Lipid raft involvement in yeast cell growth and death

Faustino Mollinedo*

Instituto de Biología Molecular y Celular del Cáncer, Centro de Investigación del Cáncer, Consejo Superior de Investigaciones Científicas - Universidad de Salamanca, Salamanca, Spain

INTRODUCTION

Apoptosis is an intrinsic cell death process that plays critical roles in the normal development and health of multicellular organisms. However, in the last years, growing evidence suggests that apoptosis-like cell death also occurs in a number of unicellular organisms, including yeast (Mado et al., 2002; Wissing et al., 2004), have profoundly changed our view of membrane organization and membrane-regulated processes. Membrane domains can be isolated by flotation in density gradients. The association of lipid rafts with membrane rafts is small (10–200 nm), heterogeneous, highly dynamic, and pH homeostasis, which influence many cellular processes, including cell growth and death. Membrane raft constituents affect drug susceptibility, and drugs interacting with sterols alter raft composition and membrane integrity, leading to yeast cell death. Because of the genetic tractability of yeast, analysis of yeast rafts could be an excellent model to approach unanswered questions of mammalian raft biology, and to understand the role of lipid rafts in the regulation of cell death and survival in human cells. A better insight in raft biology might lead to envisage new raft-mediated approaches to the treatment of human diseases where regulation of cell death and survival is critical, such as cancer and neurodegenerative diseases.

Keywords: lipid rafts, membrane domains, ergosterol, yeast, S. cerevisiae, ion homeostasis, nutrient transporters, cell death

The notion that cellular membranes contain distinct microdomains, acting as scaffolds for signal transduction processes, has gained considerable momentum. In particular, a class of such domains that is rich in sphingolipids and cholesterol, termed as lipid rafts, is thought to compartmentalize the plasma membrane, and to have important roles in survival and cell death signaling in mammalian cells. Likewise, yeast lipid rafts are membrane domains enriched in sphingolipids and ergosterol, the yeast counterpart of mammalian cholesterol. Sterol-rich membrane domains have been identified in several fungal species, including the budding yeast Saccharomyces cerevisiae, the fission yeast Schizosaccharomyces pombe as well as the pathogens Candida albicans and Cryptococcus neoformans. Yeast rafts have been mainly involved in membrane trafficking, but increasing evidence implicates rafts in important range of additional cellular processes. Yeast lipid rafts house biologically important proteins involved in the proper function of yeast, such as proteins that control Na+, K+, and pH homeostasis, which influence many cellular processes, including cell growth and death. Membrane raft constituents affect drug susceptibility, and drugs interacting with sterols alter raft composition and membrane integrity, leading to yeast cell death. Because of the genetic tractability of yeast, analysis of yeast rafts could be an excellent model to approach unanswered questions of mammalian raft biology, and to understand the role of lipid rafts in the regulation of cell death and survival in human cells. A better insight in raft biology might lead to envisage new raft-mediated approaches to the treatment of human diseases where regulation of cell death and survival is critical, such as cancer and neurodegenerative diseases.

INTRODUCTION

Apoptosis is an intrinsic cell death process that plays critical roles in the normal development and health of multicellular organisms. However, in the last years, growing evidence suggests that apoptosis-like cell death also occurs in a number of unicellular organisms, including yeast (Mado et al., 2002; Wissing et al., 2004), have profoundly changed our view of membrane organization and membrane-regulated processes. Membrane domains can be isolated by flotation in density gradients. The association of lipid rafts with membrane rafts is small (10–200 nm), heterogeneous, highly dynamic, and pH homeostasis, which influence many cellular processes, including cell growth and death. Membrane raft constituents affect drug susceptibility, and drugs interacting with sterols alter raft composition and membrane integrity, leading to yeast cell death. Because of the genetic tractability of yeast, analysis of yeast rafts could be an excellent model to approach unanswered questions of mammalian raft biology, and to understand the role of lipid rafts in the regulation of cell death and survival in human cells. A better insight in raft biology might lead to envisage new raft-mediated approaches to the treatment of human diseases where regulation of cell death and survival is critical, such as cancer and neurodegenerative diseases.

Keywords: lipid rafts, membrane domains, ergosterol, yeast, S. cerevisiae, ion homeostasis, nutrient transporters, cell death

The notion that cellular membranes contain distinct microdomains, acting as scaffolds for signal transduction processes, has gained considerable momentum. In particular, a class of such domains that is rich in sphingolipids and cholesterol, termed as lipid rafts, is thought to compartmentalize the plasma membrane, and to have important roles in survival and cell death signaling in mammalian cells. Likewise, yeast lipid rafts are membrane domains enriched in sphingolipids and ergosterol, the yeast counterpart of mammalian cholesterol. Sterol-rich membrane domains have been identified in several fungal species, including the budding yeast Saccharomyces cerevisiae, the fission yeast Schizosaccharomyces pombe as well as the pathogens Candida albicans and Cryptococcus neoformans. Yeast rafts have been mainly involved in membrane trafficking, but increasing evidence implicates rafts in a wide range of additional cellular processes. Yeast lipid rafts house biologically important proteins involved in the proper function of yeast, such as proteins that control Na+, K+, and pH homeostasis, which influence many cellular processes, including cell growth and death. Membrane raft constituents affect drug susceptibility, and drugs interacting with sterols alter raft composition and membrane integrity, leading to yeast cell death. Because of the genetic tractability of yeast, analysis of yeast rafts could be an excellent model to approach unanswered questions of mammalian raft biology, and to understand the role of lipid rafts in the regulation of cell death and survival in human cells. A better insight in raft biology might lead to envisage new raft-mediated approaches to the treatment of human diseases where regulation of cell death and survival is critical, such as cancer and neurodegenerative diseases.

Keywords: lipid rafts, membrane domains, ergosterol, yeast, S. cerevisiae, ion homeostasis, nutrient transporters, cell death
of a protein with cholesterol-rich rafts is strengthened when it becomes detergent-soluble after depletion of cholesterol from the membrane by the use of methyl-b-cyclodextrin or other agents. Nevertheless, the physiological existence of rafts has been challenged by a number of criticisms (Munro, 2003; Lichtenberg et al., 2005; Lingwood and Simons, 2007; Simons and Gerl, 2010), in particular regarding the use of detergents that could lead to artifacts and misinterpretations since, for instance, Triton X-100 has been shown to promote the formation of ordered domains in model bilayers (Heerklotz, 2003). Also, a main concern has been raised on the diverse effects that might be expected by depleting cholesterol from the membrane because cholesterol has important functions in the whole plasma membrane, apart from forming lipid rafts. On these grounds, the functional involvement of rafts as well as the raft localization of proteins based only on the use of detergents and cholesterol depletion has been challenged, and therefore caution should be taken before assigning a role of rafts in different biological processes. In addition, the evidence for the presence of rafts in the plasma membrane of living cells has, until recently, not been compelling, thus raising some doubts about the physiological existence of rafts. However, the advent of new microscopy techniques has finally demonstrated the existence of rafts in the cell. The use of stimulated emission depletion (STED) microscopy has proved that sphingolipids and glycosylphosphatidylinositol (GPI)-anchored proteins are transiently trapped in cholesterol-dependent molecular complexes in live cells (Eggeling et al., 2009). In this regard, the application of novel technologies, such as fluorescence resonance energy transfer (FRET), fluorescence polarization anisotropy (FPA), total internal reflection fluorescence (TIRF) microscopy, single quantum dot tracking, single particle tracking (SPT), and fluorescence correlation spectroscopy (FCS), have provided evidence for the localization of GPI-anchored proteins and other lipid-modified proteins in cholesterol-dependent clusters (Kusumi et al., 2004; Sharma et al., 2004; Lenz et al., 2006; Marguet et al., 2006; Vyas et al., 2008; Pinaud et al., 2009). In addition, near-field scanning optical microscopy (NSOM) in combination with quantum dots showed that T cell receptor stimulation triggers the organization of T cell receptors in nanodomains in live cells (Zhong et al., 2009; van Zanten et al., 2010). The development of the above high temporal and spatial resolution techniques has allowed to locate different molecular constituents in membrane domains with reduced mobility. The combined use of different biophysical, biochemical, and genetic technologies are now providing evidence demonstrating the existence of sterol-dependent membrane raft domains as well as their role in critical physiological functions. Despite some technical and conceptual limitations, as stated above, resistance to non-ionic detergent solubilization, together with fluctuation in gradient density centrifugation and manipulation of sterol, remain as the most widely used techniques for studying lipid rafts.

There are a wide number of reports showing that rafts in mammalian cells house proteins involved in cell survival and growth, as well as in the proper functioning of immune system receptors (Simons and Toomre, 2000; Squyre et al., 2010). In this regard, cancer cells, usually showing an increased ability to proliferate and survive, have been found to have higher levels of cholesterol (Dessi et al., 1994; Kolanjappan et al., 2003; Freeman and Solomon, 2004; Toi and Tugnoi, 2005) and cholesterol-rich lipid rafts (Li et al., 2006) than their normal counterparts. Nevertheless, in the last decade, a number of death receptors and downstream apoptotic signaling molecules have also been localized in cholesterol- and sphingolipid-rich lipid rafts in cancer cells (Gajate et al., 2003, 2005, 2007; Gajate et al., 2004; Mollinedo and Gajate, 2006; Reis-Sobreiro et al., 2009; Mollinedo et al., 2010a). The localization of the death-inducing signaling complex (DISC), a major apoptotic complex containing Fas/CD95 death receptor, Fas-associated death domain-containing protein (FADD) and procaspase-8, in lipid rafts has been shown by electron microscopy in T cell leukemic cells, when they are engaged to undergo apoptosis (Gajate et al., 2009a). In addition, raft nanodomains have been shown, following a FCS strategy, to be present in both the outer and inner leaflets of the plasma membrane and to play a crucial role in triggering the survival phosphatidylinositol-3 kinase/Akt signaling pathway, by facilitating Akt recruitment and activation upon phosphatidylinositol-3,4,5-triphosphate accumulation in the plasma membrane (Lasserre et al., 2008). On these grounds, mammalian cell lipid rafts behave as platforms that can house different, and even opposite signaling processes, such as survival and apoptosis, and therefore these membrane domains play a critical role in the modulation of cell signaling that regulates cell fate.

Lipid rafts have also been identified in yeast as membrane domains enriched in ergosterol and sphingolipids (Wachtler and Balasubramanian, 2006). The budding yeast Saccharomyces cerevisiae is one of the best characterized eukaryotic organisms. In spite of its simplicity as a free-living unicellular fungus, yeast cells are similar to higher eukaryotes regarding the cell structure and several physiological processes. Due to its genetic tractability and increasing wealth of accessible data, yeast has become a model system of choice for the study of different physiological processes occurring in mammalian cells. In this regard, yeast could be an interesting biological system to analyze the role of lipid rafts in both survival and cell death responses, despite yeast lack death receptors and most of the typical apoptotic signaling molecules present in mammalian cells.

RAFTS IN YEASTS

Regarding the lipid constituents of lipid rafts, and although the lipid levels vary between different cell types, the plasma membrane of the mammalian cell usually contains, on a molar basis, about 30–40% cholesterol and 10–20% sphingomyelin of plasma membrane lipids, while glycosphingolipids are usually present at low levels (Lange et al., 1989; van Meer, 1989). However, yeasts do not have sphingomyelin, but instead have inositol phosphosphingolipids, which may function as orthologs of mammalian sphingomyelin (Matmati and Hannun, 2008). In addition, unlike mammalian cells that have cholesterol, yeast contain ergosterol, serving the same function as cholesterol in animal cells. Ergosterol is even a better raft former than cholesterol (Xu et al., 2001). Studies on the generation of model membranes from yeast total lipid extracts led to the conclusion that formation of membrane domains depended on specific interactions between yeast sphingolipids and ergosterol.
A TPase enzyme from the plasma membrane to the vacuole (Gaigg et al., 1997). Thus, whereas lipid rafts in mammalian cells contain chol- esterol and sphingomyelin or glycosphingolipids (Simons and Ikonen, 1997), raft domains in S. cerevisiae contain ergosterol and complex sphingolipids (Kubler et al., 1996; Bagnat et al., 2000), including inositol-phosphoceramide (IPC), mannose-inositol-phosphoceramide (MIPC), and mannose (inositol phosphate)-ceramide (MIP2C) (Dickson et al., 2006).

Lipid rafts are enriched in sterols, composed of a four-ring structure with an aliphatic tail that can pack tightly with the lipid acyl chains of sphingolipids to create a compacted region of condensed bilayer termed the liquid-ordered state (Munro, 2003; Megha et al., 2006). Because of its rather unique lipid composition, lipid rafts are more resistant to extraction with cold non-ionic detergents, and therefore they were originally defined as DRMs, due to their relative insolubility in cold non-ionic detergents (London and Brown, 2000; Maxfield, 2002; Simons and Gerl, 2010). In the yeast, rafts have also been defined biochemically as DRMs and proven to be critical for protein sorting through the endoplasmic reticulum and Golgi apparatus (Bagnat et al., 2000, 2001; Bagnat and Simons, 2002). The terms lipid raft, liquid-ordered domain, and DRM are widely used indistinctly and are suggested to refer to the same chemico-biological entity. However, in a very strict way, they might have different implications and caution should be taken (Lichtenberg et al., 2003).

The yeast sphingolipid is peculiar in that it contains a saturated very long-chain fatty acid with 26 carbon atoms (Schneiter et al., 1999, 2001), which is synthesized and coupled to raft-located proteins, like the proton pumping ATPase Pma1p, already in the endoplasmic reticulum. Then, the resulting protein–lipid complex is transported and sorted as an entity to the plasma membrane. This rather long C26 fatty acid is required for proper assembly of the protein–lipid complex to transport to the membrane, as a shortening to C22 fatty acid by mutations impairs raft association of Pma1p, and induces Pma1p degradation by rerouting the ATPase enzyme from the plasma membrane to the vacuole (Gaigg et al., 2006; Toulmay and Schneiter, 2007).

Another important difference between mammalian cells and yeast cells lies in that lipid raft formation occurs primarily in the Golgi apparatus in mammals (Brown and Rose, 1992), whereas it takes place in the endoplasmic reticulum in yeast, where is suggested that proteins associate with yeast rafts (Bagnat et al., 2000). In both yeast and mammalian cells, sphingolipids and sterols are mainly present in the plasma membrane (Lange et al., 1989; Patton and Lester, 1991; Zaiser and Daum, 1995), but these molecules are synthesized in compartments of the early secretory pathway (Daum et al., 1998; Futerman and Riezman, 2005), and therefore raft-located proteins could be recruited into these domains in other subcellular structures distinct from the plasma membrane. Thus, in yeast newly synthesized Gas1p, a GPI-anchored protein, and Pma1p have been found to be recruited to lipid rafts in the endoplasmic reticulum (Bagnat et al., 2000; Lee et al., 2002).

Because a major constituent of lipid rafts is sterol, the naturally fluorescent sterol-binding antibiotic filipin has been widely used to detect regions with high sterol content in the plasma membrane of fungi. The use of this compound has led to the identification of large sterol-rich domains (SRDs) in the plasma membranes of fungi (Wachtler et al., 2003; Alvarez et al., 2007), ranging from about 3 to 15 μm². Due to the dynamic nature of lipid rafts and to their ability to aggregate, clusters of rafts in mammalian cells can be formed under different stimuli to lead to raft platforms as big as the ones reported for some fungi (Ausili et al., 2008). In this regard, it might be envisaged that SRDs in yeasts correspond to clusters of sterol-rich rafts or raft platforms, which are required for the accomplishment of specific functions. This clustering of rafts may be critical for the proper onset of certain cell functions, which might require the concentration of a great amount of proteins in specific sites of the cell, thus leading to cell movement, cytokinesis, etc. On these grounds, lipid rafts act as dynamic and mobile platforms that transport the required proteins at the proper place to act.

Sterol-rich membrane domains have been identified in several fungal species, including the budding yeast S. cerevisiae (Kubler et al., 1996; Bagnat et al., 2001), the fission yeast Schizosaccharomyces pombe (Wachtler et al., 2003), as well as the pathogenic yeasts Candida albicans (Martin and Konopka, 2004; Alvarez et al., 2007) and Cryptococcus neoformans (Siafakas et al., 2006; Alvarez et al., 2007), having being involved in a number of important processes like mating (Nichols et al., 2004; Proszynski et al., 2006), cytokinesis (Rajagopalan et al., 2003; Wachtler et al., 2003), and hyphal formation (Martin and Konopka, 2004).

Sterol-rich domains are polarized in the rod-shaped S. pombe throughout the vegetative life cycle in a cell cycle-dependent way, namely they are located to the growing cell ends during interphase, and to the medial zone where cells undergo cytokinesis, as well as at the tips of mating projections (Rajagopalan et al., 2003; Wachtler et al., 2003; Wachtler and Balasubramanian, 2006; Alvarez et al., 2007). Thus, in fission yeast, rafts localize to regions of polarized growth and to the division site. Unlike to what happens in S. pombe, sterols are distributed uniformly throughout the plasma membrane in the vegetative life cycle of the budding yeast S. cerevisiae. However, filipin-stained rafts are also detected at the tips of cells induced with mating pheromone (Bagnat and Simons, 2002; Proszynski et al., 2006; Alvarez et al., 2007). In the human pathogens C. neoformans and C. albicans, sterols are concentrated at the leading edges of mating projections, at the actively growing sites at bud tips, at sites of septation, and at the tip of hyphal growth (Martin and Konopka, 2004; Alvarez et al., 2007).

**RAFTS IN YEAST PATHOGENS**

Proteomic analysis in DRMs from C. albicans led to the identification of 29 proteins, including the well-known lipid raft marker in S. cerevisiae Pma1p (Insenser et al., 2006). Surprisingly, only three proteins (~10%) were typically located in the plasma membrane, whereas most of raft-located proteins were usually present in internal membranes, including proteins located in mitochondrial (31%), Golgi (7%), and endoplasmic reticulum (7%) membranes. This could support the existence of raft domains in the membranes of mitochondria, endoplasmic reticulum, and Golgi, as reported by different researchers (Bini et al., 2003; Mielczyn et al., 2005;
The proteins located in *C. albicans* rafts were involved in a number of biological processes, including lipid metabolism, cell wall biogenesis, protein metabolism, electron transport, and ATP synthesis (Insenser et al., 2006). In addition, heat shock proteins were also present (Insenser et al., 2006), similarly to what has been observed in mammalian cells (Neto-Miguel et al., 2008). Likewise, ATP synthesis was also located at the raft membranes in *C. albicans* (Insenser et al., 2006), and a similar raft location for this enzyme has been found in proteomic studies conducted in mammalian cells (Rae et al., 2004), leading to the suggestion that this protein might be located in plasma membrane rafts as well as in mitochondria (Rae et al., 2004). Furthermore, it is worth mentioning the presence in the *C. albicans* lipid rafts of a series of proteins involved in lipid metabolism and multidrug efflux, such as Erg1p and Scerp7p, involved in the lipid metabolism of major raft components (ergosterol and ceramide; Insenser et al., 2006), Bt2p, a translocase that moves sphingolipid long chain bases from the inside to the outside membrane (Wang et al., 2012), and the ATP-binding cassette (ABC) multidrug transporter CaCdr1p (Parrja et al., 2008). The presence of cytosolic proteins in the *C. albicans* rafts suggest that protein–protein interactions could play a major role in bringing soluble proteins to the raft domains.

*Candida albicans*-associated bloodstream infections are linked to the ability of this yeast to form biofilms (Mukherjee et al., 2005). These latter are aggregates of microbial cells, adhering to each other, which are embedded within a self-produced matrix of extracellular polymeric substances, usually made up of extracellular DNA, proteins, and polysaccharides, and represent a common mode of microbial growth. Microbes growing as biofilm are highly resistant to commonly used antimicrobial drugs. *Candida* biofilms associated with indwelling devices provide a protected niche for the fungal cells, where they can evade the host immune system, and are especially problematic due to their inherent resistance to commonly used antifungal agents (Chandra et al., 2007). The microbial cells growing in a biofilm are physiologically distinct from planktonic cells of the same organism, which are single-cells floating in a liquid medium. Biofilm formation by *Candida* species is believed to contribute to invasiveness of these fungal species, and there is a correlation between *C. albicans* biofilms and fungal pathogenesis. By using lipidomics, a significant difference was observed in the lipid profiles of *C. albicans* biofilms and planktonic cells. Biofilms contained higher levels of phospholipid and sphingolipid than planktonic cells. In the early phase, levels of lipid in most classes were significantly higher in biofilms compared to planktonic cells. The unsaturation index of phospholipids decreased with time, with this effect being particularly strong for raft membranes in *C. albicans* (de Nadal et al., 2006), and a similar raft location for this enzyme has been found in proteomic studies conducted in mammalian cells (Bae et al., 2004), leading to the suggestion that this protein might be located in plasma membrane rafts as well as in mitochondria (Bae et al., 2004). Furthermore, it is worth mentioning the presence in the *C. albicans* lipid rafts of a series of proteins involved in lipid metabolism and multidrug efflux, such as Erg1p and Scerp7p, involved in the lipid metabolism of major raft components (ergosterol and ceramide; Insenser et al., 2006), Bt2p, a translocase that moves sphingolipid long chain bases from the inside to the outside membrane (Wang et al., 2012), and the ABC multidrug transporter CaCdr1p (Parrja et al., 2008). The presence of cytosolic proteins in the *C. albicans* rafts suggest that protein–protein interactions could play a major role in bringing soluble proteins to the raft domains.

*Candida albicans*-associated bloodstream infections are linked to the ability of this yeast to form biofilms (Mukherjee et al., 2005). These latter are aggregates of microbial cells, adhering to each other, which are embedded within a self-produced matrix of extracellular polymeric substances, usually made up of extracellular DNA, proteins, and polysaccharides, and represent a common mode of microbial growth. Microbes growing as biofilm are highly resistant to commonly used antimicrobial drugs. *Candida* biofilms associated with indwelling devices provide a protected niche for the fungal cells, where they can evade the host immune system, and are especially problematic due to their inherent resistance to commonly used antifungal agents (Chandra et al., 2007). The microbial cells growing in a biofilm are physiologically distinct from planktonic cells of the same organism, which are single-cells floating in a liquid medium. Biofilm formation by *Candida* species is believed to contribute to invasiveness of these fungal species, and there is a correlation between *C. albicans* biofilms and fungal pathogenesis. By using lipidomics, a significant difference was observed in the lipid profiles of *C. albicans* biofilms and planktonic cells. Biofilms contained higher levels of phospholipid and sphingolipid than planktonic cells. In the early phase, levels of lipid in most classes were significantly higher in biofilms compared to planktonic cells. The unsaturation index of phospholipids decreased with time, with this effect being particularly strong for biofilms. Inhibition of the biosynthetic pathway for sphingolipid (MejCPC) by myriocin or aurobasidin A, and disruption of the gene encoding inositolphosphotransferase 1 (IPT1) abrogated the ability of *C. albicans* to form biofilms, suggesting that lipid rafts might be involved in biofilm formation (Lattif et al., 2011). In addition, lipid rafts have been found to be important platforms for the concentration of certain virulence factors at the cell surface of pathogenic fungi, to allow efficient access to enzyme substrates and/or to provide rapid release to the external environment. Thus, rafts from the fungal pathogen *C. neoformans* contain the virulence determinant phospholipase B1 (Pbp1p), a GPI-anchored protein, and the antioxidant virulence factor CurZn superoxide dismutase (Sodlp; Suttakas et al., 2006). The enzyme Pbp1p contains phospholipase B (PLB), lyso phospholipase (LPL), and LPL transacylase (LPLA) activities (Chen et al., 1997a,b), and therefore it might affect raft lipid composition.

**LIPID RAFTS AND ION HOMEOSTASIS IN YEAST**

The maintenance of ion homeostasis in response to changes in the environment is vital to all living cells. In yeast cells, the active transport of inorganic ions and nutrients relies on the existence of an electrochemical gradient of protons across the plasma membrane. In *S. cerevisiae*, this electrochemical gradient is mainly generated by the essential H+–ATPase gene, Pma1p, which encodes one of the most abundant proteins in the yeast plasma membrane (Serrano et al., 1986). This Pma1p–mediated electrochemical gradient is balanced by the activity of a number of symporters and antiporters, but the high-affinity potassium uptake through the plasma membrane transporters Trk1p and Trk2p, the former being the most biologically relevant potassium transporter, is the major consumer of the gradient (Gaber et al., 1988; Ko and Gaber, 1991; Madrid et al., 1999). Potassium transport into yeast cells results in plasma membrane depolarization, leading to Pma1p stimulation and a concomitant cytosolic alkalization (Rodriguez-Navaarro, 2006). Thus, the regulation of both Pma1p and Trk1p is critical for the modulation of the electrical membrane potential and intracellular pH. Thus, Pma1p and the high-affinity potassium transporters Trk1p and Trk2p are the major determinants of yeast membrane potential and internal pH, and thus should be co-ordinately regulated. The plasma membrane protein ATPase Pma1p is a resident raft protein (Bagnat et al., 2001). The major K+ transporters, Trk1p and Trk2p, have also been reported to be present in lipid rafts (Zeng et al., 2004; Yenush et al., 2005). Intracellular pH plays a critical role in modulating the activity of many cellular systems, including those regulating cell death, both in yeasts (Ludovico et al., 2001, 2002; Sokolov et al., 2006) and mammalian cells (Perez-Sala et al., 1995; Gottlieb et al., 1996; Meisenholder et al., 1996).

In *S. cerevisiae*, intracellular pH and K+ concentrations affect many cellular activities, including cell growth and death, and thereby they must be tightly controlled through the regulation of the H+–pumping ATPase Pma1p and the major K+ transporters Trk1p and Trk2p (Yenush et al., 2002, 2005). Pma1p is an electrogenic pump with an optimal pH of 6.5 and therefore is well suited to set the intracellular pH at a neutral value (Portillo, 2000), and together with Trk1p, both systems are the major regulators of cell volume, turgor, membrane potential, and pH in yeast. Potassium accumulation through Trk1p can be harmful to the cell, leading to an increase in turgor pressure and the risk of cell lysis. Trk1p is activated by Hal4p and Hal5p kinases and inhibited by the Pyr1p and Pyr2p phosphatases. Hal4p is a negative regulatory subunit of the Pyr1p Ser/Thr phosphate (de Nadal et al., 1996), and it has been shown that the inhibition of Pyr1p by Hal4p is pH dependent (Yenush et al., 2005). Interestingly, Pyr1p–Hal4p interactions could play a major role in bringing soluble proteins to the raft domains.
interaction would act as an intracellular pH sensor, and a model has been proposed for the modulation of H\(^++\) and K\(^+\) homeostasis through the regulation of Trk1p activity by intracellular pH (Yenush et al., 2005). At a relatively alkaline pH the interaction between Hal3p and Ppz1p would be destabilized, and hence the Ppz1p phosphatase would act on Trk1p to decrease the potassium uptake into the cell. Thus, following accumulation of K\(^+\), cells use then a concomitant increase in intracellular pH through the extrusion of protons by Pma1p to downregulate potassium transport.

Ppz1p has been mostly located at the plasma membrane, although some of this protein was also present in internal non-vacuolar membranes, likely the endoplasmic reticulum and/or the nuclear membrane (Yenush et al., 2005). However, unlike Trk1p, Ppz1p was not found in DRMs (Yenush et al., 2005). Nevertheless, because the raft-located Trk1p has been shown to interact with Ppz1p (Yenush et al., 2005), it cannot be ruled out that lipid rafts might transiently be a place where these proteins could interact each other, as shown for other non-raft proteins in mammalian cells that are translocated and recruited in lipid rafts to regulate cell death signaling (Gajate et al., 2004; Gajate and Mollinedo, 2007; Nieto-Miguel et al., 2008; Reis-Sobreiro et al., 2009).

The above data on yeast are of major importance for mammalian cells, as regulation of potassium transport and intracellular pH homeostasis is implicated in many diseases (Shieh et al., 2000), including cancer, and it is plausible to envisage that similar transduction pathways connecting pH and potassium homeostasis might play a critical role in human disease and constitute interesting therapeutic opportunities.

In addition, the plasma membrane Na\(^+\)/H\(^+\) antiporter Nha1p from the budding yeast S. cerevisiae, which plays an important role in intracellular Na\(^+\) as well as pH homeostasis, by mediating the exchange of Na\(^+\) for H\(^+\) across the plasma membrane, has also been shown to associate with lipid rafts (Mitsui et al., 2009). In lcb1-100 mutant cells, which are temperature-sensitive for sphingolipid synthesis, newly synthesized Nha1p failed to localize to the plasma membrane at the non-permissive temperature, but the addition of phytosphingosine or the inhibition of endocytosis in lcb1-100 cells restored the targeting of Nha1p to the plasma membrane (Mitsui et al., 2009).

Thus, Pma1p, Trk1p, and Nha1p, regulating membrane potential and intracellular pH, are located in lipid rafts in S. cerevisiae (Figure 1), and they are critical in the modulation of ion homeostasis, by keeping the major monovalent cations (H\(^+\), K\(^+\), and Na\(^+\)), mainly through proteins that extrude H\(^+\) and Na\(^+\) and import K\(^+\) ions (Figure 1), at the appropriate narrow range of ion concentrations for the proper function of distinct biological processes.

**NUTRIENT TRANSPORTERS IN YEAST LIPID RAFTS**

Table 1 shows a number of proteins that have been located in lipid rafts in *S. cerevisiae*, including proteins involved in ion homeostasis, nutrient transport, mating, stress response, and actin cytoskeleton organization.

### Table 1

| Ion Transporter | Protein Name | Localization |
|-----------------|--------------|--------------|
| Arginine/H\(^+\) symporter | Can1p (arginine permease) | Lipid rafts |
| General amino acid permease | Gap1p | DRMs |
| Hexose transporter | Foc1p | Lipid rafts |

Several nutrient transporters have been located in lipid rafts in *S. cerevisiae* (Table 1). The arginine/H\(^+\) symporter Can1p (arginine permease) has been found to be present in lipid rafts in *S. cerevisiae* (Malinska et al., 2003). Double labeling experiments with Can1p-GFP and Pma1p-RFP-containing yeast cells showed that these proteins were located in two different non-overlapping membrane domains (Malinska et al., 2003), suggesting the presence of distinct rafts in the same yeast cell. The general amino acid permease Gap1p is present at the plasma membrane and is also associated with DRMs, and in the absence of sphingolipid synthesis Gap1p fails to accumulate at the plasma membrane and is missorted to the vacuole (Lauwers and Andre, 2006). Likewise, the hexose

**FIGURE 1** Ion homeostasis in yeast. This scheme portrays the major proteins, Pma1p, Trk1p, Trk2p, and Nha1p, involved in maintaining ion homeostasis in *S. cerevisiae*.
Table 1: Proteins associated with lipid rafts in S. cerevisiae.

| Gene   | Description                                      | Biological Process                                    | Major Localization                      | Technical Reference                      |
|--------|--------------------------------------------------|------------------------------------------------------|----------------------------------------|-----------------------------------------|
| PMA1   | Plasma membrane ATPase                           | Proton transport, pH regulation                      | Plasma membrane                        | DRMGC Bagnat et al. (2001)              |
| TRK1   | Potassium transporter                            | Cellular potassium ion homeostasis                   | Plasma membrane                        | DRMGC Yenush et al. (2005)             |
| TRK2   | Potassium transporter                            | Cellular potassium ion homeostasis                   | Plasma membrane                        | RTE Zeng et al. (2004)                 |
| NHA1   | Na$^+$ /H$^+$ antiporter                         | Ion homeostasis                                      | Plasma membrane                        | DRMGC Mitsui et al. (2009)             |
| TAT2   | Tryptophan transporter                           | Tryptophan transport                                 | Plasma membrane                        | DRMGC Umebayashi and Nakano (2003)     |
| CAN1   | Arginine permease                                | Arginine transport                                   | Plasma membrane                        | DRMGC Malinska et al. (2003)           |
| GAP1   | General amino acid permease                      | Amino acid transport                                 | Plasma membrane                        | DRMGC Lauwers and Andre (2006)         |
| HXT1   | Low-affinity glucose transporter                 | Glucose transport, hexose transport                 | Plasma membrane                        | DRMGC Lauwers and Andre (2006)         |
| FUR4   | Uracil permease                                  | Uracil transport                                     | Plasma membrane                        | DRMGC Hearn et al. (2003)              |
| FUS1   | Cell fusion                                      | Mating Mating projection tip                         | DRMGC Bagnat and Simons (2002)         |
| SHO1   | Transmembrane osmosensor                         | Osmosensor activity, mating                          | DRMGC Bagnat and Simons (2002)         |
| STE6   | Transmembrane osmosensor                         | Osmosensor activity, mating                          | DRMGC Bagnat and Simons (2002)         |
| SLM1   | Membrane protein related to Hsp30p               | Stress response, negative regulation of Pma1p        | DRMGC Bagnat et al. (2000)             |
| NCE2   | Non-classical export                             | Plasma membrane organization                         | DRMGC Bagnat et al. (2000)             |

DRMGC, non-ionic detergent-resistant membrane (DRM) fractions at 4°C followed by flotation in density gradient centrifugation; RTE, resistance to Triton X-100 extraction.
transporter Hxt1p (low-affinity glucose permease) present at the cell surface was also associated with DRMs (Lauwers and Andre, 2006). The plasma membrane protein uracilH+ symporter Fur4p (uracil permease) has also been reported to be associated with lipid rafts in S. cerevisiae (Umebayashi and Nakano, 2003). The amount of this protein in plasma membrane is highly regulated. Under stress conditions, including heat stress and high concentrations of uracil in the culture medium, Fur4p is degraded by a process that includes phosphorylation, ubiquitination, endocytosis, and transport to the vacuole where the protein is eventually hydrolyzed (Galanc et al., 1998; Marzahl et al., 2002). Because rafts act as platforms for the integration and modulation of signaling pathways and processes, it could be envisaged that the raft location of Fur4p might be critical for its regulation.

The plasma membrane localization of the tryptophan permease Tap2p is regulated by the external tryptophan concentration and is dependent on lipid rafts. In wild-type cells, Tap2p is transported from the Golgi apparatus to the vacuole at high tryptophan level, and to the plasma membrane at low tryptophan level. However, Tap2p is missorted to the vacuole at low tryptophan concentration in the erg6Δ deleted mutant (ERGΔ gene encodes S-adenosylmethionine Δ24 methyltransferase, acting in the last steps of ergosterol biosynthesis by converting zymosterol to fecosterol; Umebayashi and Nakano, 2003), and following yeast treatment with the ergosterol biosynthesis inhibitor zaragozic acid, might be less obvious in yeast. However, lipid rafts seem to be critical structures and scaffolds for the proper function of proteins whose activities are required for the normal function of a yeast cell, including yeast survival and growth (Figure 2), such as proteins involved in iron homeostasis and nutrient transport (Table 1). In this regard, it might be envisaged that redistribution and displacement of raft-located proteins to non-raft domains might lead to a failure in yeast functioning and eventually to cell death. In mammalian cells, lipid rafts usually house proteins involved in survival signaling and growth, and thereby their presence is expected to play a role in the proliferation and survival of cancer cells. However, as indicated above, recent evidence in the last few years has also shown the presence of rafts enriched in death receptors and apoptotic molecules, leading to the emergence of the prosapopotic CASMER. Thus, at least two major different raft domains leading to survival and cell death can apparently be formed in mammalian cells. However, because yeast cells lack death receptors and the classical mammalian-like caspases, yeast rafts are supposed to be involved only in rather positive outcomes that keep the yeast cell alive. According to this notion, it might be envisaged that lipid raft disruption could facilitate and prompt yeast cell death.

**RAFTS AND CELL DEATH IN YEAST**

Dysregulation of ion homeostasis mediates cell death, and this represents the mechanistic basis by which a growing number of amphipathic but structurally unrelated compounds elicit antifungal activity (Zhang et al., 2012). Pma1p is displaced from lipid rafts and delivered and degraded to the vacuole upon S. cerevisiae incubation with edelfosine (Zaremberg et al., 2005), an amphipathic antitumor ether phospholipid that affects and reorganizes lipid rafts (Gajate et al., 2004, 2009b; Mollinedo and Gajate, 2010a,b). These CASMERs may reduce the apoptotic signal threshold by facilitating and stabilizing protein–protein interactions and cross-talk between signaling pathways, and thereby act as a membrane-signaling platforms to launch and catalyze the transmission of apoptotic signals (Mollinedo and Gajate, 2010a,b). The protein composition of these CASMERs includes death receptors and downstream signaling molecules (Gajate and Mollinedo, 2005; Gajate et al., 2009b; Mollinedo and Gajate, 2010a,b). On these grounds, lipid rafts play a major role in the generation of apoptotic signals in mammalian cells. In contrast to mammalian cells, yeast lacks death receptors and most of the typical apoptotic molecules, so the involvement of rafts in the cell death process might be less obvious in yeast. However, lipid rafts seem to be critical structures and scaffolds for the proper function of proteins whose activities are required for the normal function of a yeast cell, including yeast survival and growth (Figure 2), such as proteins involved in iron homeostasis and nutrient transport (Table 1). In this regard, it might be envisaged that redistribution and displacement of raft-located proteins to non-raft domains might lead to a failure in yeast functioning and eventually to cell death.

In mammalian cells, lipid rafts usually house proteins involved in survival signaling and growth, and thereby their presence is expected to play a role in the proliferation and survival of cancer cells. However, as indicated above, recent evidence in the last few years has also shown the presence of rafts enriched in death receptors and apoptotic molecules, leading to the emergence of the prosapopotic CASMER. Thus, at least two major different raft domains leading to survival and cell death can apparently be formed in mammalian cells. However, because yeast cells lack death receptors and the classical mammalian-like caspases, yeast rafts are supposed to be involved only in rather positive outcomes that keep the yeast cell alive. According to this notion, it might be envisaged that lipid raft disruption could facilitate and prompt yeast cell death.

The cationic amphipathic and antiangiogenic drug amiodarone interacts with lipid membranes to exert their biological effect. In S. cerevisiae, toxic levels of amiodarone trigger a transient membrane hyperpolarization, likely through its ability to intercalate into the lipid bilayer (Herbert et al., 1988) altering lipid fluidity (Rosa et al., 2000), which is followed by depolarization, coincident with influx of Ca2+ and H+ that can overwhelm cellular homeostasis and lead to cell death (Maresova et al., 2009). Amiodarone has been shown to have potent fungicidal activity. (Ausili et al., 2008; Busto et al., 2008), because of the complementarity of the molecular geometries of sterols and edelfosine (Busto et al., 2008). Edelfosine induces apoptotic cell death in a wide number of human cancer cells (Mollinedo et al., 1997, 2004, 2010a,b; Gajate and Mollinedo, 2002, 2007; Gajate et al., 2012) through raft reorganization and redistribution of the raft protein content (Gajate and Mollinedo, 2001, 2007; Gajate et al., 2004, 2009a). In human hematopoietic cancer cells, edelfosine treatment leads to the recruitment of apoptotic molecules into raft platforms, thus leading to the emerging concept of an apoptotic "liquid-ordered" plasma membrane platform named as "cluster of apoptotic signaling molecule-enriched rafts" (CASMERs; Gajate and Mollinedo, 2005; Gajate et al., 2009b; Mollinedo and Gajate, 2010a,b). In human hematopoietic cancer cells, edelfosine treatment leads to the recruitment of apoptotic molecules into raft platforms, thus leading to the emerging concept of an apoptotic "liquid-ordered" plasma membrane platform named as "cluster of apoptotic signaling molecule-enriched rafts" (CASMERs; Gajate and Mollinedo, 2005; Gajate et al., 2009b; Mollinedo and Gajate, 2010a,b).
against not only *S. cerevisiae*, but also for species of *Cryptococcus, Candida, Fusarium*, and *Aspergillus* (Courchesne, 2002). Using a genome-wide screen in a *S. cerevisiae* single-gene deletion library, 36 yeast strains with amiodarone hypersensitivity were identified, including mutants in transporters (*PMR1, PDR5*, vacuolar H⁺-ATPase), ergosterol biosynthesis (*ERG3, ERG6, ERG24*), intracellular trafficking (*VPS45, RCY1*), and signaling (*YPD1, PTC1*; Gupta et al., 2003). The fact that azole resistant mutants in the ergosterol biosynthesis pathway of *S. cerevisiae* (*erg3Δ, erg6Δ, and erg24Δ*) exhibited hypersensitivity to amiodarone, suggests that the drug may be particularly effective for treatment againstazole-resistant fungal strains (Gupta et al., 2003), which might be defective in raft-mediated processes. In addition, low doses of amiodarone and an azole (miconazole, flucona-zole) are strongly synergistic and show potent fungicidal effects in combination (Gupta et al., 2003). These data suggest that lipid raft disruption might favor amiodarone cytotoxic action against yeast.

The initial hyperpolarization seems to be critical for the amiodarone cytotoxic effect. Glucose increases membrane potential by increasing H⁺ pumping activity of the plasma membrane ATPase Pma1p (Serrano, 1983). Downregulation of the H⁺ pump activity of the yeast plasma membrane upon glucose removal (Serrano, 1983) was accompanied by an attenuation of amiodarone-induced 

![Diagram](https://via.placeholder.com/150)

**FIGURE 2** | Putative involvement of lipid rafts in different yeast functions.

frontiers in oncology | molecular and cellular oncology October 2012 | Volume 2 | Article 140 | 8

**Cytotoxicity of the antitumor ether phospholipid edelfos-ine against *S. cerevisiae* has been shown to be enhanced in yeast mutants defective for *LCB1*, an essential serine palmitoyltransferase that catalyzes the first step in sphingolipid synthesis,
and ERG3, a sterol C5 desaturase involved in the final steps of ergosterol synthesis (Zarenberg et al., 2005). On the other hand, S. cerevisiae mutants affected in sphingolipid and ergosterol biosynthesis, namely \( \text{ipt1} \Delta, \text{sur1} \Delta, \text{shl1} \Delta \), and erg1\( \Delta \) deletion mutants, are resistant to the azole antifungics micafungin, mainly due to the role of lipid rafts in mediating intracellular accumulation of micafungin in yeast cells (Pouya et al., 2009). Taken together these data suggest a major role of lipid rafts in the cytotoxicity of drugs in yeast.

In this regard, a number of data support that absence of ergosterol, which is one of the major constituents of membrane rafts, has a direct effect on drug susceptibility and morphogenesis of C. albicans. Low doses of amiodarone have been reported to be synergistic with fluconazole in fluconazole-resistant C. albicans (Gama et al., 2010). Ergosterol deficiency in erg1\( \Delta \) mutants led to defects in growth and increased susceptibilities to drugs, including fluconazole, ketoconazole, cycloheximide, nystatin, amphotericin B, and terbinafine in C. albicans (Pouya et al., 2005a,b). Reduced drug efflux activity of the erg1\( \Delta \) mutant was associated with poor surface localization of Cdr1p, suggesting that enhanced passive diffusion and reduced efflux mediated by the ABC transporter Cdr1p increases drug susceptibility. Additionally, conditional erg1\( \Delta \) mutant strains were unable to form hyphae in various media in C. albicans (Pouya et al., 2005a,b). Likewise, the loss of \( \text{ACT1} \) in the C. albicans \( \text{ipt1} \Delta \) mutant, a sphingolipid biosynthetic gene, resulted in increased sensitivity to drugs like 4-nitroquinoline oxide, terbinafine, \( \alpha \)-phenanthroline, fluconazole, itraconazole, and ketoconazole. The increase in drug susceptibilities of \( \text{ipt1} \Delta \) mutant cells was linked to an altered sphingolipid composition, which appeared to be due to the impaired functionality of Cdr1p, a major drug efflux pump of C. albicans that belongs to the ABC superfamily (Prasad et al., 2005a,b). Taken together, the above data indicate that an altered composition of sphingolipid or ergosterol, the major constituents of membrane rafts, affects drug susceptibility and morphogenesis in C. albicans.

**ACTIN CYTOSKELETON, RAFTS, AND STRESS RESPONSE**

Wild-type S. cerevisiae cells depolarize actin following salt stress and repolarize after a period of adaptation (Mollinedo et al., 1999). Two proteins are mainly involved in this process, namely Wick1p for actin depolarization and the amphiphysin-like protein Rvs161p for actin repolarization (Balguerie et al., 2002). Thus, rvs161\( \Delta \) mutant was able to depolarize actin in response to NaCl stress, but was unable to repolarize afterward, whereas \( \text{wic1} \Delta \) mutants was impaired in depolarizing actin (Balguerie et al., 2002). RVS161/\( \text{END3} \) gene, the budding yeast homolog of amphiphysin (Navadon et al., 1995; Youn et al., 2010), is associated, in part, with lipid rafts (Balguerie et al., 2002), and co-localizes with actin patches (Balguerie et al., 1999), thus suggesting a link between rafts and actin cytoskeleton in S. cerevisiae. Rvs161p is suggested to locate in rafts through a putative interaction with a raft-bound protein, as Rvs161p with no GPI signal anchor or transmembrane domain, and therefore it cannot be directly integrated in rafts. The SLG1/WSC1 gene product has also been reported to be partially present in DRMs (Lodder et al., 1999). Clustering of Slg1p/Wsc1p is enhanced under stress conditions, either heat or hypo-osmotic shock, as assessed by single-molecule atomic force microscopy, suggesting its relevance in stress response (Heinisch et al., 2010). Thus, lipid rafts could function as platforms for actin depolarization and actin repolarization in response to stress in S. cerevisiae.

In yeast, nutrient starvation leads to entry into stationary phase. RVS161 (RVS for Reduced Viability upon Starvation) was identified as a critical gene to respond properly to carbon, nitrogen, and sulfur starvation conditions in S. cerevisiae, and it has been implicated in the control of cellular viability. Thus, rvs161\( \Delta \) mutant cells die during the stationary phase and show sensitivity to high salt concentrations (Crouzet et al., 1991). Rvs161\( \Delta \) displays a phenotype similar to that shown for the mutant actin cytoskeleton disorganization, random budding of the diploids, loss of polarity, and sensitivity to salt. In addition, rvs161\( \Delta \), together with mutations in the actin gene, ACT1, leads to synthetic lethality (Breton and Asfie, 1998), thus suggesting that actin and Rvs161p are linked in a common functional pathway that is critical for yeast viability under stress conditions. These data might suggest a role of lipid rafts as platforms for the interaction of proteins that are required for yeast survival.

Rvs167p, another amphiphysin-like protein that interacts with Rvs161p to regulate actin cytoskeleton, endocytosis, and viability following starvation and osmotic stress, has been reported to localize to Rvs161p-containing lipid rafts (Germann et al., 2005). Several protein networks involved in diverse cellular functions, including endocytosis/vesicle traffic, converge on Rvs161p-Rvs167p complex (Boo et al., 2000), and thereby Rvs161p-Rvs167p complex might act as a docking platform for proteins involved in the regulation of different biological processes requiring actin cytoskeleton (Germann et al., 2005). In addition, the rvs mutants accumulate late secretory vesicles at sites of membrane and cell wall construction, and are synthetic-lethal with the \( \text{slt2} \Delta /\text{mpk1} \Delta \) mutation, which affects the MAP kinase cascade controlled by Fic1p and is required for cell integrity (Breton et al., 2001). These data support the idea that the RVS proteins, and thereby lipid rafts, are involved in the late targeting of vesicles whose cargos are required for cell wall construction.

Actin-linking proteins ein3, moesin, RhoA, and RhoGDI were shown to be recruited into clusters of Fas/CD95-enriched rafts in human leukemic cells upon treatment with the anticancer drug aphidin (Gajate and Mollinedo, 2005). Disruption of lipid rafts and interference with actin cytoskeleton prevented Fas/CD95 clustering and apoptosis, suggesting a major role of actin cytoskeleton in the formation of Fas/CD95 clusters and in the aggregation of proteins in lipid raft clusters in human cancer cells, thus regulating raft-associated signaling events (Gajate and Mollinedo, 2005). There is increasing evidence of structural of rafts by the cortical actin cytoskeleton, including evidence that the actin cytoskeleton associates with rafts, and that many of the structural and functional properties of rafts require an intact actin cytoskeleton (Chichili and Rodgers, 2009; Aying et al., 2012).

In yeast, stabilization of actin by addition of jasplakinolide, by point mutations in the \( \text{ACT1} \) gene, or by deletion of certain genes that regulate F-actin, leads to cell death. Yeast mutant lacking the gene \( \text{END3} \) shows stabilized actin and elevated levels of ROS, this phenotype being dependent on downstream elements of...
the Ras/cAMP pathway (Gourlay and Ayscough, 2006). Following yeast treatment with methyl-β-cyclodextrin, which depletes sterols from plasma membrane and disrupts lipid rafts, and manumycin A, that blocks prenylation, Bax2 membrane association and the level of ROS were reduced, and cell death progression was inhibited (Du and Ayscough, 2009). These data suggest that lipid rafts in yeast could be somehow related to providing platforms for the generation of stable complexes that could launch pro-cell death signals. This could open the possibility that lipid rafts in yeast could provide appropriate membrane domains for pro-cell death signaling molecules, as it has been recently described in mammalian cells, and not only for survival and growth signaling. However, this pro-cell death view of rafts in yeast remains to be elucidated, and further studies as well as the molecular characterization of the putative processes involved will be required.

**LIPID RAFTS AND EISOSONES**

Studies on the budding yeast *S. cerevisiae* have revealed that fungal plasma membranes are organized into different subdomains. Pma1p (plasma membrane H^+/-ATPase) and Can1p (H^+/-arginine symporter) have been located in lipid raft membrane domains, but, as stated above, these proteins occupy two different non-overlapping membrane microdomains (Malinsky et al., 2003). Thus, at least two different types of rafts can be distinguished in the yeast plasma membrane. Similarly to what was observed with Can1p, a family of integral membrane proteins, including Sur7p, Yol184p, and Yol222p, were visualized in cortical patches in *S. cerevisiae* (Young et al., 2002). Current evidence suggests the existence of at least two subcellular compartments in the yeast plasma membrane, namely a raft-based membrane compartment represented by a network-like structure housing Pma1p, and another raft-based membrane compartment that houses a number of proton symporters (Can1p, Fur4p, Tat2p). These two raft domains, also named as the membrane compartment occupied by Pma1p (MCP) and membrane compartment occupied by Can1p (MCC; Malinsky et al., 2004; Grossmann et al., 2007), apparently require different lipids to keep their respective protein compositions (Figure 3). The proper sorting of Pma1p has been reported to be more dependent on sphingolipids, ceramide, and the C26 acyl chain that forms part of the ceramide (Lee et al., 2002; Gaigg et al., 2005, 2006). However, sterols are required for the correct targeting of Tat2p (Umebayashi and Nakano, 2003). Thus, these data suggest the putative existence of raft domains more enriched in either sphingolipids or sterol-rich in the yeast membrane, each one containing a specific set of proteins, and this compartmentalization or lateral segregation seems to be dependent on the membrane potential (Grossmann et al., 2007). Plasma membrane depolarization caused reversible dispersion of the H^+ symporters previously present in 300-nm patches (Grossmann et al., 2007). In addition, yeast plasma membrane seems to contain an additional subdomain named eisosomes (from the Greek “eisos,” meaning into or portal, and “soma,” meaning body), which are immobile protein complexes, composed mainly of the cytoplasmic proteins Pil1p and Lap1p at the plasma membrane that mark static sites of endocytosis (Walther et al., 2006). Pil1p and Lap1p form punctuate clusters (eisosomes) on the cytoplasmic surface of the plasma membrane at MCC sites (Walther et al., 2006) and associate with the plasma membrane via their BAR domains (named for the Bin/Amphiphysin/Rvs proteins), that bind membranes and promote curvature (Zimmerberg and McLaughlin, 2004). Eisosomes form at the sites of invaginations in the plasma membrane, being flanked by Can1p-rich MCC domains at the upper edges of the furrows, whereas Pil1p is located at the bottom of the furrow (Stradalova et al., 2009; Douglas et al., 2011) and Sur7p, a protein involved in endocytosis, seems to be at the boundary between MCC and eisosomes (Figure 3). Microscopic and genetic analyses link these stable, ultrastructural assemblies, named eisosomes, to the endocytosis of both lipid and protein cargoes in cells, and are mainly composed of BAR domain proteins (Douglas et al., 2011; Olivera-Couto et al., 2011). Eisosomes have been suggested to function as organizing sites for endocytosis (Toret and Drubin, 2006). Thus, degradation of the arginine permease Can1p induced by excess of its substrate required first Can1p release from MCC patches, and only then it was endocytosed (Grossmann et al., 2008), thus suggesting that the protein is to a large extent unavailable for endocytosis and subsequent degradation as long as it stays in the protective area of MCC (Malinsky et al., 2010). In this regard, rapidly moving endocytic marker proteins avoid raft domains, and consequently the raft domain-accumulated proton symporters show a reduced state of substrate-induced endocytosis and turnover (Malinsky et al., 2010). Genetic analysis of the MCC/eisosome components indicates that these domains broadly affect overall plasma membrane organization (Douglas et al., 2011). The analysis of the major constituents of eisosomes, i.e., BAR proteins, is of major importance also in mammalian cells. BAR family proteins contribute to a range...
of cellular functions characterized by membrane and cytoskeletal remodeling, inducing membrane curvature and recruitment of effectors proteins, with important consequences in several human disorders, including cancer cell invasiveness, as well as immune and neurologic disorders (Chen et al., 2012).

PERSPECTIVES

Recognition of the presence of distinct domains at the cell membrane has been one of the most significant scientific achievements in the last decades. Lipid raft membrane domains are gaining momentum in current biology, and they seem to regulate a wide number of critical processes. Lipid rafts are variable in size and composition, and can change in a highly dynamic way both by recruiting and expelling components to smaller raft units, forming large clusters. The presence of these membrane domains in all eukaryotic cells opens new ways to study the physiological role of rafts in distinct biological systems. The existence of lipid rafts in yeast has provided an excellent way to study the role of these membrane domains in different biological processes, due to the remarkable yeast genetic tractability. Thus, changes in ergosterol and sphingolipid composition, the major raft constituents in yeast, by disrupting key metabolic genes could interact each other. Understanding the dynamic structure of lipid rafts in yeast, as well as their mobility, composition, and biological role, will be of an inestimable value in getting a better insight into the role of these membrane domains in survival and cell death signaling. This insight will recognize the importance of membrane lipid in cellular functions, highlighting lipid rafts as a new and promising therapeutic target (Mollinedo and Gajate, 2006), and could be useful for the search of novel anti-fungal agents and, following extrapolation to mammalian cells, to hopefully set up the underlying bases for the treatment of human diseases. Membrane compartmentalization in lipid rafts plays a key role in signaling process in mammalian cells, and this aspect might also be true in yeast as a plethora of processes seem to involve proteins located in these yeast membrane domains. Thus, a better knowledge of the yeast lipid raft composition and function might help us to gain insight in the regulation of critical processes regarding cell fate, which might be extrapolated to other organisms and could be valuable to conceive new approaches in the treatment of human diseases where cell death and survival are critical, such as cancer and neurodegenerative diseases.

ACKNOWLEDGMENTS

I apologize in advance to my colleagues whose work I could not cite because of the length restrictions of this review. Our laboratory is supported by the following funding agencies: Spanish Ministerio de Economía y Competitividad (SAF2011-35018, and RD06/0020/1037 from Red Temática de Investigación Cooperativa en Cáncer, Instituto de Salud Carlos III, co-funded by Fondo Europeo de Desarrollo Regional of the European Union), European Community’s Seventh Framework Programme FP7-2007-2013 (grant HEALTH-F2-2011-256986, PANACREAS), and Junta de Castilla y León (CSH/02A11-2, and CSE211A12-2).

REFERENCES

Abrahams, A. D., Douglas, L. M., and Konopka, J. B. (2007). Sterol-rich plasma membrane domains in fungi. Environ. Microbiol. 9, 797–803.
Aznar, A., Turuenda, A., Aranda, F. J., Molina, E., Gajate, C., Corbalan-Garcia, S., et al. (2008). Edpholins: a family of amphipathic α-helices that interacts with the plasma membrane. J. Phys. Chem. B 112, 11843–11856.
Arlington, G., Bredalen, S. J., Hall, M. L., Hammond, G. R., Vica, L., Pacheco, J., et al. (2012). Anillin, Dyf-1, and Dyf-3 assemble actin patches in response to osmotic stress in C. elegans. Mol. Biol. Cell 23, 3410–3424.
Bagnat, M., Keranen, S., Shevchenko, A., Shevchenko, A., and Simon, K. (2000). Yeast plasma membrane proteins are localized in rafts and dependent on sterol content. J. Cell Sci. 113, 869–880.
Bae, T. J., Kim, S. M., Kim, J. W., Kim, B. W., Choo, H. J., Lee, J. W., et al. (2004). Lipid raft, sphingolipids, and sphingosine kinase are required for actin reorganization following salt stress. J. Biol. Chem. 279, 1021–1031.
Balgord, A., Straton, P., Bogen, M., and Aigle, M. (1999). Bov1p, the budding yeast homolog of amphiphysin, colocalizes with actin patches. J. Cell Sci. 112, 2529–2537.
Ben, L., Pacini, S., Liberatore, S., Valeriani, S., Pellagrin, M., Rapisardi, R., et al. (2015). Extreme reorganization of the lipid raft proteome following hyperosmotic stress. J. Biol. Chem. 290, 301–309.
Bon, E., Reedere-Neumoser, P., Durzens, P., Jouve, M., Teix, E. A., and Aigle, M. (2000). A network of proteins around Bov1p and Ral1p6p, two proteins related to the yeast actin cytoskeleton. Li. Sci. 16, 1229–1241.
Bosson, B., Iqbalzadeh, M., and Cordummann, A. (2008). Gene expression in Saccharomyces cerevisiae encodes an O-acetyltransferase involved in remodeling of the GPI anchor. Mol. Biol. Cell 17, 2639–2645.
Brown, A. M., and Aigle, M. (1998). Genetic and functional relationship between Rsp5, myosin and actin in Saccharomyces cerevisiae. Curr. Genet. 34, 280–286.
Brown, A. M., Schaufuss, J., and Aigle, M. (2001). The yeast Bov1p and Bov1p7 proteins are involved in actin cytoskeleton remodeling and in cell integrity. Proc. Natl. Acad. Sci. U.S.A. 98, 3254–3259.
Bzug, M., and Simon, K. (2002). Cell-surface polarization during yeast mating Proc. Natl. Acad. Sci. U.S.A. 99, 14315–14320.
Bulgarelli, D., Baghat, M., Bogen, M., Aigle, M., and Brown, A. M. (2002). Bov1p6p and sphingolipids

www.frontierlife.org

October 2012 | Volume 2 | Article 140 | #11
Brown, D. A., and Rose, J. K. (1982). Sorting of GPI-anchored proteins to glycosphingolipid-enriched membrane subdomains during transport to the apical cell surface. J. Cell Biol. 95, 533–544.

Bruno, J. V., del Caño-Juárez, E., Godí, F. M., Molinillo, F., and Alonso, A. (2008). Combination of the anti- tumour cell other lipid ceramide with sphingolipid induces ceramide cell loss effects of the drug. J. Chem. Biol. 1, 89-94.

Carmona-Gutierrez, D., Eisenberg, T., Carmona-Gutierrez, D., Eisenberg, T., Chen, S. C., Wright, L. C., Santander, M., Busto, J. V., del Canto-Janéz, E., Goñi, G., and Mollinedo, F. (2009). Apoptosis in yeast triggers, pathways, subdomains. Curr. Drug Delivery 6, 173–257.

Carmona-Gutierrez, D., Reutenburg, A., Heimbach, P., Bauer, M. A., Braun, R. J., Bueckelin, C., et al. (2011). Ceramide triggers metacaspase-independent mitochondrial cell death in yeast. Cell Cycle 10, 3975-3979.

Chandra, J., McCormick, T. S., Inamura, Y., Mihalco, P. K., and Glentouna, M. A. (2007). Interaction of Candida albicans with adherent human peripheral blood mononuclear cells increases C. albicans biofilm formation and results in differential expression of pro- and anti-inflammatory cytokines. Infect. Immun. 75, 2632–2639.

Chen, Z., Maitland, M., Zhou, J., Zhang, S., Xu, H., C., and Serrell, T. C. (1997a). Phospholipase activity in Cryptococcus neoformans: a new virulence factor? J. Infect. Dis. 175, 415–422.

Chen, Z., C., Wright, L. C., Santander, R. T., Maitland, M., Meran, V. R., Kuchel, P. W., et al. (1997b). Identification of extracellular phospholipase B, sphingomyelinase, and acylphosphatase in Cryptococcus neoformans var. grubii. Fungal Genet. Biol. 24, 445–467.

Douglas, L., M., Wong, H. X., Li, L., and Kompa, J. B. (2011). Membrane compartment occupied by the phosphatidylcholine and sphingomyelin subdomains of the fungal plasma membrane. Membranes (Basel) 1, 396–411.

Du, W., and Angusworth, K. R. (2005). Membrane beta-lysophosphatidylcholine reduces accumulation of reactive oxygen species and cell death in yeast. Free Radic. Biol. Med. 40, 1478–1487.

Eggertsen, L., Hirt, T., Mudal, S. B., Schwarzmann, G., Sundhoff, B., Poljakova, S., et al. (2009). Direct observation of the rotational dynamics of membrane lipids in a living cell. Nature 457, 1159–1162.

Ferrera, C., and Lucas, C. (2008). The yeast O-acetyl-O-sphinganine Gtpase interferes with lipid metabolism with direct consequences on the sphingolipid-starch-related domains integrity/assembly. Biochim. Biophys. Acta 1778, 2640–2653.

Ferreira, I., E., Bois, A., Vandercappellen, J., A. P., Toulmé, A., and Schories, B., et al. (2009). Membranes rafts are involved in an intracellular micromolecular accumulation in yeast cells. J. Biol. Chem. 284, 52085–52095.

Freeman, M. R., and Solomon, K. R. (2004). Cholesterol and prostate cancer. J. Cell Biol. 161, 54–60.

Futerman, A. H., and Riezman, H. (2005). The ins and outs of sphingolipid metabolism. Trends Cell Biol. 15, 312–318.

Gaber, R. F., Cuppel, D. M., Kennedy, B. K., Vidal, M., and Reed, J. (1989). The yeast gene ERG5 is required for normal membrane function but is not essential for biosynthesis of the cell-cycle-stimulating sterol. Mol. Cell. Biol. 9, 3447–3456.

Gajate, C., Del Caño-Juárez, E., and Fink, G. (1991). TRIM induces a novel, broad-based fungal plasma membrane protein required for high-affinity potassium transport in Saccharomyces cerevisiae. Mol. Cell. Biol. 11, 2860–2853.

Gagge, B., Toméck, B., Corbins, L., and Schmitter, B. (2001). Synthesis of sphingolipids with very long chain fatty acids but not ergosterol is required for sorting of newly synthesized plasma membrane ATPase to the cell surface of yeast. J. Biol. Chem. 276, 22325–22332.

Gagge, B., Toméck, B., and Schmitter, B. (2000). Very long-chain fatty acid-containing lipids rather than sphingolipids per se are required for raft association and stable surface transport of newly synthesized plasma membrane ATPase in yeast. J. Biol. Chem. 275, 3145–3146.

Gajate, C., Del Caño-Juárez, E., Arenas, A. U., Amat-Gaertner, F. G., Geijo, E., Santos-Benito, A. M., et al. (2004). Intracellular triggering of Fas aggregation and recruitment of apoptotic molecules into Fas-enriched rafts in a selective tumor cell apoptosis. J. Exp. Med. 200, 353–365.

Gajate, C., Gómez-Gómez, F., and Molinillo, F. (2009a). Involvement of raft aggregates enriched in Fas/CDCP1–death-inducing signaling complex in the antiapoptotic action of edelfosine in Jurkat cells. Mol. Cell. Biol. 29, e544. doi: 10.1128/MCB.005944-09.

Gajate, C., Gómez-Gómez, F., and Molinillo, F. (2008b). Lipid raft connection between extrinsic and intrinsic apoptotic pathways. Biochim. Biophys. Acta 1784, 780–784.

Gajate, C., Mateo-de la-Serna, M., Duker, E. L., Fontenot, R. K., Alvarez, J., and Molinillo, F. (2012). Antitumor activity of the sphingomyelinase analog edelfosine induces apoptosis in pancreatic cancer by targeting sphingolipid metabolism. Oncogene 31, 2627–2639.

Gajate, C., and Molinillo, F. (2001). The antitumor ether lipid ET-18-OCH3 induces apoptosis through translocation and capping of Fas/CeDAP into membrane rafts in human leukemic cells. Blood 98, 3800–3803.

Gajate, C., and Molinillo, F. (2002). Biological mechanisms of action and preclinical/clinical prospective of the antitumor ether phospholipid ET-18-OCH3 (Edelfosine), a proapoptotic agent in tumor cells. Curr. Drug Metab. 3, 491–525.

Gajate, C., and Molinillo, F. (2005). Cryptococcus-mediated death receptor and ligand concentration in lipid rafts forms apoptosis-promoting clusters in cancer chemotherapy. J. Biol. Chem. 280, 13641–13647.

Gajate, C., and Molinillo, F. (2007). Edelfosine and perifosine induce selective apoptosis in multiple myeloma by recruitment of death receptors and downregulation signaling molecules into lipid rafts. Blood 109, 711–719.

Galan, J. M., Cantegril, B., Garnier, C., Namy, O., and Hagemann-Toupin, R. (1998). ER degradation of a mutant yeast plasma membrane protein by the ubiquitin-proteasome pathway. J. Biol. Chem. 273, 525–532.

Garnar, S., Roche, E. M., Zhang, Y. Q., Park, S., Ras, R., and Parkin, D. S. (2010). Mechanism of the synergistic effect of amiodarone and fluconazole in Candida albicans. Antimicrob. Agents Chemother. 54, 2753–2761.

Germann, M., Swain, E., Bergman, L., and Nicks, J. T. Jr. (2005). Characterizing the targeting and stabilizing pathway that remodels affected for viability upon nutrient stress and the yeast cytoskeleton: triggers, pathways, subroutines. Trends Cell Biol. 15, 547–555.

Göppert, A. U., Amat-Guerri, F., Geijo, E., Santos-Benito, A. M., et al. (2004). Sensitization of a novel, broad-based fungal plasma membrane protein required for high-affinity potassium transport in Saccharomyces cerevisiae. Mol. Cell. Biol. 24, 1753–1761.

Grossmann, G., Opekarova, M., Malinova, G., Foster-Meckl, I., and Tannner, W. (2012). Sorting of GPI-anchored proteins to lipid rafts and cell death.
(2007). Membrane potential governs lateral segregation of plasma membrane proteins and lipids in yeast. EMBO J. 26, 1–8.
Gupta, S. S., Ton, V. K., Bontadini, V., Bili, S., Cunningham, K., and Bac, R. (2010). Antithrombogenic activity of amiodarone is mediated by disruption of calcium homeostasis. J. Biol. Chem. 285, 2063–2069.
Heinemann, H. (2002). Lipid rafts promote domain formation in lipid raft mixtures. Biochim. Biophys. Acta 1527, 213–230.
Kaijser, L., Kron, H., Jaffredic, B., and Staehelin, L. (1997). Single-molecule atomic force microscopy reveals clustering of the yeast plasma-membrane sensor Wei1. Plant Physiol. 114, 1101–1105. doi: 10.1104/pp.114.3.1101
Kornelius, F., Conchonaud, F., Herzer, S., and Zerial, M. (2008). Reduced drug resistance in yeast with disrupted lipid rafts. Mol. Biol. Cell 19, 3737–3747.
Kulshrestha, A., and San Roman, J. A. (2010). Yeast multidrug resistance pump, Mdr1p, confers reduced drug resistance in yeast. J. Biol. Chem. 285, 1994–2000.
Lange, T., Huang, M. H., Ramas, B. V., and Stoic, T. L. (1999). Plasma membranes contain half the phospholipid and 90% of the cholesterol and sphingolipids in cultured human endothelial cells. J. Biol. Chem. 274, 3796–3793.
Lasserre, R., Guo, X. J., Conchonaud, F., Hamon, Y., Hotter, O., Bernard, A. M., et al. (2008). Raft nanodomains contribute to ΔψK/ER plasma membrane recruitment and activation. Nat. Chem. Biol. 4, 538–547.
Latt, A. A., Mukherjee, P. K., Chandra, J., Roth, M. R., Welti, C., Maliniak, C. A., et al. (2013). Lipid rafts in fungal cell membranes: recent findings and future directions. J. Cell Sci. 126, 4266–4273.
Lazzeri, R., Guo, X. J., Conchonaud, F., Hamon, Y., Hotter, O., Bernard, A. M., et al. (2008). Raft nanodomains contribute to ΔψK/ER plasma membrane recruitment and activation. Nat. Chem. Biol. 4, 538–547.
Latt, A. A., Mukherjee, P. K., Chandra, J., Roth, M. R., Welti, C., Maliniak, C. A., et al. (2013). Lipid rafts in fungal cell membranes: recent findings and future directions. J. Cell Sci. 126, 4266–4273.
Nichols, C. B., Fraser, J. A., and Heit-Munro, S. (2003). Lipid rafts: elusive or illusive?
Mollinedo, F., Gajate, C., Martin-Martín, B., Benitez, A., Mollinedo, F., and Gajate, C. (2010b). Selective induction of apoptosis in cancer cells by the other lipid ET-18-OCH3 (edelfosine): molecular structure requirements, cellular uptake, and protection by Bcl-2 and Bel-1 XL Cancer 57, 1520–1528.
Mollinedo, F., and Gajate, C. (2006). FadCD95 death receptor and lipid rafts: new targets for apoptosis-driven cancer therapy. Drug Resist. Updat 9, 53–73.
Mollinedo, F., and Gajate, C. (2010a). Lipid rafts and diesters of apoptotic signaling molecular-membranous rafts in cancer cells. Protein expression activity of FadCD95 death receptor. Curr. Med. Chem. 17, 3521–3530.
Mourtzou, S., and Rous, B. (2008). Fugaci-dial activity of amosidase is tightly coupled to calcium influx. FEBS Lett. 585, 425–431.
Mukherjee, P. K., Zhou, C., Munir, B., and Ghannoum, M. A. (2005). Candida albicans: a self-designed post-translational environment. Med. Mycol. 43, 191–200.
Musa, S. (2003). Lipid rafts shutdown or illusive? Curr. Biol. 13, 377–388.
Nicholls, C. B., Fraser, J. A., and Hoverman, J. (2004). PI(4,5)P2 kinase Ste20 and PI(4,5)P2 cell polarity at different stages of membrane pro- cessing in neutrophils. Mol. Biol. Cell 15, 4487–4496.
Nieto-Miguel, T., Gajate, C., Gonzalez-Camacho, F., and Mollinedo, E. (2008). Proapoptotic role of Hsp90 by its interaction with c-kin- terminal kinase in lipid rafts in edelfosine-modulated antiluemic therapy. Oncogene 27, 1779–1787.
Olivera-Guindo, A., Grau, M., Harapé, L., and Aguadé, F. (2011). The exosome core is composed of RIL domains. Mol. Biol Cell 22, 2360–2372.
Perris, A., Krishnamurthy, S., Prasad, T., Erritzø, F., and Prasad, R. (2007a). Squamous epidermis ocluded by ERG1 affects meiosis and sprawl susceptibilities of Candida albicans. J. Antimicrob. Chemother. 59, 905–913.
Perris, R., Pinarut, S. L., and Prasad, R. (2008). Multidrug transporters CaGDSr and CaGDS in Candida albicans display different lipid specificities: both ergosterol and sphingolipids are essential for targeting of CaGDSr to membrane rafts. Antimicrob. Agents Chemother. 52, 698–704.
Perris, R., Prasad, T., and Prasad, R. (2008b). Multidrug transporters CaGDSr and CaGDS in Candida albicans display different lipid specificities: both ergosterol and sphingolipids are essential for targeting of CaGDSr to membrane rafts. Antimicrob. Agents Chemother. 52, 698–704.
Pettigrew, K., and Tornquist, G. (2012). Monolayer spreading of a single-quantum dot tracking. Nano Lett. 12, 557–564.
Pike, I. J. (2006). Rafts defined: a report for understanding lysosome-membrane interaction tandem mass spectrometry (ESI-MS/MS) analysis of the lipid molecular species composition of yeast subcellular membranes reveals distinct molecular species on route to the plasma membrane. J. Biol. Chem. 281, 21084–21094.
Portillo, F. (2000). Regulation of plasma membrane H-+ATPase in fungi and plants. Biochim. Biophys. Acta 1469, 31–42.
Prasad, T., Saini, G., Prasad, T., Erritzø, F., and Prasad, R. (2007b). Squamous epidermis ocluded by ERG1 affects meiosis and spread susceptibilities of Candida albicans. J. Antimicrob. Chemother. 59, 905–913.
Presty, T. J., Klemm, R., Bignard, M., Guin, K., and Simmen, K. (2006). Plasma membrane polarization during mitaging in yeast cells. J. Cell Biol. 175, 861–877.
Rajagopalan, S., Wachter, V., and Bala-sbabaraman, M. (2005). Cytoskeleton in fission yeast: a story of rings, rafts and walls. Trends Genet. 21, 413–418.
Ribeiro, G. F., Corte-Real, M., and Madeira, V. M. (2000). Lipid rafts and clusters of apoptotic mitochondria: a well-designed pro- opening. Mol. Biol. Cell 11, 356–363.
Pike, I. J., and Laster, R. L. (1993). The phosphophanolipid sphingolipids of Saccharomyces cerevisiae are highly localized in the plasma membrane. J. Biol. Chem. 268, 3101–3108.
Perera, C., Silva, R. D., Sarabia, L., Johansson, S., Sosa, J. L., and Corte-Real, M. (2008). Mitochondria-dependent apoptosis in yeast. Biochim. Biophys. Acta 1783, 1286–1292.
Peters, D., Cellino-Ensebar, D., and Mollinedo, F. (1995). Intracellular alkalisation suppresses leucovorin-induced apoptosis in HEL-60 cells by the maximisation of a pH-dependent endo-susceptibility. J. Biol. Chem. 270, 6253–6257.
Pinto, J., Saito-Young, D., and Haber, J. E. (1989). Defective H2-ATPase of hyporesistant mutant-derivatives of Saccharomyces cerevisiae. J. Biol. Chem. 264, 24540–24546.
Pikulev, D. I. (2006). Rafts defined: a report for understanding lysosome-membrane interaction tandem mass spectrometry (ESI-MS/MS) analysis of the lipid molecular species composition of yeast subcellular membranes reveals distinct molecular species on route to the plasma membrane. J. Biol. Chem. 281, 21084–21094.
Pike, I. J. (2006). Rafts defined: a report for understanding lysosome-membrane interaction tandem mass spectrometry (ESI-MS/MS) analysis of the lipid molecular species composition of yeast subcellular membranes reveals distinct molecular species on route to the plasma membrane. J. Biol. Chem. 281, 21084–21094.
Pike, I. J. (2006). Rafts defined: a report for understanding lysosome-membrane interaction tandem mass spectrometry (ESI-MS/MS) analysis of the lipid molecular species composition of yeast subcellular membranes reveals distinct molecular species on route to the plasma membrane. J. Biol. Chem. 281, 21084–21094.
Pike, I. J. (2006). Rafts defined: a report for understanding lysosome-membrane interaction tandem mass spectrometry (ESI-MS/MS) analysis of the lipid molecular species composition of yeast subcellular membranes reveals distinct molecular species on route to the plasma membrane. J. Biol. Chem. 281, 21084–21094.
Pike, I. J. (2006). Rafts defined: a report for understanding lysosome-membrane interaction tandem mass spectrometry (ESI-MS/MS) analysis of the lipid molecular species composition of yeast subcellular membranes reveals distinct molecular species on route to the plasma membrane. J. Biol. Chem. 281, 21084–21094.
Pike, I. J. (2006). Rafts defined: a report for understanding lysosome-membrane interaction tandem mass spectrometry (ESI-MS/MS) analysis of the lipid molecular species composition of yeast subcellular membranes reveals distinct molecular species on route to the plasma membrane. J. Biol. Chem. 281, 21084–21094.
Pike, I. J. (2006). Rafts defined: a report for understanding lysosome-membrane interaction tandem mass spectrometry (ESI-MS/MS) analysis of the lipid molecular species composition of yeast subcellular membranes reveals distinct molecular species on route to the plasma membrane. J. Biol. Chem. 281, 21084–21094.
Pike, I. J. (2006). Rafts defined: a report for understanding lysosome-membrane interaction tandem mass spectrometry (ESI-MS/MS) analysis of the lipid molecular species composition of yeast subcellular membranes reveals distinct molecular species on route to the plasma membrane. J. Biol. Chem. 281, 21084–21094.
Pike, I. J. (2006). Rafts defined: a report for understanding lysosome-membrane interaction tandem mass spectrometry (ESI-MS/MS) analysis of the lipid molecular species composition of yeast subcellular membranes reveals distinct molecular species on route to the plasma membrane. J. Biol. Chem. 281, 21084–21094.
Pike, I. J. (2006). Rafts defined: a report for understanding lysosome-membrane interaction tandem mass spectrometry (ESI-MS/MS) analysis of the lipid molecular species composition of yeast subcellular membranes reveals distinct molecular species on route to the plasma membrane. J. Biol. Chem. 281, 21084–21094.
Pike, I. J. (2006). Rafts defined: a report for understanding lysosome-membrane interaction tandem mass spectrometry (ESI-MS/MS) analysis of the lipid molecular species composition of yeast subcellular membranes reveals distinct molecular species on route to the plasma membrane. J. Biol. Chem. 281, 21084–21094.
Pike, I. J. (2006). Rafts defined: a report for understanding lysosome-membrane interaction tandem mass spectrometry (ESI-MS/MS) analysis of the lipid molecular species composition of yeast subcellular membranes reveals distinct molecular species on route to the plasma membrane. J. Biol. Chem. 281, 21084–21094.
Vyas, N., Goswami, D., Manonmani, T., Ulha, J., Faria-Oliveira, F., Lucas, C., van Zanten, T., Cambi, A., and Toulmay, A., and Schneiter, R. (2007). Tosi, M. R., and Tugnoli, V. (2005). www.frontiersin.org

Tozet, C. P., and Drubin, D. G. (2006). Mollinedo, Yeast lipid rafts and cell death

VijayRaghavan, K., et al. (2008). Cell is essential for long-range signaling.

Ergosterol is required for targeting

Gene.

is hampered by the deletion of GUP1

Ferreira, C. (2012). Programmed cell death in Saccharomyces cerevisiae is hampered by the deletion of GUP1 gene. BMC Microbiol. 12, 80. doi: 10.1186/1471-2180-12-80

Umemura, K., and Nakano, A. (2005). Ergosteryl is required for targeting of tryptophan permease to the yeast plasma membrane. J. Cell Biol. 161, 1117–1131.

van Moer, G. (1989). Lipid traffic in animal cells. Annu. Rev. Cell Biol. 5, 247–275.

van Zanten, T. S., Cambi, A., and Garcia-Parajo, M. F. (2010). A nanometer scale optical view on the compartmentalization of cell membranes. Baculum. Bacteriol. Acta 1798, 777–797.

Vyas, N., Gweon, D., Maronnani, A., Sharma, P., Rangarath, H. A., VijayRaghavan, K., et al. (2008). Nanoscale organization of hedgehog is essential for long-range signaling. G3 135, 1214–1227.

Wachter, V., and Bala-Subramaniam, M. R. (2003). Sterol-rich plasma membrane domains in the fission yeast Schizosaccharomyces pombe. J. Cell Biol. 161, 1117–1131.

This article was submitted to Frontiers in Molecular and Cellular Oncology, a specialty of Frontiers in Oncology. Copyright © 2012 Mollinedo. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and subject to any copyright notices concerning any third-party graphics etc.