The major plant plasma membrane H\(^{+}\)-ATPases fall into two gene categories, subfamilies I and II. However, in many plant tissues, expression of the two subfamilies overlaps, thus precluding individual characterization. Yeast expression of PMA2 and PMA4, representatives of the two plasma membrane H\(^{+}\)-ATPase subfamilies in *Nicotiana plumbaginifolia*, has previously shown that (i) the isoforms have distinct enzymatic properties and that (ii) PMA2 is regulated by phosphorylation of its penultimate residue (Thr) and binds regulatory 14-3-3 proteins, resulting in the displacement of the autoinhibitory C-terminal domain. To obtain insights into regulatory differences between the two subfamilies, we have constructed various chimeric proteins in which residues upstream of the phosphorylated Thr. It is Ser in PMA2 (as in most members of subfamily I) and His in PMA4 (as in most members of subfamily II). Substitution of His by Ser in PMA4 resulted in an enzyme showing increased phosphorylation status, 14-13-3 binding, and ATPase activity, as well as improved yeast growth. The reverse substitution of Ser by His in PMA2 resulted in the failure of this enzyme to complement the absence of yeast H\(^{+}\)-ATPases. These results show that the two plant H\(^{+}\)-ATPase subfamilies differ functionally in their regulatory properties.

The plant plasma membrane H\(^{+}\)-ATPase (PMA)\(^1\) is an electrogenic pump, which couples ATP hydrolysis to proton transport out of the cell. The resulting electrochemical gradient is then used by secondary transporters, acting as uniporters or as proton symporters and antiporters, to move ions and metabolites across the plasma membrane (for review, see Refs. 1–3).

The 33 plant H\(^{+}\)-ATPases cDNA clones obtained as yet have been classified into two different gene subfamilies (I and II), which diverged before the separation of monocot and dicot plants (4, 5). However, the fact that members of both subfamilies are simultaneously expressed in the same organ (4, 6) hinders the determination of the enzymatic and regulatory properties of individual isoforms in plant material. This limitation was overcome by the heterologous expression of H\(^{+}\)-ATPases from *Arabidopsis thaliana* (7) and *Nicotiana plumbaginifolia* (8) in the yeast *Saccharomyces cerevisiae*. PMA2 and PMA4, the two most widely expressed *N. plumbaginifolia* genes, each representing one of the two subfamilies, were able to replace the yeast H\(^{+}\)-ATPase genes, while still allowing yeast growth. However, clear differences were observed between the two PMAs in terms of proton pumping, pH dependence of ATPase activity, and growth capacity of the yeast at low pH (9).

The plant H\(^{+}\)-ATPase is regulated by an autoinhibitory domain located in its C-terminal region (10). In the plant, phosphorylation of the penultimate residue (a threonine) is required for the binding of regulatory 14-3-3 proteins (11, 21, 27). These highly conserved proteins function as a dimer in all eukaryotic cells and serve diverse regulatory functions (for review, see Ref. 12). However, studies performed using plant material have not attempted to differentiate between the different isoforms, and yeast expression of plant H\(^{+}\)-ATPases was therefore also used to address their regulatory features. For instance, deletions or point mutations within the inhibitory C-terminal region of H\(^{+}\)-ATPases from *Arabidopsis* (13, 14) and *N. plumbaginifolia* (15, 16) resulted in enzyme activation and improved yeast growth. Using this heterologous system, it was recently demonstrated that, as with H\(^{+}\)-ATPases expressed in the plant, phosphorylation of the H\(^{+}\)-ATPase penultimate residue (Thr) is necessary for 14-3-3 binding and ATPase activation (17, 20). In the case of PMA2, the phosphorylation and complex formation with 14-3-3 proteins was readily observed in yeast in the absence of fuscoecin, a fungal toxin often used to stabilize the plant H\(^{+}\)-ATPase-14-3-3 complex (17). Given the kinetic differences between PMA2 and PMA4, it was important to determine whether these representatives of the two subfamilies were regulated in the same way. Here, we show that, in contrast to PMA2, PMA4 was poorly phosphorylated and bound 14-3-3 proteins only weakly. This regulatory difference could be linked to a single residue located two positions upstream of the phosphorylated Thr.

**EXPERIMENTAL PROCEDURES**

**Strains**—YAK2 (8) is a yeast strain in which the H\(^{+}\)-ATPase genes, *PMA1* and *PMA2*, have been deleted. This strain is kept alive by yeast *PMA1* under the control of the *GAL1* promoter in a *URA3* centromeric vector.

\(^1\) The abbreviations used are: PMA, plasma membrane H\(^{+}\)-ATPase; PCR, polymerase chain reaction; MOPS, 4-morpholinopropanesulfonic acid.
plasmid PRS-316 (18). The strain YAKpma2Ntagged was previously described (17).

For His tagging of PMA4, plasmid 2μmpMA1/pma4, containing wild-type *N. plumaginifolia* pma4 cDNA (9), was modified by PCR to introduce a sequence coding for 6 histidine residues at the N terminus (between codons 2 and 3). Two primers: T4-PCR2, 5'-GGGGCTAGGC-ACCTCTCTCTTACCCATATACGATCTGAG-3′ and T4-PCR3, 5'-GACCAGAGGTTTCTCTTGTG-3′ were used to generate the PCR product. The fragment was digested with BamHI and then used to replace the corresponding fragment in plasmid 2μmpMA1/pma4 (9) to give 2μmpMA1/pma4Ntagged.

We produced four chimeras in which the 110 residues at the C-terminal region of PMA2 were progressively substituted by the corresponding region from PMA4. Recombinant DNA molecules were created by sequential PCR amplifications (Table I). 2μmpMA1/pma2Ntagged (17) served as the template for the PCR1 series and 2μmpMA1/pma4 for the PCR2 series. For each chimera, both PCR products (which partly overlapped) were combined and amplified as a single fragment using primers PH2-1 and PH4-2. The new fragments were digested with BglII/EcoRI, then used to replace the corresponding fragment in plasmid 2μmpMA1/pma4Ntagged (17).

Plasmid 2μmpMA1/pma2Ntagged (17) was modified by PCR to replace Ser-953 with Ala (PMA2-S953A), Asn (PMA2-S953N), or His (PMA2-S953H). N-tagged PMA2-E14D was modified by PCR to replace Ser-953 with His (PMA2-E14D/S953H). His-S953 in PMA4 was replaced in plasmid 2μmpMA1/pma4Ntagged and chimera (PMA2-S953H) by Ser (PMA4-H953S) and (PMA2-1/3H953S). All constructs were checked by sequencing.

Transformants with the different constructions were obtained as follows: YAK2 was transformed by the plasmid bearing the plant H^+-ATPase gene under the control of the PMA1 promoter and was plated on MMGHA-S, MMGHA-U, or MMGHA-H. Transforms were replicated on MMG with glucose 6% or 5% supplemented with 0.1% 5-fluoro-orotic acid to counterselect the ura3 plasmid PRS-316 and obtain a yeast strain expressing only a plant plasma membrane H^+-ATPase.

**Media and Growth Conditions**—Yeast cells were grown either in rich medium containing 2% (w/v) glucose and 2% (w/v) yeast extract (YGlu) or in minimal medium containing 0.7% (w/v) yeast nitrogen base without amino acids (Difco) and 0.15% (w/v) drop mix (19), supplemented with all amino acids and nucleotides required for growth. Solid media contained 2% agar. The 5-fluoro-orotic acid medium was prepared as described by Treco (19). The pH of the medium was adjusted with KOH/HCl to pH 6.5 for PMA2, pH 5.5 for PMA4 or to another pH when indicated. For growth comparison, liquid cultures at the early exponential stage (15–30 × 10^6 cells/ml) were diluted in sterile water to 1 × 10^6 cells/ml and then spread on different plates.

**Solubilization and Purification of His-tagged H^+-ATPase**—Plasma membranes were prepared according to Morsomme *et al.* (16). Solubilization and purification of the His-tagged H^+-ATPase were performed according to Maudou *et al.* (17).

**Electrophoresis and Western Blotting**—Electrophoresis analysis and Western blotting were performed as described in Morsomme *et al.* (16). Quantification was performed using a 32P-protein A (Amersham Pharmacia Biotech) detected by PhosphorImager® (Bio-Rad Molecular Imaging® System GS-S25).

**ATPase Assays**—ATPase assays were performed at 32 °C in a reaction mixture consisting of 50 mM MES/MOPS/Tris, 6 mM MgATP, 1 mM free Mg^2+ (MgCl2), 10 mM sodium azide (mitochondrial ATPase inhibitor), 20 mM KNO3 (vacuolar ATPase inhibitor), and 0.2 mM molybdate (phosphatase inhibitor) at pH 6.4. The reaction was started by adding plasma membrane proteins (14 μg) to 160 μl of reaction mixture. After 3, 6, and 9 min, 50-ul aliquots were mixed with 60 μl of 5% trichloroacetic acid to stop the reaction.

**RESULTS**

**PMA4 Is Weakly Phosphorylated and Shows Weak 14-3-3 Binding**—Heterologous expression in yeast has been previously used to express *N. plumaginifolia* PMA2 and PMA4 separately, making it possible to study individual enzymatic and physiological properties. His tagging of PMA2 permitted its easy purification (17). To compare the solubilized isoforms, a Hisn tag was added to the N-terminal region (between residues 2 and 3) of PMA4. In addition, we tagged an activated PMA4 mutant (A129P) bearing a mutation (Ala-129 replaced by Pro) that resulted in a more active enzyme and improved yeast growth at low pH (data not shown), similar to two other activated PMA4 mutants described previously (T861D and Gln882stop, Ref. 9). We chose to use A129P in this study because, contrary to the other PMA4-activated mutants, the amino acid substitution is not localized in the regulatory C-terminal region. However, we must be aware that this does not exclude a three-dimensional proximity.

No significant differences in growth rate or ATPase activity were observed between yeast strains expressing tagged or untagged PMA4,2 showing that, as with PMA2 (17), His tagging did not interfere with enzyme performance. After purification on a Ni^{2+}-NTA column, PMA4 was found to interact weakly with the two yeast 14-3-3 proteins (BMH1 and BMH2) compared with PMA2 (Fig. 1A). This was not because of a loss of 14-3-3 proteins during purification because Western blotting analysis of membranes also revealed the low abundance of these regulatory proteins compared with the strain expressing PMA2 (Fig. 1B). The difference in binding amount of 14-3-3 proteins between the two H^+-ATPase isoforms was not because of a difference in the total amount of 14-3-3 proteins in the yeast cells expressing either PMA2 or PMA4 because the same amount of 14-3-3 proteins was found in a total yeast extract of both strains (data not shown). As the binding of 14-3-3 to PMA2 depends on phosphorylation of Thr-955 (17), we determined the phosphorylation status of PMA4 using anti-phosphothreonine antibodies. In contrast to PMA2, phosphothreonine in PMA4 was only weakly detectable (Fig. 1B). A129P, a PMA4-activated mutant, displayed higher 14-3-3 binding and phosphorylation status than the corresponding wild-type protein (Fig. 1B), suggesting that the mutation resulted in increasing accessibility of the C-terminal region to phosphorylation.

**Defining the PMA2 C-terminal Inhibitory Domain**—To define putative differences between the inhibitory C-terminal region of PMA2 and PMA4, we constructed four chimeras in
which the 110-residue C-terminal region of PMA2 was progressively replaced by the corresponding region of PMA4. These chimeras were defined on the basis of (i) sequence alignment of the C-terminal domains of the two isoforms and (ii) information on mutated residues in this region (Fig. 2A). We defined three zones (Fig. 2B), the first two (residues 851–871 and 879–895) containing all but one of the identified PMA2-activating mutations in the C-terminal region (15, 16) and the third (residues 896–915) was highly divergent between the two isoforms. The chimeras used are shown in Fig. 2C. As shown in Fig. 3, chimera (PMA211), in which the entire PMA2 C-terminal region was replaced with that from PMA4, showed much better growth than PMA2, suggesting that the PMA2 inhibitory domain could not be functionally replaced by the corresponding region from PMA4. A chimera retaining the first zone in which activating PMA2 mutants were found (chimera PMA213⁄4) still showed stimulation of growth compared with PMA2, but to a lesser extent. Retention of both activating mutant zones (chimera PMA211⁄2) reduced growth to the level observed for wild-type PMA2. Finally, when only the third C-terminal region was replaced (chimera PMA211⁄3), no growth was observed when the transformant was transferred to a medium containing 5-fluoro-orotic acid to eliminate the plasmid bearing the yeast
PMA1 gene. We therefore conclude that the first half of the C-terminal region represents the major part of the autoinhibitory domain but that residues between positions 895 and 915 also contribute to some extent. We speculate that in (PMA2 + 1⁄3) the last third of the PMA4 C-terminal region prevented phosphorylation and 14-3-3 binding and therefore did not allow release of the inhibitory effect by the more N-terminal PMA2 C-terminal region. This hypothesis was evaluated by site-directed mutagenesis.

The Nature of the Fourth Residue from the C terminus (C^-4) Determines Phosphorylation—As shown in the PMA2/PMA4 alignment (Fig. 2a), chimera (PMA2 + 1⁄3) contained only four residues that differ from the PMA2 sequence. Among these, the fourth residue from the C terminus (C^-4, position 953) appeared of interest as it was the only one that was highly conserved within, but different between, the two plant H^+--ATPase subfamilies: Ser (55%) or Ala (45%) in subfamily I (here represented by PMA2) and His (87.5%) or Asn (12.5%) in subfamily II (here represented by PMA4).

We therefore mutated His-953 in chimera (PMA2 + 1⁄3) to Ser (PMA2 + 1⁄3-H953S). After transfer on a 5-fluoroorotic acid medium, complementation of the yeast strain, free from its own H^+--ATPase gene, was restored (Fig. 4a) to a level comparable with that observed in the wild-type PMA2. To confirm the importance of Ser-953, we mutated this residue in PMA2 into His, Ala, or Asn (PMA2-S953H, PMA2-S953A, or PMA2-S953N, respectively) and found that PMA2-S953A supported yeast growth to the same extent as PMA2 (Fig. 4B), whereas PMA2-S953H (data not shown) and PMA2-S953N (Fig. 4B) did not, showing that Ser-953 or Ala-953 is indeed a key residue for the first subfamily. Because His-953 is only 2 residues distant from the phosphorylated Thr-955, the failure of PMA2-S953H or PMA2-S953N to support yeast growth could be interpreted as an interference with phosphorylation and thus 14-3-3 binding, preventing release of the autoinhibitory effect of the more upstream region. This could not be checked directly on PMA2-S953H, because this strain was not viable after removing the yeast H^+--ATPase PMA1. So we mutated Ser-953 into His in the activated mutant PMA2-E14D, previously shown to grow better than PMA2 (15, 16), yielding PMA2-E14D/S953H, and found that this strain supported yeast growth to a lesser extent than the original E14D (Fig. 5A). It also displayed lower phosphorylation status and 14-3-3 binding (Fig. 5B). The variation was smaller for the latter, suggesting that 14-3-3 binding might be the limiting step. As a final proof, we prepared the reciprocal substitution (His to Ser) in PMA4 (PMA4-H949S) and found that, compared with PMA4, PMA4-H949S showed increased phosphorylation status and 14-3-3 binding, a higher ATPase activity, and better yeast growth (Fig. 6).

DISCUSSION

In this study, N. plumbaginifolia PMA2 and PMA4 were chosen as representatives of the two major plant H^+--ATPase subfamilies. Their direct characterization in plant material has been hampered by their partially overlapping expression in many plant tissues (4). Heterologous expression in yeast is a good alternative, because it has previously allowed us to compare their kinetics (9) and to show that PMA2 is activated in yeast, as in plants, by phosphorylation of its penultimate residue (Thr) and binds regulatory 14-3-3 proteins (17). Moreover, 14-13-3 binding was observed in the absence of fusicoicin, a fungal toxin often used to artificially stabilize the H^+--ATPase/14-3-3 complex. This therefore validates the yeast model.

The first aim of this study was to delineate the autoinhibitory C-terminal region. Replacing the entire PMA2 C-terminal region with that from PMA4 fully activated the enzyme and resulted in much better yeast growth. This demonstrates that the autoinhibitory C termini of PMA2 and PMA4 are not interchangeable. Progressive reduction of contribution of the PMA4 C-terminal region was correlated to a decrease of yeast growth or even lethality when only the last 41 residues of PMA2 were replaced by those from PMA4. From these data, we can conclude that the inhibitory domain covers more than 50 residues and includes the first-half of the C-terminal region in which 19 single-point PMA2 mutations, which result in activated ATPase and better yeast growth, have been localized (15, 16). Our data show that the inhibitory domain extends to the region encompassing residues 895–915, because adding back this region of PMA2 (PMA2 + 1⁄3) resulted in an enzyme that no longer sustained yeast growth. Our results extend those recently obtained for the A. thaliana AHA2 isoform by Axelsen et al. (14), who used alanine-scanning mutagenesis to identify two regions, corresponding to PMA2 Lys-871 to Leu-893 and Asn-911 to Gln-926 and including positions that when mutated release enzyme inhibition. Comparison of the PMA2-PMA4 chimera H^+--ATPases showed that the region upstream of Lys-871 and that between 895 and 915 also contain inhibitory sequences. This is in agreement with the previous identification of eight activated mutants within this region (15, 16). Thus, the inhibitory effect and, therefore, the region presumably interacting with the rest of the enzyme seems to be spread.
over a large part of the C-terminal region.

The second finding of this paper concerns the regulatory phosphorylation site of the H\textsuperscript{+}-ATPase family. Sequence comparison of all plant H\textsuperscript{+}-ATPase cDNAs available in the database shows that they can be grouped into two subfamilies and a similar dichotomy is observed in the first half of the C-terminal region. In contrast, the last 30 C-terminal residues are highly homologous except for the fourth residue from the C-terminus (PMA2-953 or PMA4-949), which is Ser or Ala in subfamily I and His or Asn in subfamily II. This amino acid is 2 residues removed from the penultimate Thr, which is phosphorylated by a kinase and therefore belongs to the 14-3-3 binding site defined at the H\textsuperscript{+}-ATPase C terminus (11, 17, 20, 21). The significance of the difference at this position is supported by two arguments. Firstly, the divergence between the two subfamilies is ancient, because it preceded the separation between dicot and monocot species and yet the consensus has been strictly retained within each subfamily. Secondly, we showed that these differences have functional consequences on PMA regulation and yeast growth. For instance, converting Ser-953 of PMA2 into Ala (the alternative residue for subfamily I) did not cause any change, whereas converting it into either His or Asn (subfamily II residues) prevented yeast growth. The observation that PMA2-S953H did not support yeast growth whereas PMA4 (which also displayed His at C\textsuperscript{-4}) did, was expected because we showed previously that PMA4 has a higher intrinsic ATPase activity than PMA2 (9). To bypass the lethality of PMA2-S953H, mutation of Ser-953 was obtained in an activated mutant (PMA2-E14D). In this case, His at C\textsuperscript{-4} decreased phosphorylation of Thr-955 and binding of 14-3-3 proteins, and reduced yeast growth. Reciprocal results were obtained when His-949 of PMA4 was mutated to Ser. We can therefore conclude that the differences observed between the two subfamilies at residue C\textsuperscript{-4} are functionally significant. As the position C\textsuperscript{-4} is only two residues upstream from the phos-
phorylated Thr, the most direct interpretation of these data is to consider that the Ser/His substitution modifies the recognition site for kinase. A major difference between Ala/Ser and His/Asn is that the latter are larger and thus possibly interfere with kinase binding or catalysis. Interestingly, another case has been reported recently (22) in which the presence of either a Ser or Asn residue seems to be related to different 14-3-3 binding properties. The barley lypoxygenase isoform 13-lox interacts with 14-3-3 proteins, whereas the 9-lox isoform does not. Sequence analysis revealed a putative 14-3-3 binding site (RKPSD5SKP) in 13-lox, which differed by a single residue in 9-lox, where the Ser two positions N-terminal to the putative phosphorylated Ser in 13-Lox was replaced by Asn.

An additional fact has to be considered. PMA2-E14D and PMA4-A129P, both activated mutants, were more phosphorylated and bound more 14-3-3 proteins than their respective wild-type enzymes. As it was shown previously that the PMA2-E14D H+ -ATPase had a C-terminal region more accessible to trypsin cleavage (16), we can suggest that in the wild type, the C-terminal region is not fully accessible to the kinase. Two parameters have to be taken into account: (i) the accessibility to the C-terminal region and (ii) the phosphorylation consensus sequence. Both effects seem to be additive because E14D (more accessible C-terminal region and Ser at 953) conferred a better growth than PMA2 (Ser at 953) or E14D/S953H (more accessible C-terminal domain). Weak accessibility and poor consensus (PMA2-S953H) combined did not allow yeast growth.

In most cases, 14-3-3 proteins recognize their target through a specific phosphorylated sequence. The two most widespread motifs are RSXpXP (23) and RX(Y/F)XPSP (24). The plant H+-ATPase is an exception, because the 14-3-3 dimer interacts with the last part of the C-terminal region (11, 17, 20, 21), which does not contain the above sequence. Within the last 30 residues, which are highly conserved between both subfamilies, position C+4 is the only one showing a clear difference between subfamilies. We therefore propose that the formation of the H+-ATPase/14-3-3 complex, which depends on the phosphorylation of Thr at position C-2, is differentially regulated between the two subfamilies, the consensus being QQ(S/A)YpTV for subfamily I and QQ(S/H)YpTV for subfamily II.

How can we apply the data obtained in the yeast system to plants? The existence of several isoforms expressed in various plant organs complicates the comparison of their individual regulatory properties. For instance, in spinach, the formation of a H+-ATPase/14-3-3 complex is increased by fusicoccin, a fungal toxin that makes 14-3-3 binding irreversible. After tryptic cleavage, two H+-ATPase C-terminal peptides were identified, one with Ala and the other with Asn at position C+2 (11). Although H+-ATPase genes have not been cloned from spinach, we can suggest that these two peptides belong to subfamilies I and II, respectively, and that both plant H+-ATPase types can be phosphorylated and interact with 14-3-3 proteins. However, being a rather extreme approach, fusicoccin is not a physiological regulator and is therefore not a good tool for revealing possible differential regulation between H+-ATPase subfamilies. The case might be different with blue light activation, which, in guard cells from Vicia faba, led to the identification of two H+-ATPase isoforms, VHA1 and VHA2, that were phosphorylated in the C-terminal region (25). In this case, both isoforms belong to subfamily II, suggesting that only this subfamily was responding to this environmental factor in this cell type.

More generally, how could differential regulation be explained? At least two models can be proposed. In the first one, different kinases would be involved, each of which specifically recognizes members of only a single subfamily, allowing completely independent transducing systems. In the second model, a single kinase would be involved and, if behaving like in the yeast enzyme, would favor the phosphorylation of subfamily I members unless the modification of subfamily II members (e.g. previous phosphorylation by another kinase at another H+-ATPase site) renders their C terminus more accessible to the kinase. This hypothesis is realistic, because we showed that a single point mutation (A129P) of PMA4 resulted in increased phosphorylation and 14-3-3 binding.

It is clear that any progress toward the understanding of differential regulation will require tools that allow members of a single family to be specifically studied. Genetic methods that silence the expression of a whole subfamily might be one approach. However, the silencing of pma4 in tobacco induced pleiotropic effects and therefore might disturb the regulatory systems (26). A better approach might be to express in the plant single H+-ATPase isoforms tagged in such a way (e.g. His6) that they can be specifically followed.

REFERENCES
1. Sussman, M. R. (1994) Annu. Rev. Plant Physiol. Plant Mol. Biol. 45, 211–234
2. Palmgren, M. G., and Harper, J. F. (1999) J. Exp. Bot. 50, 883–893
3. Morsonne, P., and Boutry, M. (2000) Biochim. Biophys. Acta 1465, 1–16
4. Moriau, L., Michelet, B., Bogaerts, P., Lambert, L., Michel, A., Oufattole, M., and Boutry, M. (1999) Plant J. 19, 31–41
5. Oufattole, M., Arango, M., and Boutry, M. (2000) Planta 210, 715–722
6. Ewing, N. N., and Bennett, A. B. (1994) Plant Physiol. 106, 547–557
7. Palmgren, M. G., and Christensen, G. (1994) J. Biol. Chem. 269, 3027–3033
8. de Kerchove d’Exaerde, A., Supply, P., Dufour, J. P., Bogaerts, P., Thines, D., Goffau, A., and Boutry, M. (1995) J. Biol. Chem. 270, 23828–23837
9. Luo, H., Morsonne, P., and Boutry, M. (1999) Plant Physiol. 119, 627–634
10. Palmgren, M. G., Sommarin, M., Serrano, R., and Larsson, C. (1991) J. Biol. Chem. 266, 20470–20475
11. Olsson, A., Svennelid, F., Ek, B., Sommarin, M., and Larsson, C. (1998) Plant Physiol. 118, 551–555
12. Finnie, C., Borch, J., and Collinge, D. B. (1999) Plant Mol. Biol. 40, 545–554
13. Regenberg, B., VillaJla, J. M., Lanfermeijer, F. C., and Palmgren, M. G. (1995) Plant Cell 7, 1655–1666
14. Axelsen, K. B., Venema, K., Jahn, T., Baumgaard, L., and Palmgren, M. G. (1999) Biochemistry 38, 7227–7234
15. Morsonne, P., de Kerchove d’Exaerde, A., De Meeester, S., Thines, D., Goffau, A., and Boutry, M. (1996) EMBIO J. 15, 5513–5526
16. Morsomme, P., Dambly, S., Maudoux, O., and Boutry, M. (1998) J. Biol. Chem. 273, 34887–34842
17. Maudoux, O., Batoke, H., Oecking, C., Gevaert, K., Vandekerckhove, J., Boutry, M., and Morsonne, P. (2000) J. Biol. Chem. 275, 17762–17770
18. Sikorski, R. S., and Hieter, P. (1989) Genetics 122, 19–27
19. Treco, D. A., and Lundblad, V. (1994) in Current Protocols in Molecular Biology (Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K., eds) Vol. 2, pp. 13.1.1–13.1.7, John Wiley & Sons, Inc., New York, NY
20. Fuglsang, A. T., Visconti, S., Drumm, K., Jahn, T., Stensballe, A., Mattei, B., Jensen, O. N., Aducu, P., and Palmgren, M. G. (1999) J. Biol. Chem. 274, 36774–36780
21. Svennelid, F., Olsson, A., Piotrowski, M., Ottman, C., Larsson, C., Oecking, C., and Sommarin, M. (1999) FEBS Lett. 489, 48–52
22. Treco, D. A., Tanner, J. W., Allen, P. M., and Shaw, A. S. (1996) Cell 84, 889–897
23. Yaffe, M. B., Rittering, K., Volinia, S., Caron, P. R., Aitken, A., Jeffers, L., Gamblin, S. J., Smerdon, S. J., and Cantley, L. C. (1997) Cell 91, 961–971
24. Kinosita, T., and Shimazaki, K. (1999) EMBIO J. 18, 5548–5558
25. Zhao, R., Dielen, V., Kinet, J. M., and Boutry, M. (2000) Plant Cell 12, 535–546
26. Camoni, L., Iori, V., Marra, M., and Aducu, P. (2000) J. Biol. Chem. 275, 9919–9923