Lipid-dependent surface transport of the proton pumping ATPase: A model to study plasma membrane biogenesis in yeast

Alexandre Toulmay, Roger Schneiter*

Department of Medicine, Division of Biochemistry, University of Fribourg, Chemin du Musée 5, CH-1700 Fribourg, Switzerland

Abstract

The proton pumping H^+-ATPase, Pma1, is one of the most abundant integral membrane proteins of the yeast plasma membrane. Pma1 activity controls the intracellular pH and maintains the electrochemical gradient across the plasma membrane, two essential cellular functions. The maintenance of the proton gradient, on the other hand, also requires a specialized lipid composition of this membrane. The plasma membrane of eukaryotic cells is typically rich in sphingolipids and sterols. These two lipids condense to form less fluid membrane microdomains or lipid rafts. The yeast sphingolipid is peculiar in that it invariably contains a saturated very long-chain fatty acid with 26 carbon atoms. During cell growth and plasma membrane expansion, both C26-containing sphingolipids and Pma1 are first synthesized in the endoplasmatic reticulum from where they are transported by the secretory pathway to the cell surface. Remarkably, shortening the C26 fatty acid to a C22 fatty acid by mutations in the fatty acid elongation complex impairs raft association of newly synthesized Pma1 and induces rapid degradation of the ATPase by rerouting the enzyme from the plasma membrane to the vacuole, the fungal equivalent of the lysosome. Here, we review the role of lipids in mediating raft association and stable surface transport of the newly synthesized ATPase, and discuss a model, in which the newly synthesized ATPase assembles into a membrane environment that is enriched in C26-containing lipids already in the endoplasmatic reticulum. The resulting protein−lipid complex is then transported and sorted as an entity to the plasma membrane. Failure to successfully assemble this lipid−protein complex results in mistargeting of the protein to the vacuole.

Keywords: Protein transport; Membrane microdomains (rafts); Secretory pathway; Plasma membrane; Very long-chain fatty acids; *Saccharomyces cerevisiae*

1. Introduction

The plasma membrane constitutes the interface of the cell with its environment. This membrane thus protects the cell against a potential harsh and hostile environment. At the same time, nutrient transporters located at the plasma membrane must allow controlled uptake of small molecules and essential cofactors that may be present in that environment. The action of these transporters relies on an electrochemical gradient across the membrane, which is maintained by abundant ion pumps such as the Na^+/K^+-ATPase in mammalian cells or the proton pumping H^+-ATPase in yeast. Efficient maintenance of the resulting electrochemical gradient, however, requires specialized lipids in that membrane, particularly sterols and sphingolipids. These two lipids form condensed complexes that aggregate into membrane microdomains or lipid rafts, which may not only locally increase the permeability barrier of the membrane, but also play more direct roles in protein and membrane sorting along the exocytic and endocytic pathways. The fact that the plasma membrane grows and expands through the biogenesis of its components, that is both lipids and integral membrane proteins, in the endoplasmatic reticulum (ER), suggests that biogenesis and surface transport of proteins and lipids may be coupled. Evidence for such a coupling between protein, lipid biogenesis, and transport, however, is rare.

Here, we review recent results that indicate an important role of lipids in oligomerization, raft association, and surface

Abbreviations: COPII, coat protein complex II; ER, endoplasmatic reticulum; GPI, glycosylphosphatidylinositol; PI, phosphatidylinositol.

* Corresponding author. Tel.: +41 26 300 8654; fax +41 26 300 9735.
E-mail address: roger.schneiter@unifr.ch (R. Schneiter).

transport of the proton pumping ATPase in *Saccharomyces cerevisiae* and discuss a model in which the newly translocated protein acquires a lipid microenvironment that corresponds to that of its future host membrane already in the ER. In such a model, Pma1 is then transported together with its ''lipid shell'' enriched in C26-containing lipids to the plasma membrane where it can stably integrate only if its lipid shell has been properly formed prior to its arrival at the plasma membrane.

2. Biogenesis and transport of the proton pumping H^+-ATPase

The proton pumping H^+-ATPase, Pma1, belongs to the P-type of cation transporting ATPases, which includes the Na^+/K^+-ATPase and Ca^{2+}-ATPase of the mammalian plasma membrane [1]. A characteristic feature of P-type ATPases is that a strictly conserved aspartate residue is phosphorylated during the reaction cycle. Pma1 is a 100-kDa abundant and long-lived polytopic membrane protein of the yeast plasma membrane. Its activity is essential for regulating intracellular pH and for uptake of nutrients by plasma membrane symporters. Due to its abundance at the cell surface, where Pma1 accounts for more than 25% of all the proteins, the protein constitutes a major cargo of the secretory pathway. Thus Pma1 serves as an excellent model to study plasma membrane biogenesis, that is the coordinated synthesis, assembly and transport of both the protein and lipid constituents of the future plasma membrane.

As with other integral membrane proteins destined for the plasma membrane, Pma1 is biosynthetically inserted into the membrane of the ER, from where it is transported by vesicular carriers to its final destination [2,3]. Already in the ER, Pma1 forms a large 1.8-MDa homo-oligomeric complex that resists extraction by detergents [4]. This protein–lipid complex is then packaged into a larger subclass of COPII (coat protein complex II) transport vesicles that contain both Sec24 and its homologue, Lst1 as coat components [5]. Even though *LST1* is not essential, cells lacking *LST1* are sensitive to low pH because of a reduced flux of Pma1 out of the ER [5]. During transport, Pma1 is not modified by glycosylation or proteolysis, but the protein becomes phosphorylated on multiple serine and threonine residues [2,3]. The significance of these phosphorylations is still unknown, but there is evidence that at least one of these phosphorylations that occurs at or near the plasma membrane is important for glucose-dependent activation of the enzyme [3,6].

From the Golgi complex, Pma1 is transported to the cell surface by a branch of the secretory pathway that does not intersect with endosomes [7,8]. At the cell surface, Pma1 becomes stabilized by a poorly characterized mechanism and occupies detergent-resistant domains that are distinct from those occupied by the arginine/H^+ symporter Can1 [9,10]. From the plasma membrane, Pma1 is finally recycled and turned-over by endocytic delivery to the vacuole.

Because of its importance for cell viability, Pma1 is subject to extensive studies aimed at understanding its mechanism of action as well as its potential as a drug target for antifungal agents. Numerous mutants that affect its catalytic activity, quality control in the ER and/or trafficking to the cell surface have been generated and characterized [11,12].

Two of these mutants are of special interest as they affect the stability, targeting, and detergent solubility of the mutant enzyme during its transport to the surface. The first of these, Pma1-7 is a temperature-sensitive mutant allele of Pma1 that is mistargeted from the Golgi to the vacuole under non-permissive conditions without reaching the cell surface [13]. Increasing the residence time of Pma1-7 in the ER, as is the case in mutants lacking the ER membrane protein Sop4, partially restores cell surface delivery of the mutant ATPase [14]. The molecular function of Sop4, however, remains to be established. At the Golgi apparatus, sorting of Pma1-7 is controlled by a ubiquitin-dependent quality control system composed of an ubiquitin ligase complex containing Rsp5 and Bul1,2 [15]. From the Golgi, rerouting of the mutant Pma1-7 to the cell surface is restored upon inactivation of the biosynthetic pathway to the vacuole or by sorting defects within the endosomal system [16,17]. Interestingly, surface delivery of Pma1-7 can also be restored by overexpression of Ast1 a peripheral membrane protein that directly interacts with Pma1 and induces clustering of Pma1-7 into SDS/Triton X-100 resistant oligomers [13,18].

The second ATPase allele, Pma1-10, is also temperature-sensitive, but in this case, the mutant protein follows the secretory pathway to the cell surface but fails to become stabilized there. Instead, it is endocytosed in an ubiquitin-dependent manner and delivered to the vacuole for degradation. Pma1-10 is hypophosphorylated and fails to associate with detergent insoluble domains at the non-permissive temperature. Indicating that phosphorylation and lipid raft association may play important roles in maintaining protein stability at the plasma membrane [19,20]. This view is consistent with the fact that disruption of membrane microdomains at the plasma membrane by incubation with the anti-cancer drug edelfosine, an alkylglycerophosphocholine, disrupts raft association of plasma membrane localized Pma1 and induces its vacuolar turnover [21].

3. Coupling of H^+-ATPase biogenesis to sphingolipid synthesis

Similar to the synthesis of integral membrane proteins, the synthesis of sphingolipids commences in the ER, where serine palmitoyltransferase catalyzes the condensation of serine with palmitoyl-CoA to form a long-chain base. The long-chain base then condenses with a C26 very long-chain fatty acid to form ceramide, a reaction that is catalyzed by the ER localized ceramide synthase. From the ER, ceramide is transported both by vesicular and non-vesicular routes to the Golgi apparatus, where it is converted to sphingolipids [22,23]. Mature sphingolipids are then transported to the plasma membrane, where they are highly enriched [24,25] (Fig. 1).

Work by the Schekman and Chang laboratories has established that the biogenesis of Pma1 depends on ongoing

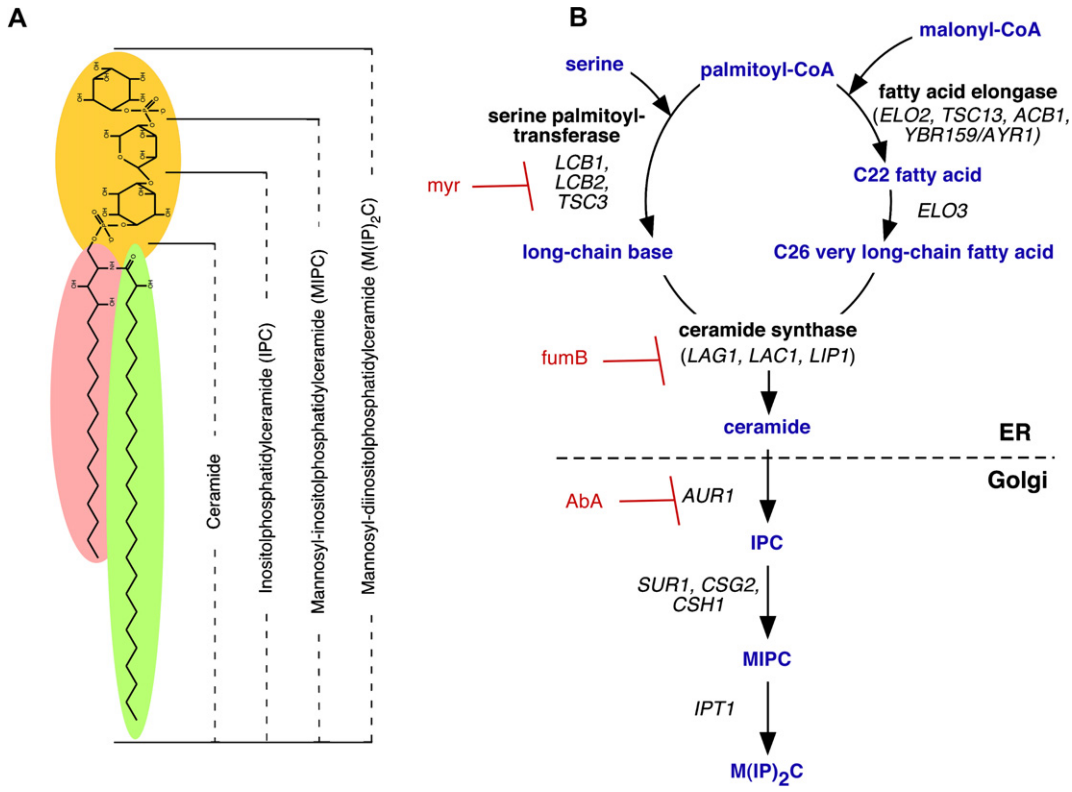

Fig. 1. Structure of yeast sphingolipids and outline of the sphingolipid biosynthetic pathway. (A) Structure of the yeast sphingolipid. The long-chain base is marked in pink with the amide-bound C26 fatty acid in green. The hydrophilic head group is marked in yellow. Ceramide and the three major sphingolipids, inositolphosphatidylceramide (IPC), mannosyl-inositolphosphatidylceramide (MIPC), and mannosyl-diinositolphosphatidylceramide ($M(IP)_2C$) are indicated to the right. (B) Schematic diagram of the sphingolipid pathway. Inhibitory drugs are indicated in red. See the reviews cited in the text for further details. Abbreviations are: myr, myriocin; fumB, fumonisin B1; AbA, aureobasidin A.

sphingolipid synthesis. Using either a temperature-sensitive allele of serine palmitoyltransferase, *lcb1-100*, or myriocin to block serine palmitoyltransferase activity, these groups showed that ongoing long-chain base synthesis is required for oligomerization of Pma1 in the ER membrane and for its association with lipid rafts [4]. In the absence of long-chain base synthesis, monomeric, non-raft associated Pma1 is still exported from the ER but it is mistargeted to the vacuole and degraded [4,18]. Inhibition of the conversion of ceramide to sphingolipids by the use of aureobasidin A, an inhibitor of phosphatidylinositol:ceramide phosphoinositol transferase (IPC synthase, encoded by *AUR1*), however, does not affect Pma1 oligomerization, indicating that long-chain base or ceramide synthesis, but not sphingolipid synthesis per se, is required for oligomerization of Pma1 [4]. The formation of an oligomeric complex in the ER is consistent with the fact that co-expression of dominant-negative mutant forms of Pma1 results in the retention of both the mutant and the wild-type proteins in the ER [26]. Formation of mixed oligomers between the normal and mutant proteins could lead to the recognition of the entire complex as misfolded and subject to ER quality control. Such a model is supported by the observation that assembly of the multimeric Pma1 complex is dependent on long-chain base synthesis and that a block in long-chain base synthesis relieves the dominant lethal phenotype of a Pma1 mutant [27].

We became interested in the role of lipids in surface transport of Pma1 because we observed that Pma1 was rapidly degraded in cells that fail to elongate the ceramide-bound C22 fatty acid to the mature C26 very long-chain fatty acid, as is the case in cells lacking *ELO3*, a component of the ER localized acyl chain elongase [28,29]. Interestingly, this rapid turnover of Pma1 in the *elo3Δ* mutant correlates with a lack of the newly synthesized protein to acquire detergent resistance [29] (Fig. 2). Turnover of Pma1 in *elo3Δ* is dependent on ongoing endocytosis, indicating that the protein reaches the plasma membrane first, but that it fails to become stabilized there and instead is endocytosed and delivered to the vacuole for degradation [29]. Thus the wild-type ATPase in the *elo3Δ* mutant background behaves similarly as the conditional Pma1-10 allele with regard to its increased turnover that correlates with a failure to acquire detergent resistance [19].

Interestingly, *elo3Δ* mutant cells also affect the biogenesis of the two populations of post-Golgi vesicles. In wild-type cells these two vesicle populations have different densities. The high-density vesicles contain invertase and acid phosphatase and intersect with the endosomal system, whereas the low-density vesicles contain Pma1 and glucanase and are directly transported to the cell surface without intersection with endosomes [7]. Elongase mutants on the other hand fail to generate these two vesicle populations and instead missort invertase and acid phosphatase into the Pma1 containing

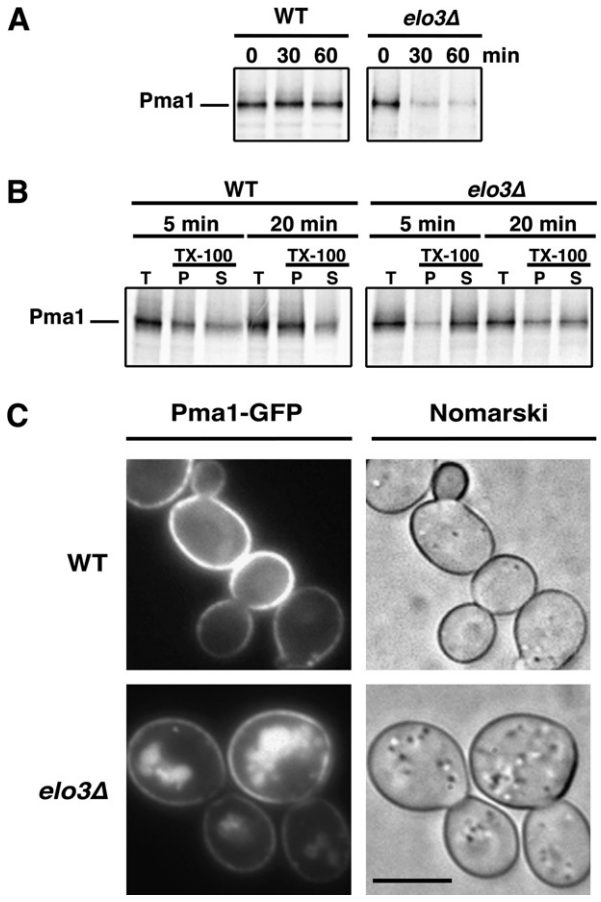

Fig. 2. C26 fatty acids are required for biogenesis of Pma1. (A) Newly synthesized Pma1 is rapidly degraded in *elo3Δ*. Pulse−chase analysis of the stability of Pma1 in wild-type (WT) and elongase (*elo3Δ*) mutant cells. Cells were cultivated at 24 °C, shifted to 37 °C for 15 min prior to pulse-labeling and immunoprecipitation of Pma1p. (B) Newly synthesized Pma1 fails to acquire detergent resistance in *elo3Δ*. Association of newly synthesized Pma1p with lipid rafts was examined by a pulse−chase experiment, in which samples (T, total) taken at 5 or 20 min after addition of chase are extracted with Triton X-100 and separated into a soluble (S) and detergent insoluble (P) fraction. (C) Pma1 is mistargeted to the vacuole in *elo3Δ*. Cells expressing functional GFP-tagged version of Pma1 were cultivated at 24 °C, and then incubated at 37 °C for 2 h before examination by fluorescence microscopy (bar, 5 μm).

vesicle [30]. Whether the stability of Pma1, once delivered to the plasma membrane, is affected by the mixing of the two vesicle populations is presently not known.

More precise analysis of the requirement for sphingolipids in surface transport and stabilization of Pma1 then revealed that all mutations that affect C26 synthesis result in rapid turnover of newly synthesized Pma1 [31]. Increased turnover of Pma1 in these mutants is always accompanied by a lack of the newly synthesized protein to acquire detergent resistance [31]. Remarkably other mutations that affect the structure of the sphingolipid head group or its hydroxylation pattern did not affect rafts association or turnover of Pma1 [31]. These results thus suggested that the synthesis of C26-containing lipids rather than ceramide or sphingolipids per se are important for stable delivery of newly synthesized Pma1 to the cell surface and for its raft association. To test this hypothesis, we took advantage of a strain that is viable even without synthesizing long-chain base or ceramide and sphingolipids [32]. This so-called ‘‘suppressor strain’’, bears a dominant mutation in an acyltransferase, Slc1, that allows the enzyme to synthesize unusual C26-containing phosphatidylinositol (PI) species. These C26-containing PIs thus replace the essential function of sphingolipids and structurally and functionally mimic sphingolipids [33]. Remarkably, analysis of Pma1 stability in this suppressor strain revealed that newly synthesized ATPase is stably delivered to the cell surface and that it acquires detergent resistance [34]. Shortening the C26 fatty acid on these suppressor lipids by means of an *elo3Δ* mutation, however, neutralized the suppressor activity of these lipids, resulting in the rapid turnover of Pma1 and the newly synthesized Pma1 failed to acquire detergent resistance [34]. These results thus strongly indicate that lipids containing C26 fatty acids, either ceramide or glycerophospholipid bound are important for stable biogenesis and raft association of Pma1.

Sphingolipids associate with sterols to form membrane domains that resist detergent extraction [35]. To test whether synthesis of the fungal sterol, ergosterol, is important for Pma1 biogenesis and acquisition of detergent resistance, the stability of newly synthesized Pma1 was examined in viable mutants in the post-squalene part of the ergosterol biosynthetic pathway. Pulse−chase analysis revealed that Pma1 was stable in all the viable mutants that affect ergosterol biosynthesis. Even a total block of the ergosterol synthesis induced by terbinafine, an inhibitor of squalene epoxidase, did not affect the stability of newly made Pma1 [31]. These results thus suggest that the lipid microdomains that Pma1 acquires to resist detergent extraction do not need ergosterol for their formation and hence are distinct from the classical sterol- and sphingolipid-rich domains that are known from mammalian cells [35]. This notion is further supported by the observation that the ceramide backbone on the yeast sphingolipids is dispensable for the function of sphingolipids in Pma1 biogenesis since Pma1 is stable in cells synthesizing C26-containing suppressor lipids instead of ceramide and sphingolipids. On the other hand, genetic interactions between mutations in *ELO3* and defects in certain sterol modifications, indicate that in vivo, sphingolipid−sterol interactions are affected by the C26 acyl chain on the sphingolipid [29].

4. What is the function of C26-containing lipids?

C26-containing lipids are a hallmark of the yeast plasma membrane. Synthesis of these lipids, however, occurs in the ER where both the fatty acid elongase and the ceramide synthase are located. Thus, similar to the integral membrane proteins that are destined to the cell surface, C26-containing ceramide/sphingolipids must travel from the ER to the plasma membrane. The fact that these lipids affect detergent solubility of newly synthesized Pma1 already upon ER exit would indicate that lipids and protein may already assemble at their site of synthesis and are then co-transported to the surface [4]. A failure to properly assemble this protein−lipid complex results either in a diversion of surface destined vesicle to the

vacuole, a failure in stabilization of the complex upon arrival at the plasma membrane, or both [18,19,27].

Our observations would indicate that C26-containing lipids are essential for the formation of functional lipid−protein complexes. The precise function that C26 fulfills in this assembly, however, remains to be defined. It has been suggested that the length of the transmembrane domain of proteins along the secretory pathway may increase to match bilayers of increasing ''thickness'' [23]. In such a model, the abundance of C26-containing lipids may determine the thickness of membranes along the secretory pathway and thereby affect the sorting of integral membrane proteins. Biophysical studies with pure lipid bilayers, on the other hand, indicate that lipids with highly asymmetric acyl chains can interdigitate into the hydrophobic core of the opposite half of the bilayer and thus do not necessarily increase the thickness of the bilayer [36]. In addition, membrane proteins themselves have recently been shown to modulate bilayer thickness by more than 10% [37], and observation that is consistent with the proposition that the thickness of the lipid component of a biological membrane must not naturally match that of the embedded proteins [38]. Based on these considerations, we believe it is premature to correlate acyl chain length with bilayer thickness in biological membranes. Alternative functions of the C26 acyl chains could be to interdigitate into the opposite leaflet thereby (i) coupling the two halves of the bilayer to lower the energy required to deform this potentially stiff cholesterol-rich membrane and (ii) increase acyl chain packing density to prevent permeability by small molecules.

Better understanding of the role that these asymmetrical C26-containing lipids have on protein sorting to the plasma membrane is important because *elo3Δ* mutant cells not only affect Pma1 stability but also show delayed ER to Golgi transport of the GPI-anchored protein Gas1 and defective surface transport of an artificial cargo protein [30,39]. We have previously characterized the biophysical properties of an inositol glycerophospholipid with a C26 fatty acid in position *sn-1* and shown that this lipid is very potent in stabilizing highly curved membrane structures [40]. Even though we currently do not know whether a phosphatidylinositol with a C26 acyl chain in position *sn-2*, as made in strains producing the C26-containing suppressor lipids, or a C26-containing ceramide would exhibit similar biophysical properties, it is tempting to speculate that the effect on membrane curvature is a general property of these highly asymmetrical lipids. One of the essential functions these lipids may fulfill could thus be to stabilize highly curved membrane domains that are transiently formed during vesicle budding and fusion along the secretory pathway. Biophysical experiments to compare the properties of such highly asymmetric lipids will hopefully be instructive to better define the function of these lipids in vivo.

Acknowledgements

We thank Rashi Tiwari for careful proofreading of this manuscript, and the Swiss National Science Foundation (631-065925) for financial support. We apologize for the primary literature that could not be cited due to space limitations.

References

[1] W. Kühlbrand, Biology, structure and mechanism of P-type ATPases, Nat. Rev. Mol. Cell Biol. 5 (2004) 282−295.
[2] C.L. Holcomb, W.J. Hansen, T. Etcheverry, R. Schekman, Secretory vesicles externalize the major plasma membrane ATPase in yeast, J. Cell Biol. 106 (1988) 641−648.
[3] A. Chang, C.W. Slayman, Maturation of the yeast plasma membrane [H+]ATPase involves phosphorylation during intracellular transport, J. Cell Biol. 115 (1991) 289−295.
[4] M.C. Lee, S. Hamamoto, R. Schekman, Ceramide biosynthesis is required for the formation of the oligomeric H+-ATPase Pma1p in the yeast endoplasmic reticulum, J. Biol. Chem. 277 (2002) 22395−22401.
[5] K.J. Roberg, M. Crotwell, P. Espenshade, R. Gimeno, C.A. Kaiser, *LST1* is a SEC24 homologue used for selective export of the plasma membrane ATPase from the endoplasmic reticulum, J. Cell Biol. 145 (1999) 659−672.
[6] A. Goossens, N. de La Fuente, J. Forment, R. Serrano, F. Portillo, Regulation of yeast H(+)-ATPase by protein kinases belonging to a family dedicated to activation of plasma membrane transporters, Mol. Cell Biol. 20 (2000) 7654−7661.
[7] E. Harsay, R. Schekman, A subset of yeast vacuolar protein sorting mutants is blocked in one branch of the exocytic pathway, J. Cell Biol. 156 (2002) 271−285.
[8] S. Gurunathan, D. David, J.E. Gerst, Dynamin and clathrin are required for the biogenesis of a distinct class of secretory vesicles in yeast, EMBO J. 21 (2002) 602−614.
[9] M. Bagnat, S. Keranen, A. Shevchenko, K. Simons, Lipid rafts function in biosynthetic delivery of proteins to the cell surface in yeast, Proc. Natl. Acad. Sci. USA 97 (2000) 3254−3259.
[10] K. Malinska, J. Malinsky, M. Opekarova, W. Tanner, Visualization of protein compartmentation within the plasma membrane of living yeast cells, Mol. Biol. Cell 14 (2003) 4427−4436.
[11] P. Morsomme, C.W. Slayman, A. Goffeau, Mutagenic study of the structure, function and biogenesis of the yeast plasma membrane H(+)-ATPase, Biochim. Biophys. Acta 1469 (2000) 133−157.
[12] T. Ferreira, A.B. Mason, C.W. Slayman, The yeast Pma1 proton pump: a model for understanding the biogenesis of plasma membrane proteins, J. Biol. Chem. 276 (2001) 29613−29616.
[13] A. Chang, G.R. Fink, Targeting of the yeast plasma membrane [H+]ATPase: a novel gene AST1 prevents mislocalization of mutant ATPase to the vacuole, J. Cell Biol. 128 (1995) 39−49.
[14] W.J. Luo, X.H. Gong, A. Chang, An ER membrane protein, Sop4, facilitates ER export of the yeast plasma membrane [H+]ATPase, Pma1, Traffic 3 (2002) 730−739.
[15] M. Pizzirusso, A. Chang, Ubiquitin-mediated targeting of a mutant plasma membrane ATPase, Pma1-7, to the endosomal/vacuolar system in yeast, Mol. Biol. Cell 15 (2004) 2401−2409.
[16] W. Luo, A. Chang, Novel genes involved in endosomal traffic in yeast revealed by suppression of a targeting-defective plasma membrane ATPase mutant, J. Cell Biol. 138 (1997) 731−746.
[17] W. Luo, A. Chang, An endosome-to-plasma membrane pathway involved in trafficking of a mutant plasma membrane ATPase in yeast, Mol. Biol. Cell 11 (2000) 579−592.
[18] M. Bagnat, A. Chang, K. Simons, Plasma membrane proton ATPase Pma1p requires raft association for surface delivery in yeast, Mol. Biol. Cell 12 (2001) 4129−4138.
[19] X. Gong, A. Chang, A mutant plasma membrane ATPase, Pma1-10, is defective in stability at the yeast cell surface, Proc. Natl. Acad. Sci. USA 98 (2001) 9104−9109.
[20] Y. Liu, A. Chang, Quality control of a mutant plasma membrane ATPase: ubiquitylation prevents cell-surface stability, J. Cell Sci. 119 (2006) 360−369.

[21] V. Zaremberg, C. Gajate, L.M. Cacharro, F. Mollinedo, C.R. McMaster, Cytotoxicity of an anti-cancer lysophospholipid through selective modification of lipid raft composition, J. Biol. Chem. 280 (2005) 38047−38058.

[22] K. Funato, H. Riezman, Vesicular and nonvesicular transport of ceramide from ER to the Golgi apparatus in yeast, J. Cell Biol. 155 (2001) 949−959.

[23] T.P. Levine, C.A. Wiggins, S. Munro, Inositol phosphorylceramide synthase is located in the Golgi apparatus of *Saccharomyces cerevisiae*, Mol. Biol. Cell 11 (2000) 2267−2281.

[24] R. Schneiter, Brave little yeast, please guide us to Thebes: sphingolipid function in *S. cerevisiae*, Bioessays 21 (1999) 1004−1010.

[25] R.C. Dickson, C. Sumanasekera, R.L. Lester, Functions and metabolism of sphingolipids in *Saccharomyces cerevisiae*, Prog. Lipid Res., in press.

[26] S.L. Harris, S. Na, X. Zhu, D. Seto-Young, D.S. Perlin, J.H. Teem, J.E. Haber, Dominant lethal mutations in the plasma membrane H(+)-ATPase gene of *Saccharomyces cerevisiae*, Proc. Natl. Acad. Sci. USA 91 (1994) 10531−10535.

[27] Q. Wang, A. Chang, Sphingoid base synthesis is required for oligomerization and cell surface stability of the yeast plasma membrane ATPase, Pma1, Proc. Natl. Acad. Sci. USA 99 (2002) 12853−12858.

[28] C.S. Oh, D.A. Toke, S. Mandala, C.E. Martin, *ELO2* and *ELO3*, homologues of the *Saccharomyces cerevisiae ELO1* gene, function in fatty acid elongation and are required for sphingolipid formation, J. Biol. Chem. 272 (1997) 17376−17384.

[29] M. Eisenkolb, C. Zenzmaier, E. Leitner, R. Schneiter, A specific structural requirement for ergosterol in long-chain fatty acid synthesis mutants important for maintaining raft domains in yeast, Mol. Biol. Cell 13 (2002) 4414−4428.

[30] D. David, S. Sundarababu, J.E. Gerst, Involvement of long chain fatty acid elongation in the trafficking of secretory vesicles in yeast, J. Cell Biol. 143 (1998) 1167−1182.

[31] B. Gaigg, B. Timischl, L. Corbino, R. Schneiter, Synthesis of sphingolipids with very long chain fatty acids but not ergosterol is required for routing of newly synthesized plasma membrane ATPase to the cell surface of yeast, J. Biol. Chem. 280 (2005) 22515−22522.

[32] R.C. Dickson, G.B. Wells, A. Schmidt, R.L. Lester, Isolation of mutant *Saccharomyces cerevisiae* strains that survive without sphingolipids, Mol. Cell Biol. 10 (1990) 2176−2181.

[33] R.L. Lester, G.B. Wells, G. Oxford, R.C. Dickson, Mutant strains of *Saccharomyces cerevisiae* lacking sphingolipids synthesize novel inositol glycerophospholipids that mimic sphingolipid structures, J. Biol. Chem. 268 (1993) 845−856.

[34] B. Gaigg, A. Toulmay, R. Schneiter, 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., in press.

[35] K. Simons, W.L. Vaz, Model systems, lipid rafts, and cell membranes, Annu. Rev. Biophys. Biomol. Struct. 33 (2004) 269−295.

[36] S.W. Hui, T.J. Mason, C.-H. Huang, Acyl chain interdigitation in saturated mixed chain phosphatidylcholine bilayer dispersions, Biochemistry 23 (1984) 5570−5577.

[37] K. Mitra, I. Ubarretxena-Belandia, T. Taguchi, G. Warren, D.M. Engelman, Modulation of the bilayer thickness of exocytic pathway membranes by membrane proteins rather than cholesterol, Proc. Natl. Acad. Sci. USA (2004) 4083−4088.

[38] O.G. Mouritsen, M. Bloom, Models of lipid−protein interactions in membranes, Annu. Rev. Biophys. Biomol. Struct. 22 (1993) 145−171.

[39] T.J. Proszynski, R.W. Klemm, M. Gravert, P.P. Hsu, Y. Gloor, J. Wagner, K. Kozak, H. Grabner, K. Walzer, M. Bagnat, K. Simons, C. Walch-Solimena, A genome-wide visual screen reveals a role for sphingolipids and ergosterol in cell surface delivery in yeast, Proc. Natl. Acad. Sci. USA 102 (2005) 17981−17986.

[40] R. Schneiter, B. Brügger, C.M. Amann, G.D. Prestwich, R.F. Epand, G. Zellnig, F.T. Wieland, R.M. Epand, Identification and biophysical characterization of a very-long-chain-fatty-acid-substituted phosphatidylinositol in yeast subcellular membranes, Biochem. J. 381 (2004) 941−949.