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Review

Hans Merzendorfer* and Jürgen J. Heinisch*

Microcompartments within the yeast plasma membrane

Abstract: Recent research in cell biology makes it increasingly clear that the classical concept of compartmentation of eukaryotic cells into different organelles performing distinct functions has to be extended by microcompartmentation, i.e., the dynamic interaction of proteins, sugars, and lipids at a suborganellar level, which contributes significantly to a proper physiology. As different membrane compartments (MCs) have been described in the yeast plasma membrane, such as those defined by Can1 and Pma1 (MCCs and MCPs), *Saccharomyces cerevisiae* can serve as a model organism, which is amenable to genetic, biochemical, and microscopic studies. In this review, we compare the specialized microcompartment of the yeast bud neck with other plasma membrane substructures, focusing on eisosomes, cell wall integrity-sensing units, and chitin-synthesizing complexes. Together, they ensure a proper cell division at the end of mitosis, an intricately regulated process, which is essential for the survival and proliferation not only of fungal, but of all eukaryotic cells.

Keywords: bud neck; cell wall; chitin synthesis; CWI sensors; cytokinesis; eisosomes.

***Corresponding authors: Hans Merzendorfer,** Department of Biology/Chemistry, University of Osnabrück, Animal Physiology, Barbarastr. 11, 49076 Osnabrück, Germany; and **Jürgen J. Heinisch,** Department of Biology/Chemistry, University of Osnabrück, Genetics, Barbarastr. 11, 49076 Osnabrück, Germany, e-mail: merzendorfer@biologie.uni-osnabrueck.de; heinisch@biologie.uni-osnabrueck.de

Introduction

A key achievement in the evolution of eukaryotic cells is the compartmentation of cellular functions within membrane-enclosed organelles of characteristic shapes. Examples of such functions are the energy conversion in the mitochondria or chloroplasts, bulk protein degradation in the lysosomes or vacuoles, protein transport in the endoplasmic reticulum (ER) and the Golgi network, and the maintenance and expression of genetic information in the nucleus, the defining feature of the eukaryotic cells (see Shibata et al., 2009; Wilson and Dawson, 2011, and references therein). Although providing a defined local environment that is protected from potentially interfering substances, the disadvantage of such an organization is the limited availability of reaction partners, which requires their transport across lipid bilayers. The other extreme for physiological processes is the diffusion-controlled recruitment of reaction partners from a pool within the cytoplasm or an organelle. In recent years, it is becoming increasingly evident that cells have developed yet another strategy to allow for a fast adaptation to changing physiological needs: the formation of suborganellar, highly dynamic, yet defined functional units, which are introduced in this review series as microcompartments. Thus, structures like the nuclear bodies involved in different mechanisms of gene expression control (Mao et al., 2011) or cytoplasmic stress granules and P-bodies for mRNA storage and processing (Buchan et al., 2011) can easily acquire and release components in response to extra- and intracellular signals. This provides a means to rapidly increase the local concentrations of reaction partners and to disassemble as rapidly, if their functions are no longer required. Such functionally specialized microcompartments cannot only be formed in the soluble pool of cellular components but also within membranes. A prominent topic is the ongoing debate on the role of 'lipid rafts' in the concentration of signal transduction components within and at the plasma membrane (reviewed in Simons and Gerl, 2010). Examples for which biological roles have been established include the signaling in the immune response (Chichili and Rodgers, 2009), the formation of caveolae in integrin signaling (Singh et al., 2010), and also the polar accumulation of chemotaxis sensors in bacteria (reviewed in Kirby, 2009).

Also in this respect, the yeast *Saccharomyces cerevisiae* has proven its value in studying the basic cellular functions in eukaryotes. Thus, the lateral compartmentalization of proteins within the plasma membrane can be readily observed (Malinska et al., 2003). Using

the 'awesome power of yeast genetics' (Forsburg, 2001), in combination with biochemical and biophysical approaches, the underlying molecular mechanisms in the formation and physiological role of these rafts are being studied. These data have been reviewed recently (Malinsky et al., 2010) and will be updated and discussed here briefly. In subsequent parts of this review, we will focus on two examples of specialized yeast plasma membrane microcompartments, namely, the clustering of cell wall integrity sensors and the role of the yeast bud neck during cytokinesis, culminating in actomyosin ring (AMR) constriction and the formation of the chitinous primary septum.

Microcompartments in and beneath the yeast plasma membrane

Despite the ongoing discussion on the existence and relevance of the lipid rafts in higher eukaryotes, it is generally agreed that at least a functional compartmentation in the plasma membranes exists, which ensures that different signaling processes and biochemical reactions occur at the right place and time. As in other fundamental biological processes, the yeast *S. cerevisiae* turned out to be a valuable model to study such lateral compartmentation.

Plasma membrane compartments

The mammalian lipid rafts are thought of as small, freely diffusing, highly dynamic membrane patches being 20–80 nm in diameter (Simons and Gerl, 2010), whereas the yeast disposes of two defined and stable plasma membrane compartments (Figure 1) (reviewed in Malinsky et al., 2010):

1. In early works, a punctuated distribution was observed for GFP fusions to the arginine permease encoded by *CAN1*. Accordingly, these patches were named MCC, for membrane compartment of Can1, with an appearance of 50–80 dots per cell and a diameter of approximately 300 nm. These microcompartments also house two other proton-dependent permeases for uracil and tryptophan, Fur4 and Tat2, respectively, as well as four proteins of the Sur7 family (Young et al., 2002; Malinska et al., 2004). The MCCs are associated with the eisosomes (discussed below) at the inner leaflet of ergosterol-enriched regions of the plasma membrane, which invaginate in furrow-like structures (Grossmann et al., 2008; Stradalova et al., 2009). It has been speculated that the transmembrane protein Nce102, which also resides in the MCCs, functions as a sensor to link the membrane's sphingolipid content with the formation of the eisosomes (Fröhlich et al., 2009; see below for more details on the eisosomes).

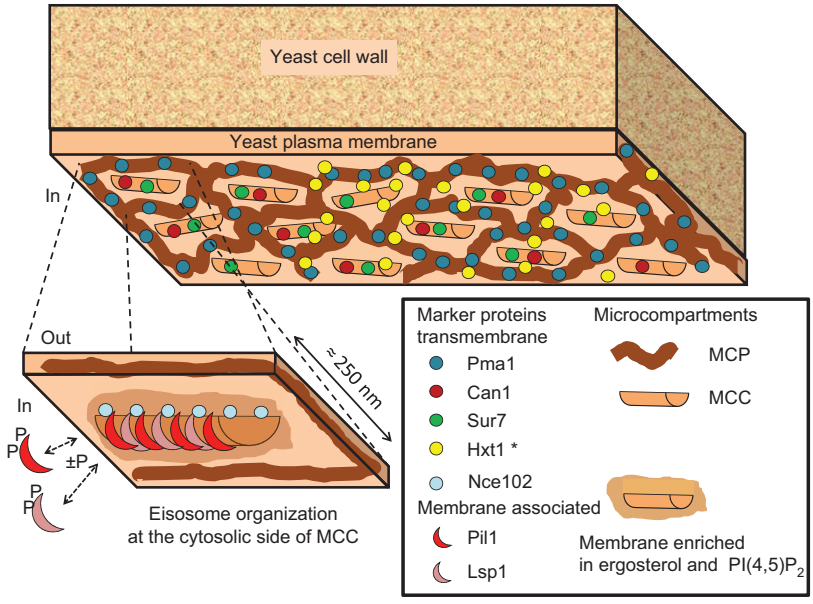

Figure 1 Organization of the yeast plasma membrane and its associated structures (cell wall and eisosomes).
MCP, membrane compartment of Pma1; MCC, membrane compartment of Can1. Colored dots represent transmembrane proteins and their distribution. *Note that a recent study places Hxt1 primarily into the MCP compartment (Spira et al., 2012). See text for further details.

2. The proteins marking the MCC patches are mutually exclusive with the surrounding network, defined by the presence of the plasma membrane ATPase Pma1. Consequently, this network was termed MCP, for membrane compartment of Pma1 (Malinska et al., 2003).
3. In addition, more dynamic punctuate structures with somewhat smaller diameters than the MCCs have been detected, which localize to the regions that are different from both the MCCs and the MCPs and are defined by the presence of the TORC2 complex (hence the name MCT, for membrane compartment of TORC2; Berchtold and Walther, 2009). It should be noted that besides these characteristic determinants, other membrane proteins may be more uniformly distributed within the plasma membrane, such as the hexose transporter Hxt1 or the general amino acid permease Gap1 (Malinsky et al., 2010). However, the latter distribution has been doubted in a recent publication, in which the distribution of 46 different plasma membrane proteins has been studied with fluorescence tags at high resolution (Spira et al., 2012). The authors come to several important conclusions: i) apart from the MCC compartment, which is clearly organized in patches, they find different proteins distributed in a gradient from more patch-like to more network-like organizations. Even within the latter, different proteins are localized into different subdomains. ii) The lipid composition within the plasma membrane, in conjunction with the transmembrane domain sequence of the proteins, is a major determinant of microcompartmentation, i.e., the nonrandom distribution of the plasma membrane proteins in yeast. iii) According to the kinetics of the proteins within their subdomains, neither secretion nor endocytosis seems to contribute significantly to the patchwork organization. iv) The analyses of Can1 in mutants, changing its membrane distribution, provided a first hint that the localization in the right microdomain is of functional importance, as the resistance levels to canavanine were significantly altered by mislocalization.

Eisosomes

A physiological role for the plasma membrane microcompartments seemed to emerge when a colocalization of the MCC marker protein Sur7 with one of the main proteins of the eisosomes, Pil1, was detected (Figure 1) (Grossmann et al., 2008). The eisosomes were then speculated to be static complexes regulating endocytic events (Walther et al., 2006; Karotki et al., 2011). They were first found in *S. cerevisiae* and later on also in other ascomycetous fungi. The eisosomes consist of some 13 proteins, with the closely related Pil1 and Lsp1 as their major constituent components. The latter are among the most abundant cytosolic proteins (with approximately 100 000 molecules of each per cell) and form furrow-like invaginations of the yeast plasma membrane harboring the nine marker proteins of the MCC, which, oddly enough, are especially resistant to endocytosis (Grossmann et al., 2008). Consistent with this observation, the position of the eisosomes in *Ashbya gossypii*, a filamentous fungus closely related to *S. cerevisiae*, did not coincide with the bulk of the endocytic traffic at the hyphal tip, but they rather accumulated in the subapical tip regions (Seger et al., 2011). *In vivo* photobleaching experiments in *S. cerevisiae* suggest that the organization of the MCC compartments within the yeast plasma membrane has no influence on the intracellular vesicle trafficking, also opposing a function in endocytosis (Brach et al., 2011). In fact, both endocytosis and exocytosis were found to be restricted to the MCP microcompartment, independent of the position of the neighboring MCC patches. Further work by Karotki et al. (2011) demonstrated that both Pil1 and Lsp1 can self-assemble *in vitro* into tubules, which in their ultrastructure strikingly resemble the half tubes covered *in vivo* by these proteins at the MCC plasma membrane invaginations (Figure 1). Contact to the plasma membrane is mediated by the banana-shaped BAR domains (for Bin1, Amphiphysin and Rvs proteins) of Pil1 and Lsp1, which probably trigger membrane curvature (Karotki et al., 2011). Their binding requires $PI(4,5)P_2$ within the target membrane. It has, therefore, been proposed that the eisosomes, or at least their constituent Pil1 and Lsp1 proteins, could form part of a fungal membrane cytoskeleton.

Both the formation and localization of the eisosomes and their overlaying MCC compartment are strictly regulated. Their *de novo* formation has been observed in dependence of the cell cycle in newly forming buds, occurring in waves toward the bud tip. The major components, Pil1 and Lsp1, are phosphorylated by a dual pair of phosphoinositide-dependent protein kinases, Pkh1 and Pkh2, which leads to the eisosome disassembly (Walther et al., 2007). In accordance with the lipid interaction partner of Pil1 and Lsp1, decreasing the levels of $PI(4,5)P_2$ results in the formation of fewer eisosomes at the plasma membrane, whereas increasing the levels elongate the membrane furrows (Karotki et al., 2011). The lipid composition of the plasma membrane is apparently also monitored by the MCC transmembrane protein Nce102, which signals

the two Pkh kinases (Fröhlich et al., 2009). As expected, the deletion of *PIL1* leads to a loss of the MCC compartments, with a homogenous distribution of the respective membrane proteins within the plasma membrane and the accumulation in one or a few clusters of the 'eisosome remnants', which are only detectable in the later stages of cell growth (Brach et al., 2011). Surprisingly, the loss of the closely related Lsp1 function does not cause a disassembly of the eisosomes and the MCC microcompartment, indicating subtle functional differences. Apart from somewhat increased sensitivities to different stresses, like heat and oxidative stress, *pil1* null mutants lack a strong phenotype. Thus, despite the detailed molecular analyses and the emerging structural role of the eisosomes as putative components of a yeast plasma membrane cytoskeleton, their exact physiological function remains obscure.

Compartmentation of cell wall integrity sensors

A similar example of microcompartmentation within the yeast plasma membrane is the punctuated and sometimes polarized distribution of the CWI (for cell wall integrity) sensors (Straede and Heinisch, 2007). In brief, the CWI-mitogen activated protein kinase (MAPK) signaling pathway is triggered by stress at the yeast cell surface (either at the cell wall or at the plasma membrane), which is recognized by a five-membered family of transmembrane sensors (namely, Wsc1 to Wsc3, Mid2, Mtl1; Figure 2) (reviewed in Jendretzki et al., 2011). The interaction of the cytoplasmic tail of these sensors with the GDP/GTP exchange factor (GEF) Rom2 presumably activates the small GTPase Rho1, which then interacts with the sole yeast protein kinase C (Pkc1). This, in turn, triggers a phosphorylation cascade through a conserved MAPK module (consisting consecutively of the MAPKKK Bck1, a dual pair of the MAPKKs Mkk1 and Mkk2, and the MAPK Mpk1/Slt2). Finally, the phosphorylation of the transcription factor Rlm1 activates the expression of some 30 genes involved in cell wall synthesis and composition. Mpk1/Slt2 also phosphorylates a subunit of a dimeric transcription factor (Swi4–Swi6), which coordinates the cell wall remodeling with the cell cycle progression (reviewed in Levin, 2011). The mutants affecting the signaling chain usually display an osmoremedial cell lysis phenotype due to a weakened cell wall at the bud tip.

Of the CWI sensors, Wsc1 and Mid2 are supposed to perform the major functions, as single or combined deletions of the genes encoding the other sensors result in comparatively mild phenotypes (reviewed in Rodicio and Heinisch, 2010). Mid2-GFP fusions appear in a punctuated, rather stable, pattern in the plasma membrane of vegetative cells, but move into the shmoo projections during the yeast mating process (Hutzler et al., 2008; Wilk et al., 2010). On the other hand, Wsc1-GFP is a highly dynamic sensor, which constantly travels through endocytosis and accumulates in the plasma membrane at sites of cell wall remodeling, i.e., in the newly forming bud and at the bud neck during cytokinesis (Piao et al., 2007; Wilk et al., 2010). With regard to the above-described lateral organization of the plasma membrane, preliminary results from the Heinisch group indicate that Mid2 (and Wsc2) may colocalize with Pma1 in the MCP network, whereas Wsc1 may reside in the MCC microcompartment (Wilk, 2010). Although data from fluorescence microscopy for the latter localization are still somewhat ambiguous due to the highly mobile nature of the Wsc1 sensor, a MCC localization would also be consistent with data from single-molecule AFM (see below), as well as with the detection of a *wsc1/slg1* deletion in a genetic screen for defective eisosome formation (Fröhlich et al., 2009).

Among the CWI sensors, Wsc1 has been studied most extensively. Thus, a His-tagged, elongated version of the sensor has been constructed, which transverses the cell wall and can be detected at the yeast cell surface by scanning with a modified AFM tip (Heinisch et al., 2010a). Several important conclusions were drawn from the application of this single-molecule technique: i) The sensor displays the mechanical properties of a nanospring, consistent with the hypothesis of its function as a mechanosensor (Dupres et al., 2009). These nanospring properties are conferred by the highly mannosylated serine/threonine-rich region of the extracellular region, which is also present in all other members of the CWI sensors. This supports a structural model in which the CWI sensors possess two points of anchorage at the yeast cell surface, one constituted by the single transmembrane domain, and the other by a head group (see below), which should be flexibly attached to the cell wall to allow for sensor recycling (Figure 2). ii) Wsc1 does not distribute evenly at the cell surface, but rather accumulates in patches of approximately 250 nm in diameter, consistent with the size of the MCC microcompartments (Heinisch et al., 2010b). The number of sensors, as well as cluster formation, increases under stress conditions such as heat or low osmolarity. It is important to note that due to technical constraints, only the Wsc1 sensors in nonbudding, round mother cells can be detected by this method. iii) The clustering is mediated by the cysteine-rich extracellular domain (CRD) of the protein, forming a head group near the N-terminal end

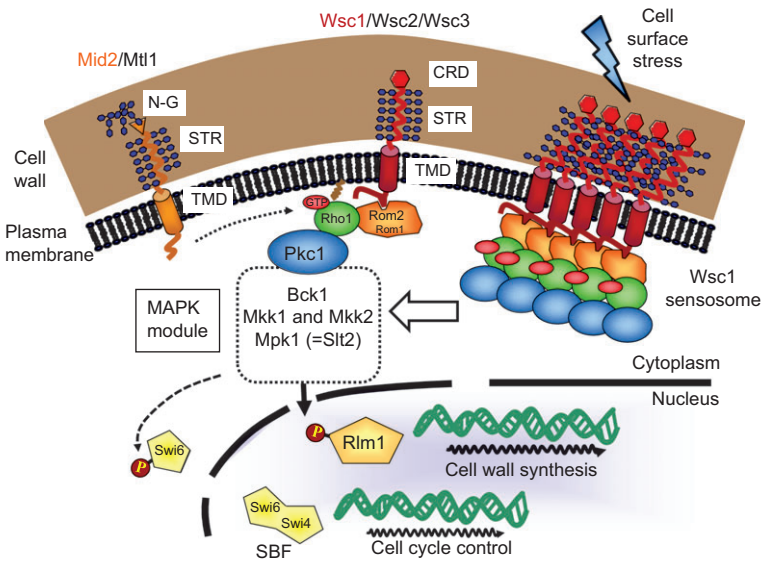

Figure 2 The cell wall integrity signaling pathway in *S. cerevisiae* and sensor structures.
Cell wall integrity signaling components, including the two sensor families as described in the text, are depicted. Cell surface stress induces sensor clustering (as demonstrated for Wsc1) and formation of a Wsc1 sensosome (Heinisch et al., 2010b) depicted in the upper right-hand corner. TMD, transmembrane domain; STR, serine/threonine-rich region (blue hexagonals designate O-mannosylations); CRD, cysteine-rich domain; N-G, N-glyosylated asparagine. For a detailed description of the CWI components see text and Levin (2011).

of the mature sensor. Its function presumably depends on the formation of disulfide bridges, whose exact nature remains to be determined (Heinisch et al., 2010b; Dupres et al., 2011). The CRD has also been found as a so-called 'WSC domain' in some 80 proteins from viral, bacterial, fungal, or mammalian origin (Ponting et al., 1999). A prominent example is the human polycystin-1, whose heritable defects cause polycystic kidney disease (Harris and Torres, 2009). Although the domain has been speculated to interact with polysaccharide chains, its exact molecular function has not yet been determined for any of the proteins studied (reviewed in Heinisch and Dufrene, 2010).

For Mid2 and Mtl1, which lack a CRD sequence, an N-glycosylated asparagine residue near the N-terminus has been suggested to fulfill the second anchor function in the yeast cell wall (Rodicio and Heinisch, 2010).

Interestingly, cysteine-to-alanine mutations in the CRD of the Wsc1 sensor not only abolish clustering but also impair the *in vivo* signaling function to the downstream MAPK cascade (Heinisch et al., 2010b). On the other hand, sensors lacking the entire CRD sequence can rescue some of the *wsc1* deletion phenotypes upon strong overexpression (Lodder et al., 1999). Together with the AFM results, this indicates a physiological role for sensor clustering: in analogy to the situation in bacterial chemotaxis (Kirby, 2009), the accumulation of sensors within a plasma membrane microcompartment would more efficiently recruit the downstream components of the CWI signaling cascade (such as Rom2 and Rho1) to form a Wsc1 sensosome, which then triggers the appropriate cellular response leading to a reinforcement of the cell wall (Figure 2) (Heinisch et al., 2010b). An increase in the sensor density by overproduction of the CRD-less protein would have a similar effect of the local accumulation of the intracellular signaling components and could, thus, functionally substitute for a lack of signal-induced cluster formation.

Cytokinesis and the yeast bud neck microcompartment

The yeast bud neck is a special microcompartment, whose structural components and dynamic organization serve as a model for a number of fundamental biological processes related to cytokinesis far beyond yeasts (Versele and Thorner, 2005).

The site of cell division, like the budding site in yeast, is established by the members of the conserved septin protein family, which recruit further cytokinesis components. In yeast, cell polarity is marked by a rearrangement of the actin cytoskeleton. An AMR structure, which is also found in mammalian cell division, is formed in coordination with the final steps of mitosis in the yeast

cell cycle and ensures the timely and proper constriction of the plasma membrane and thereby the separation of the cytosols of the mother and daughter cells. New cell wall has to be deposited in two steps: a primary, chitinous septum is formed in conjunction with the constriction of the AMR, whereas glucan and mannoproteins are added as the secondary septa from both mother and daughter cells only after the separation of the cytoplasmata. Finally, a carefully controlled degradation of the cell wall polysaccharides ensures cell separation, before a new round of the cell cycle occurs. These events are summarized in Figure 3 and have been extensively reviewed with regard to their molecular mechanisms and regulation, and the homologous functions in fungal and mammalian cytokinesis. For some excellent recent reviews, see Roncero and Sanchez (2010), Seiler and Justa-Schuch (2010), Merlini and Piatti (2011), Bi and Park (2012), and Howell and Lew (2012).

In the following, we will focus on the relation of yeast cytokinesis to CWI signaling and how microcompartmentation is important for chitin synthesis and delivery.

Cell wall integrity signaling at the yeast bud neck

As the CWI signaling pathway ensures cell wall biosynthesis and wall reinforcement at the site of lesions (Rodicio and Heinisch, 2010; Levin, 2011), it is expected to be also activated at the time of budding in normal vegetative growth, when septum formation occurs. Despite this obvious relationship, little attention has been paid, so far, on the coordinated control of cytokinesis and CWI signaling. Not surprisingly, already early protein localization studies placed several upstream components of the CWI pathway at the bud neck. These include the GEF Rom2 and its target GTPase Rho1 (Figure 2) (see Yoshida et al., 2009 and references therein). The latter organizes the actin cytoskeleton via its interaction with formins and also functions as an activator of the central CWI component Pkc1, which also localizes to the bud neck during cytokinesis (Andrews and Stark, 2000; Denis and Cyert, 2005). The homologs of Rho1 and Pkc1 also show a bud neck accumulation in the related milk yeast *Kluyveromyces lactis*, indicating similar mechanisms in different

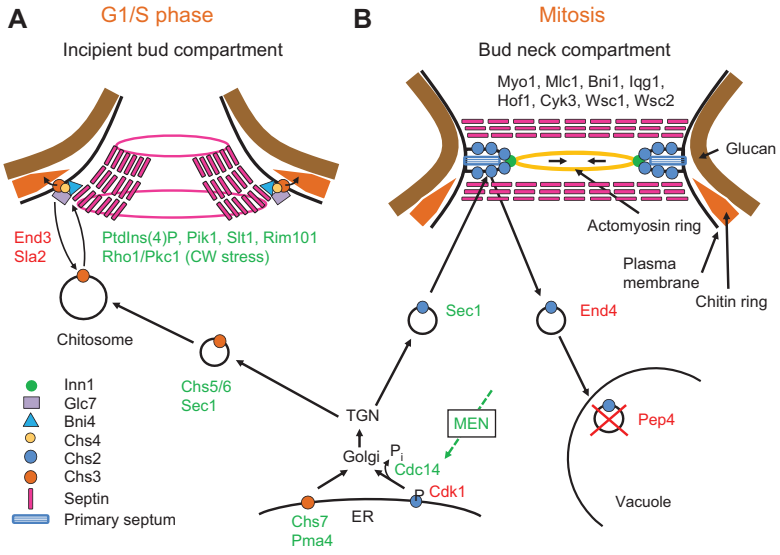

Figure 3 Chitin synthesis and compartmentation at the incipient bud and at the bud neck.
(A) Compartmentation of incipient bud at the G1/S phase. Chs3, the catalytic subunit of the CSIII complex, is recruited to the plasma membrane by the Bni4/Glc7 complex. The regulatory subunit Chs4 is tethered by Bni4, which binds to the Cdc10 of the septin ring, whose filaments are oriented along the polarity axis. Tethering of CSIII to the septin ring prevents lateral diffusion in the plasma membrane and, hence, supports formation of the chitin ring. (B) Compartmentation at the bud neck during the anaphase of mitosis. The contractile AMR assembles in between the two septin rings, whose filaments are oriented circumferential. Plasma membrane ingression, ring contraction, and primary septum formation by Chs2 are interdependent processes that require the indicated proteins. Chs2 and Chs3 enter divergent trafficking pathways upon their release from the ER, which involve different tethering factors. While Chs2 is degraded in the vacuole after endocytosis, Chs3 is sorted into chitosomes, which act as an endocytic reservoir. Proteins that promote trafficking to the plasma membrane are depicted in green; proteins that inhibit anterograde transport, promote endocytosis, or mediate degradation are shown in red. TGN, *trans*-Golgi network; ER, endoplasmic reticulum. See text for further details.

yeasts (Rodicio et al., 2006). Moreover, the CWI sensor Wsc1 accumulates in the growing bud and, later on, at the bud neck in both *S. cerevisiae* and *K. lactis* (Rodicio et al., 2008; Wilk et al., 2010).

There are some striking coincidences related to the mechanism of localization of these components: i) Rho1 is apparently recruited either by its interaction with the GEF Rom2 or with $PI(4,5)P_2$, with the latter also recruiting Pil1 and Lsp1, the main eisosome proteins (Yoshida et al., 2009; Karotki et al., 2011). Rho1 is also necessary for the accumulation of Pkc1 at the bud neck (Andrews and Stark, 2000). ii) Rom2 interacts with the cytoplasmic domain of the CWI sensors Wsc1 and Mid2, which also contributes to the recruitment of the downstream Rho1 target (Philip and Levin, 2001). iii) The plasma membrane invagination during cytokinesis requires extensive membrane bending, which could be mediated by the eisosome proteins Pil1 and Lsp1. Interestingly, the membrane furrows related to these proteins were shown to proceed in waves from the bud neck to the growing daughter cell (Karotki et al., 2011). iv) Eisosome formation is impaired in a *wsc1* deletion (Fröhlich et al., 2009). Together with being a focus of actin cable orientation and vesicular trafficking, this makes the bud neck a place of vital and highly dynamic interactions of a myriad of protein-protein and protein-lipid complexes. Although it is tempting to speculate on a functional link between the eisosome function, CWI signaling, and AMR constriction, this clearly needs further investigation.

Chitin synthesis and neck compartmentation

Chitin is a nonessential component of the yeast cell wall and forms a fibrillar network, which supports the organization of glucans (β1,3 and β1,6), mannans (β1,2, β1,3, and β1,6) and, thus, indirectly also of some cell wall proteins, which are initially linked to the plasma membrane by GPI anchors and then to cell wall glucans by transglycosylation (Klis et al., 2002; Latge, 2007). Three glycosyltransferases are responsible for chitin biosynthesis in yeast (Roncero, 2002): while Chs1 appears to have a more general function in cell wall repair (and secondary septum formation), Chs2 and Chs3 have specialized functions during cell division. At the onset of cell division, the active chitin synthase III (CSIII) complex, which contains Chs3 as the catalytic subunit, synthesizes the chitin ring, which marks the base of the incipient bud and, to some extent, ensures neck integrity (Shaw et al., 1991; Schmidt et al., 2003). Later on, in the vegetative cell cycle, as well as in sporulation, CSIII also produces chitin for the lateral cell walls or for the chitosan ascospore layer. Toward the end of mitosis, the catalytic subunit of the chitin synthase II (CSII) complex, Chs2, forms the primary septum, which is a chitinous disk that physically separates the mother from the daughter cell (Sburlati and Cabib, 1986). Chitin synthesis, thus, provides a crucial step in the formation of the bud neck microcompartment, which will be discussed in detail in the following sections.

Early compartmentation at the incipient bud site

Budding starts at the G1/S phase transition with the assembly of a septin ring at the site of the incipient bud. This is followed by the deposition of a chitin ring at the mother side of the bud neck, which reinforces the division zone, although its formation is not essential (Lesage and Bussey, 2006). The synthesis of the chitin ring depends on two factors: the activation of the CSIII complex and its proper localization at the plasma membrane of the incipient bud. The cortical scaffold at the bud neck is formed by septin filaments, which are oriented along the polarization axis and assembled into a ring-like structure (Figure 3) (Vrabioiu and Mitchison, 2006). As *CHS3* is constitutively expressed throughout the cell cycle, the encoded chitin synthase is primarily regulated at the posttranslational level (Choi et al., 1994). Accordingly, the localization and activity of Chs3 depend on the interaction with different regulatory proteins. Thus, Chs3 localizes to the septin ring at the incipient bud and remains at the bud neck until the daughter cells are approximately medium sized. Anchoring to the septin ring appears to be mediated by Chs4, a regulatory subunit of the CSIII complex, which associates with Chs3 and induces CSIII activity by a yet unknown mechanism (Trilla et al., 1997). However, Chs3 and Chs4 do not colocalize during all phases of the yeast cell cycle. Moreover, they engage in different routes of trafficking to the plasma membrane (Grabinska et al., 2007; Reyes et al., 2007; Meissner et al., 2010). Chs4 connects the CSIII activity to the septin ring by interacting with the scaffold protein Bni4, which in turn binds to Cdc10 (one of five septins) (DeMarini et al., 1997). In addition, Bni4 recruits the catalytic subunit of protein phosphatase 1 (Glc7) to the mother side of the emerging bud. Glc7 appears also to have a role in directing the CSIII complex to the bud neck (Kozubowski et al., 2003; Larson et al., 2008). In summary, the active CSIII complex is tethered to the incipient bud site in the late G1 phase by a hierarchy of proteins that assemble at the septin ring scaffold (see also Table 1 and Figure 3). The resulting incipient

Name	Localization	Activities and functions	Reference
Incipient bud compartment			
Septins (Cdc3, Cdc10, Cdc11, Cdc12, and Sph1)	Hourglass-like structure before bud emergence	Septin filaments oriented along polarity axis; they act as a scaffold to recruit cell division factors initiating bud emergence	Vrabioiu and Mitchison, 2006; McMurray and Thorner, 2009a; Roncero and Sanchez, 2010
Chs3	Ring-like structure at the incipient bud site and at the neck of small-budded cells; during cytokinesis (medium-sized buds) Chs3 dissociates from the neck region and reappears as a double ring after cytokinesis has progressed (large-budded cells)	Catalytic subunit of the CSIII complex, which synthesizes the chitin ring. Anchoring to the septins involves Chs4, Bni1, and Glc7	Chuang and Schekman, 1996; Ziman et al., 1996; Kozubowski et al., 2003; Roncero and Sanchez, 2010
Chs4	Localization is similar but not identical to that of Chs3	Regulatory subunit of the CSIII complex, tethers to the septin ring by binding to Bni4. Chs4 is transported to the plasma membrane independently from Chs3 trafficking	Trilla et al., 1997; Grabinska et al., 2007; Reyes et al., 2007; Meissner et al., 2010
Bni4	Single ring at most nonbudded and small/medium-budded cells; double ring in most large-budded cells	Regulatory subunit of Glc7, binds to the Cdc10 septin and to Chs4	DeMarini et al., 1997; Kozubowski et al., 2003; Larson et al., 2008
Glc7	Ring-like structure at the incipient bud	Type 1 serine/threonine phosphatase involved in the recruits Chs3 to the plasma membrane of the bud neck	Larson et al., 2008
Bud neck compartment			
Septins (Cdc3, Cdc10, Cdc11, Cdc12, and Sph1)	Two septin rings after ring splitting and septin reorientation in mid-budded cells	Septin filaments are oriented circumferential. They orchestrate cell division and act as a signaling platform for cytokinesis	Vrabioiu and Mitchison, 2006; McMurray and Thorner, 2009a; Roncero and Sanchez, 2010
AMR (Myo1, Mlc1, Act1)	AMR assembles during early mitosis	Myo1; type II myosin of the AMR; Mcl1, type V myosin, light chain of Myo1; Act1, forming filamentous actin	Vallen et al., 2000
Iqg1 (=Cyk1)	Ring-like structure at the bud neck	Essential for actomyosin formation but has also a cytokinetic function that is independent from the AMR; interacts with Myo1 independently of septins	Epp and Chant, 1997; Ko et al., 2007
Cyk3	Ring-like structure at the bud neck	Recruits Inn1 to the bud neck; when overexpressed, Inn1 recruitment is AMR independent	Jendretzki et al., 2009; Meitinger et al., 2010
Inn1	Localizes to the bud neck during late mitosis	Required for plasma membrane ingression concomitant with AMR constriction; binds to Hof1 and Cyk3	Jendretzki et al., 2009; Meitinger et al., 2010
Hof1	Bud neck at anaphase; initially forms a double ring that fuses after AMR constriction	Binds to the formin Bnr1 and triggers AMR constriction; then, it relocates to the septin filaments	Vallen et al., 2000; Meitinger et al., 2011
Formins (Bni1, Bnr1)	Bni1 localizes at the bud tip during bud growth and later at the bud neck; Bnr1 stably associates with septins at the bud neck	Promote actin polymerization in a Rho1 dependent manner. Bni1 is necessary for AMR constriction, while Bnr1 has a role in coupling septum formation and the actomyosin system. Bnr1 generates actin cables extending into the mother cell	Vallen et al., 2000; Gao et al., 2010; Liu et al., 2012
Chs2	Localizes to the invaginating plasma membrane at the bud neck in the anaphase	Catalytic subunit of the CSII; synthesizes the chitin disk of the primary septum	Shaw et al., 1991; Chuang and Schekman, 1996
CWI sensors (Wsc1, Wsc2)	Localize to the bud neck at anaphase	Sensing of cell surface stress; signal transduction triggers new cell wall biosynthesis	Wilk et al., 2010

Table 1 Proteins involved in the formation of incipient bud and the bud neck compartments.

bud microcompartment facilitates the morphogenesis of the bud neck. Moreover, septin-based compartmentation defines a plasma membrane region at the bud neck, which provides a barrier from the lateral plasma membranes. The anchoring to this membrane compartment by septins may separate Chs3 from the regions of endocytosis and, hence, prevent its turnover (Sacristan et al., 2012). Thus, the membrane compartmentation at the bud neck could interfere with the antero- and retrograde transport of Chs3 and, thereby, govern chitin ring formation.

Compartmentation at the bud neck

After bud emergence during the G2 phase, the septin collar splits into two separate rings at the end of the anaphase (see also Figure 3). The splitting process appears to be associated with the major conformational changes of the septins and reorientation of the septin filaments (Vrabioiu and Mitchison, 2006). The septin rings are essential for cytokinesis because they act as a permeability barrier, which prevents free diffusion of lipids and proteins between the mother cell and the daughter cell (McMurray and Thorner, 2009b). However, the diffusion barrier is apparently not essential for yeast cytokinesis but rather it may recruit proteins to the bud neck, a function which is primarily exerted by the AMR (Wloka et al., 2011). By allowing specific factors to accumulate at the division site, they create a unique cortical compartment at the bud neck. In doing so, the septins contribute to cellular asymmetry by partitioning the cellular components during cell division. Cytokinesis then starts with the ingression of the plasma membrane at the neck between the mother cell and the daughter cell, followed by chitin deposition in the resulting invagination of the plasma membrane (Figure 3). Eventually, the mother and daughter cells become separated by two plasma membranes and a chitinous disc, a structure referred to as the primary septum. The ingression of the plasma membrane is driven by the contraction of the AMR, which is sandwiched between the septin rings (Epp and Chant, 1997). As stated above, Chs2 synthesizes the chitinous disk, concordant with its localization at the invaginated plasma membrane toward the end of mitosis (Shaw et al., 1991). The contraction of the AMR and the primary septum formation seem to be interdependent processes (Schmidt et al., 2002). Mutants defective in either *chs2* or *myo1* (the gene encoding a type II myosin required for AMR formation) show striking similar cytokinesis phenotypes, i.e., they fail to contract the plasma membrane and do not form a primary septum. However, Chs2 does not directly interact with the AMR, and a potential mediator has not yet been identified. The machinery that orchestrates AMR contraction, plasma membrane ingression, and primary septum formation is only partially understood. Recently, Inn1 has been suggested to couple ring contraction to plasma membrane ingression (Sanchez-Diaz et al., 2008; Jendretzki et al., 2009). The proline-rich region of Inn1 binds to the SH3 domain of the transient actomyosin component Hof1, an F-BAR protein, which appears at the septin ring already early during cell cycle (Nishihama et al., 2009). In the late anaphase, Hof1 is phosphorylated by the components of the mitotic exit network (MEN; specifically by the Dbf2-Mob1 heterotrimer), which triggers its release from the septin filaments and a shift to the AMR, where it promotes ring contraction and membrane ingression (Meitinger et al., 2011). Besides, with Hof1, Inn1 also interacts with Cyk3, which might have a role in a cytokinesis pathway that is triggered by Iqg1 independently from the AMR (Ko et al., 2007; Jendretzki et al., 2009). Inn1-Cyk3 complex formation depends on the phosphorylation status of Inn1, which is controlled by Cdk1 and Cdc14 (Palani et al., 2012). Despite this recent progress in identifying proteins that link actomyosin contraction, the plasma membrane ingression and primary septum formation, the molecular mechanisms leading to the activation of Chs2 at the bud neck are still unknown. Yet, the dynamics and localization of Inn1, Cyk3, and Chs2 are apparently controlled by a common signaling pathway, which will be outlined in the following section.

Delivery and removal of chitin synthases to and from the neck compartment

As chitin synthesis is crucial for the proper formation of the bud neck microcompartment, the dynamics of chitin synthases at the plasma membrane of this subcellular region has been extensively studied. In this context, the different trafficking routes for Chs2 and Chs3 are of particular interest and will be described in further detail.

The intracellular localization of Chs3 varies significantly in dependence of the phases of the yeast cell cycle: during the G1/S phase transition, the protein appears at the incipient bud and at the bud neck of small-budded cells. It then accumulates in specialized endocytic vesicles, so-called chitosomes (Ruiz-Herrera et al., 1977), in the G2 phase (Chuang and Schekman, 1996), which finally deliver Chs3 to the bud neck again during mitosis

(Figure 3). The chitosomes, thus, apparently function as a *trans*-Golgi reservoir for Chs3, which is replenished by the endocytotic turnover of the enzyme (Ziman et al., 1996). Hence, Chs3 is not degraded in the vacuoles after endocytosis but rather utilized for another round of chitin synthesis. The delivery of Chs3 to the plasma membrane is controlled by protein-protein interactions that determine its trafficking route. After its *de novo* synthesis, the export of Chs3 from the ER requires the ER resident chaperone Chs7 and palmitylation by Pfa4 (Trilla et al., 1999; Lam et al., 2006). After Chs3 has passed the Golgi apparatus, it is transported from the *trans*-Golgi network (TGN) to the cell surface. This step requires Chs5 and Chs6, which are part of an exomer coat complex that transiently interacts with Chs3 and mediates its sorting into the secretory vesicles in an Arf1-dependent manner (Sanchatjate and Schekman, 2006; Wang et al., 2006). Other ways of regulation have been discussed recently by Merzendorfer (2011) and are considered in Figure 3. Notably, cell stress leads to a redistribution of Chs3 from chitosomes to the plasma membrane. This process is triggered by the upper parts of the CWI pathway involving Rho1 and Pkc1, but does not involve downstream effectors of the MAP kinase cascade (Valdivia and Schekman, 2003).

In contrast to Chs3, mRNA and protein amounts of Chs2 oscillate during the cell cycle suggesting that the enzyme levels necessary for primary septum formation are adjusted by the balance between synthesis and degradation (Choi et al., 1994). Cell cycle-dependent transcriptional regulation of *CHS2* is probably governed by the Mcm1-Ndd1-Fkh2 complex (Chen et al., 2009). After the synthesis of Chs2 at the rough ER, it is delivered to the bud neck via the secretory pathway in the late anaphase (Chuang and Schekman, 1996). This targeting is negatively controlled by the mitotic kinase Cdk1, which upon phosphorylation of Chs2 blocks its exit from the ER (Teh et al., 2009). When Cdk1 becomes inactivated toward the end of mitosis by the MEN signaling cascade, Chs2 gets dephosphorylated by Cdc14 and is ultimately delivered to the bud neck (Chin et al., 2012). Upon its release from the ER, Chs2 is transported by COPII vesicles to the bud neck region (Zhang et al., 2006). The turnover of Chs2 occurs after endocytosis, when it is directed to the vacuole for degradation by the major Pep4 protease. All yeast chitin synthases have been reported to be produced as zymogens that need to be cleaved for activation *in vitro*. As in yeast, no protease has been identified to be involved in the cleaving of the zymogenic chitin synthase so far, the physiological relevance is uncertain (Merzendorfer, 2011). In summary, chitin synthesis at the incipient bud and at the bud neck is predominantly regulated by divergent trafficking pathways. This process involves tethering factors that coordinate the formation of the chitin ring and the primary septum, in which the septin ring serves as a scaffolding and signaling platform.

Conclusions and outlook

In the past decade, the view on the regulation and compartmentation of physiological processes within eukaryotic cells has shifted away from the entire organelles to the suborganellar entities, one of which is represented by lipid rafts and their dynamic complexes, which are composed of a variety of proteins and lipids. Regarding such microcompartmentation, the yeast plasma membrane has attracted special attention, as, in contrast to higher eukaryotes, its membrane contains larger subdomains. Although their physiological importance is still elusive, these domains can be clearly distinguished by fluorescence microscopy, as they harbor a specific subset of proteins, which can be readily tagged by GFP and its derivatives. Moreover, within this review, we discussed the different types of microcompartments, which are tethered to the yeast plasma membrane: whereas the bud neck is defined in its position mainly by the septin ring, the eisosomes can occur all over the plasma membrane and are primarily formed by two BAR-domain proteins. In contrast, the sensors of the CWI pathway seem to be more flexible in their localization and, at specific time points, may be recruited to one or the other microcompartment. Yet, how the sensors are targeted to these domains as well as what the physiological significance of this compartmentation may be are questions that remain to be addressed. It should also be noted that microcompartments other than those tethered to membranes exist in yeast cells, like the protein and protein/RNA complexes mentioned in the introduction. As shown in the first sections of this review, caution has to be taken in the interpretation of localization data regarding their cellular function: thus, the eisosomes were first proposed to mark the sites of constitutive endocytosis, whereas later studies suggest a function within a fungal membrane cytoskeleton. Moreover, although cytokinesis and cell wall synthesis at the specialized microcompartment of the yeast bud neck have both been extensively studied separately, the connecting regulatory network has largely been ignored. In the light of the growing understanding of the underlying molecular mechanisms, together with the recent advances in single-molecule imaging techniques as described above, we expect to see significant progress in this respect in the coming years.

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Hans Merzendorfer studied Biology from 1988 to 1993 at the University of Munich, where he earned his doctorate in 1999 working on insect v-type ATPases. From 1999 to 2001 Hans Merzendorfer continued his work as a Postdoc at the University of Osnabrück. In 2001 he became assistant professor, and in 2006 he obtained his “Habilitation” in animal physiology. In 2007 he received a Heisenberg fellowship from the German Research Foundation. From 2008 to 2009 he joined the Department of Biochemistry at the Kansas State University. Since 2009 Hans Merzendorfer works as an adjunct (apl.) professor at the University of Osnabrück.

Jürgen Heinisch studied Biology at the University of Darmstadt and earned his PhD in 1985 working on yeast phosphofructokinase genetics. After a two-year postdoc (1986–1988) at the University of Alberta (Edmonton, Canada) he became a research assistant at the University of Düsseldorf and got his “Habilitation” in Microbiology in 1993. From 2000–2002 he held a professorship in Fermentation Technology at the University of Hohenheim and since 2003 is a full professor and head of the Department of Genetics at the University of Osnabrück.