to other lipids (154, 155). Significantly, polyunsaturated fatty acids are often found at sites of membrane curvature where they facilitate membrane deformations, suggesting a rationale for why the FLPs are enriched at membrane furrows (161). Since ubiquinone is present in other membranes in addition to the mitochondria, these studies led to a model proposing that FLPs reduce ubiquinone to its quinol form so that it can be reused to detoxify radicals and prevent oxidative damage in the PM (28). The significant role FLPs play in resisting oxidative stress is highlighted by the fact that the *pst1Δ pst2Δ pst3Δ ycp4Δ* mutant was avirulent in a mouse model of infection (28). In contrast, the well-known antioxidant protein catalase is not essential for virulence (162), indicating that the FLPs play a key role in protecting the PM from oxidation during infection.

Copper toxicity. A *C. albicans* *sur7Δ* mutant was defective in growing in macrophage phagosomes under conditions in which wild-type cells readily grew and escaped (51). Screening the *sur7Δ* mutant for susceptibility to different phagosomal conditions revealed that it was >100-fold more sensitive to killing by copper (51). Copper is an essential cofactor of many enzymes; however, it is toxic in high levels because it promotes redox reactions due to its ability to cycle between cuprous (Cu⁺) and cupric (Cu²⁺) states (163). Control studies showed that Sur7 acts independently of the known copper resistance pathways, including the Crp1 copper exporter and the Cup1 metallothionein, which appeared to function normally in the *sur7Δ* strain (29, 51). A *pil1Δ lsp1Δ* mutant strain was also much more susceptible to copper (29), but the furrows were not sufficient to promote copper resistance, as freeze-etch EM showed that the *sur7Δ* strain still developed PM furrows (73). Interestingly, the overexpression of *SUR7* in the *pil1Δ lsp1Δ* mutant rescued the copper-sensitive phenotype (29). This indicated that Sur7 plays the key role in copper resistance and that Pil1 and Lsp1 are needed to form furrows that stabilize Sur7 in the PM (29).

Further studies indicated that *C. albicans* MCC/eisosomes are needed for normal phospholipid asymmetry in the PM, which provides important protection against permeabilization by copper (29). Copper has been reported to damage membranes by forming pores, but the mechanism has been unknown (164, 165). Interestingly, *sur7Δ* cells exposed phosphatidylserine in the outer leaflet of the PM, as indicated by increased sensitivity to papuamide, an antibiotic that binds to phosphatidylserine in

FIG 6 PM Furrow shape varies across fungal species. (A) *S. cerevisiae* furrows are short and punctate and show random orientation. Scale bar represents 100 nm, the arrow indicates diagonal striations, and the asterisk indicates structure that is thought to correspond to a cluster of Pma1 proteins (170). (B) Short, punctate furrows from unidentified species of bread mold. Scale bar represents 100 nm; the arrow points to cell wall remnants. (C) Long furrows in *S. pombe*. Scale bar represents 100 nm. The arrow points to diagonal striations, and an asterisk indicates an anastomosing furrow with reduced depth. (D) Long, anastomosing furrows in *C. neoformans*. Scale bar represents 50 nm. (E) Highly dense, wavy furrows in lichenized *Candelaria concolor*. Scale bar represents 500 nm. (All images are reproduced from reference 170 with permission.)

membranes (166). This most likely indicates that *sur7* Δ cells have a defect in one or more of the phospholipid flippases that maintain lipid asymmetry by facilitating movement of phospholipids from the outer to the inner leaflet of the bilayer (29). Importantly, this suggested a mechanism for permeabilization, as previous reports indicated that copper binds phosphatidylserine with very high picomolar affinity and promotes lipid oxidation (167–169). In support of this model, deletion of *CHO1*, the phosphatidylserine synthase, rescued the copper-sensitive phenotype of *sur7* Δ cells (29). These findings led to a model wherein deletion of *SUR7* impaired phospholipid flippase activity, resulting in abnormal presentation of phosphatidylserine in the outer leaflet, where it could react with copper, leading to loss of PM integrity.

DIVERSITY OF MCC/EISOSOME STRUCTURE AND FUNCTION IN FUNGI

A landmark study by Goodenough and colleagues revealed an amazing diversity in the size and shape of PM furrows found in different fungi and algae (Fig. 6) (170). Although the width of the furrows is typically ~ 50 nm, the length is quite variable. For example, while *S. cerevisiae* and *C. albicans* form small, straight furrows that are about 200 to 300 nm in length, longer furrows are found in *S. pombe* and *Cryptococcus neoformans*, with those in *S. pombe* appearing up to 5 times longer than those in *S. cerevisiae* (77, 170, 171). Not all furrows are straight, as some species form wavy furrows, and others form

anastomosing connections between furrows (Fig. 6) (170). The short furrows appear to be oriented randomly, but the longer furrows can be either random or organized in rows. Further studies will be needed to identify the full range of furrow structures.

Although PM furrows have been detected in all fungal species that have been examined, the proteins that localize to *S. cerevisiae* MCC/eisosomes are not highly conserved. It is interesting that although *S. pombe* and *S. cerevisiae* are both *Ascomycetes*, *S. pombe* appears to have simplified MCC/eisosomes that contain fewer proteins (77). For example, *S. pombe* appears to lack a true Sur7 ortholog, and the closest homolog of Sur7, as well as Slm1, localizes to the growing tips of the cells, not to MCC/eisosomes (77). Even when orthologous proteins are present in different species, they do not always function the same. For example, Sur7 function is more important in *C. albicans* than in *S. cerevisiae* (50), whereas the opposite is true for Slm1/2 (73). In contrast to the essential roles of Slm1/2 in *S. cerevisiae*, the only *C. albicans* ortholog of Slm1/2 does not appear have a significant function.

In spite of a lack of conservation in the associated proteins, mutational analyses are beginning to reveal that MCC/eisosomes have similar functions in different species. The most highly conserved function appears to be in promoting proper morphogenesis and cell wall integrity. In addition to the defects described above for *S. cerevisiae* and *C. albicans*, cell wall and morphogenesis defects have been reported for MCC/eisosome mutants in *S. pombe* (77), *Ashbya gossypii* (74), *Beauveria bassiana* (149), and *Alternaria brassicicola* (172). Interestingly, MCC/eisosomes were prominent in conidia and spores from *Aspergillus fumigatus* and *Aspergillus nidulans*, suggesting a link to stress resistance (85, 173). MCC/eisosomes in several species have also been found to promote resistance to oxidation, as described above for *S. cerevisiae* and *C. albicans* (148, 149, 174). Consistent with these functional roles, pathogenic fungi carrying mutations in MCC/eisosome proteins have virulence defects in plants (172), insects (149), and humans (28, 51). These differences indicate that although there is broad diversity in the proteins that comprise MCC/eisosomes, there are underlying themes in the function of these domains. Thus, future studies will benefit from the insights that will come from comparative analysis of MCC/eisosomes in different fungi and algae.

CONCLUDING REMARKS

Initial studies of MCC/eisosomes focused primarily on defining their structure and protein composition. The functions of MCC/eisosomes were not always evident under ideal log-phase conditions where these domains appeared to be static. However, that view is now changing, as new studies are revealing that MCC/eisosomes respond dynamically to stress by changing furrow depth and allowing different proteins to exit or enter. It will be interesting in future studies to explore how MCC/eisosomes can act as organizational hubs to cross-coordinate appropriate responses to changes in membrane tension, nutrient regulation, cell wall integrity, oxidation, and other stressful conditions. A better understanding of MCC/eisosome functions in responding to stress will have practical impacts for areas such as industrial-scale fermentation and biotechnology. In addition, developing strategies for disrupting MCC/eisosome function may open up novel approaches for antifungal agents for human, animal, and plant fungal pathogens. The need for new antifungal strategies was highlighted at a recent colloquium convened by the American Academy of Microbiology, which focused on the growing impact of fungi on human health, food security, and biodiversity (175).

Outstanding Questions

- A high-resolution structure of MCC/eisosomes is needed to develop new testable models for MCC/eisosome function.
- How do MCC/eisosomes assemble? Although there are some general ideas, the molecular mechanisms are still poorly defined.

- How do specific proteins localize to MCC/eisosomes? Is it based solely on interactions with other proteins, or are specific lipids important too?
- What controls eisosome disassembly? This includes global MCC/eisosome disassembly in response to stress and the selective disassembly of a subset of MCC/eisosomes at sites of bud initiation in *S. cerevisiae* or septation in *S. pombe*.
- Are there other proteins recruited to MCC/eisosomes that are needed to adapt to different growth and stress conditions?
- What causes MCC/eisosome furrow flattening due to increased membrane tension? Is it the exit of Nce102, mechanical stress, increased membrane fluidity, other factors, or all of them acting together?
- How do MCC/eisosomes activate TORC2, and does Ypk1 have broader roles in adaptation to stress than just the phosphorylation of Orm1/2 to derepress sphingolipid synthesis?
- Do the ~26 members of the APC symporter family cross-regulate their functions? How do they alter MCC/eisosome shape and function?
- Why does the length and shape of PM furrows vary so much between different fungal species? Are there functional implications for the different shapes?

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