The plant plasma membrane H\textsuperscript{+}-ATPase is activated by the binding of 14-3-3 proteins to its extreme C-terminal amino acids (YTV) and phosphorylation of the penultimate threonine (YpTV) necessary for this interaction in vitro. However, in the presence of the fungal toxin fusicoccin (FC), binding of 14-3-3 proteins occurs independently of phosphorylation but still involves the YTV motif. Since FC exclusively binds to the complex consisting of both 14-3-3 homologs and the C-terminal domain of the H\textsuperscript{+}-ATPase, the toxin was used as a tool to reveal potential protein-protein interaction sites in the enzyme’s C terminus. We performed in vitro interaction studies by applying various C-terminal parts of the H\textsuperscript{+}-ATPase PMA2 from Nicotiana plumbaginifolia expressed as glutathione S-transferase fusion peptides in E. coli. Interestingly, the PMA2 region encompassing residues 905–992 is implicated in FC-dependent binding of 14-3-3 homologs. Recently, part of this region has been shown to contribute to the autoinhibitory action of the PMA2 C terminus. Site-directed mutagenesis of individual amino acids localized within this region resulted in a drastic decrease in FC-dependent binding of 14-3-3 proteins. Furthermore, by expressing the corresponding mutants of PMA2 in yeast, we observed a reduced capability of the mutant enzymes to functionally replace the endogenous H\textsuperscript{+}-ATPase. Notably, the decreased activity of the mutant enzymes was accompanied by a weakened binding of yeast 14-3-3 homologs to the plasma membrane of transformed cells. Taken together, our results suggest that a section of the autoinhibitory C-terminal domain of the PMA2 region contributes to binding of activatory 14-3-3 proteins in the absence of FC.

The plant H\textsuperscript{+}-ATPase is responsible for building up an electrochemical gradient across the plasma membrane that provides the driving force for nutrient uptake and maintenance of turgor (reviewed in Ref. 1). The C terminus of the enzyme is known to act as an autoinhibitor; proteolytic removal of cell turgor (reviewed in Ref. 1). The C terminus of the plant H\textsuperscript{+}-ATPase activity (2, 3). Recently, evidence has been obtained for the presence of individual regulatory regions within the C terminus of both the H\textsuperscript{+}-ATPase AHA2 from Arabidopsis thaliana and PMA2 from Nicotiana plumbaginifolia. By use of alanine-scanning mutagenesis, residues of AHA2 were identified that when altered gave rise to an activated enzyme after heterologous expression in yeast. These residues were concentrated into a region of the N-terminal half (region I) as well as the C-terminal half (region II) of the AHA2 C terminus (Ref. 4; see Fig. 1). By analyzing various chimeric proteins in which the C terminus of PMA2 residues 851–956, see Fig. 1) was progressively substituted by the corresponding sequence from PMA4, the autoinhibitory region of PMA2 was localized to a region between residues 851 and 915 in which three individual sections could be identified (5, see Fig. 1). The authors concluded that the inhibitory effect and, therefore, the region presumably interacting with the rest of the enzyme seem to be spread over a large part of the PMA2 C terminus (5). Consequently, solely the extreme C-terminal residues of PMA2 (residues 916–956, see Fig. 1) appear to be uninvolved in autoregulation.

The fungal phytotoxin fusicoccin (FC)\textsuperscript{1} activates the plant plasma membrane H\textsuperscript{+}-ATPase by a process involving the C-terminal domain of the pump (6, 7). To date it is well established that FC binds to and subsequently stabilizes a complex consisting of the enzyme’s C terminus and 14-3-3 proteins (8–10). Members of the eukaryotic 14-3-3 family are highly conserved hydrophilic proteins that have been implicated in the regulation of diverse physiological processes by protein-protein interactions (reviewed in Refs. 11–13). Binding of 14-3-3 homologs to their target proteins appears to occur in a sequence-specific and phosphorylation-dependent manner (binding motif RS\textsuperscript{pS}XP (Ref. 14) or RX(Y/F)XP\textsuperscript{pS}XP (Ref. 15); pS indicates a phosphorylated serine residue). However, a novel binding site for 14-3-3 proteins within the C-terminal domain of the plant H\textsuperscript{+}-ATPase has been identified that does not resemble any other 14-3-3 binding motif: 14-3-3 homologs bind to the conserved extreme C-terminal tripeptide of the enzyme (amino acids YTV), and this interaction requires phosphorylation of the penultimate threonine in vitro (YpTV; Refs. 16–18). Nevertheless, FC circumvents the need for this phosphorylation and promotes 14-3-3 binding to the unphosphorylated C-terminal domain of the H\textsuperscript{+}-pump. Since binding of FC requires the 14-3-3-C terminus (H\textsuperscript{+}-ATPase) complex, it is not surprising that FC-dependent binding of 14-3-3 homologs to the unphosphorylated enzyme still involves the YTV motif (16, 17). Hence, the FC binding site is identical to the part of the H\textsuperscript{+}-ATPase that contributes to the 14-3-3 binding site. Furthermore, binding of activatory 14-3-3 proteins and FC seems to occur in a region that is uninvolved in autoregulation, i.e. the enzyme’s extreme C-terminal end.

By performing in vitro interaction studies using C-terminal residues of the N. plumbaginifolia H\textsuperscript{+}-ATPase PMA2
pressed as GST fusion peptides in *Escherichia coli*, we identified a region within the enzyme's C terminus (comprising amino acids 905–922), which in addition to the YTV motif is required for the generation of a FC binding site. Site-directed mutagenesis point to the importance of this region for FC-dependent binding of 14-3-3 homologs. Interestingly, the region overlaps with both the autoinhibitory region II identified in AHA2 (4) and the autoinhibitory section encompassing residues 895–915 in PMA2 (Ref. 5; see Fig. 1). Notably, by expressing wild-type and mutant forms of PMA2 in yeast, we provide evidence for the relevance of this C-terminal H\(^+\)-ATPase region with respect to 14-3-3 binding independent of FC.

### EXPERIMENTAL PROCEDURES

#### Growth Media

*E. coli* was grown at 37 °C in 2YT medium (1% (w/v) yeast extract, 1.6% (w/v) tryptone, and 0.5% (w/v) NaCl) containing 100 μg ml \(^{-1}\) ampicillin.

Saccharomyces cerevisiae cells were grown at 30 °C either in rich medium, containing 2% (w/v) glucose or galactose, 2% (w/v) yeast extract, 2% (w/v) peptone, and 20 mM KH\(_2\)PO\(_4\), or in minimal medium, containing 0.67% (w/v) yeast nitrogen base without amino acids (Difco), 2% (w/v) glucose, and 20 mM KH\(_2\)PO\(_4\) supplemented with all amino acids and minerals required for growth. The medium pH was adjusted to 6.5 (KOH) or another pH when indicated.

Expression and Purification of Proteins—For expression of the *Nicotiana tabacum* 14-3-3 isoform T14c-3c as a His\(_{6}\)-tagged protein in *E. coli* M15, the corresponding cDNA (19) was amplified by PCR and cloned into the expression vector pQE-30 (Qiagen, Hilden, Germany). Two mg of native protein was purified from 100 ml of transformed bacteria using Ni\(^2+\)-nitrilotriacetic acid-agarose (Qiagen) according to the manufacturer's protocol.

For expression of the *N. plumbaginifolia* H\(^+\)-ATPase C-terminal region fused to GST in *E. coli* M15, DNA fragments encoding peptides of different length (Fig. 1) were amplified by PCR and cloned into pBluescript SK\(^{+}\) (Strategene, La Jolla, CA). Site-directed mutagenesis was performed by PCR (20). PCR products and products of mutagenesis were verified by sequencing and subsequently cloned into the GST expression vector pGEX-4T-1. When indicated GST fusion proteins were purified from transformed bacteria using GSH-Sepharose according to the manufacturer's protocol (Amersham Pharmacia Biotech, Freiburg, Germany).

#### Gel Electrophoresis and Protein Immunoblotting

Denaturing gel electrophoresis and protein Western blots were performed according to Laemmli (21). Protein blotting onto nitrocellulose was done electrophoretically overnight (4 °C, 64 mA) or within 2 h at room temperature (200 mA) as described by Towbin et al. (22). Immunodetection of GST (antiserum; Amersham Pharmacia Biotech) followed standard procedures; the secondary antibody was conjugated to alkaline phosphatase.

### RESULTS

#### FC-dependent Binding of 14-3-3 Proteins to the C Terminus of PMA2 Involves Part of Its Autoinhibitory Region—To investigate which part(s) of the *N. plumbaginifolia* H\(^+\)-ATPase PMA2 contribute(s) to the phosphorylation-independent, FC-dependent binding of 14-3-3 proteins, truncated forms of the C terminus of PMA2 were expressed as GST fusion peptides in *E. coli* (Fig. 1). Bacterial proteins were separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose, and the immobilized polypeptides were allowed to interact with purified (His)\(_{6}\)-tagged 14-3-3 protein in the presence of FC. Protein-protein interactions were visualized by use of an antibody raised against the (His)\(_{6}\) epitope (14-3-3 protein overlay). To date it is well established that the extreme C-terminal tripeptide of the plant plasma membrane H\(^+\)-ATPase (YTV) is essential for FC-dependent binding of 14-3-3 homologs to the enzyme's regulatory domain (16, 17). However, although the essential YTV is present in the C-terminal 28, 31, or 34 PMA2 residues, binding of FC and 14-3-3 could not be observed (GST+28aa, GST+31aa, and GST+34aa, corresponding to aa 929–956, 926–956, and 923–956, respectively; Figs. 1 and 2). The data indicate that the binding site for FC and 14-3-3 on PMA2 is complex and involves structural features other than the tripeptide motif.

As evident from the analysis of several other C-terminal peptides of PMA2, binding of 14-3-3 proteins in the presence of FC is affected by the length of the PMA2 peptide (Figs. 1 and 2). As already mentioned, interaction could not be detected when the C terminus of PMA2 was truncated to less than 35 amino acid residues (aa 923–956, Fig. 2). However, by lengthening the C-terminal fragment, the FC-dependent interaction with 14-3-3 proteins is enhanced in two steps. A weak, but significant interaction could be observed when the C terminus of PMA2 was extended to 35–41 amino acid residues (corresponding to aa 920–956, Fig. 2). Interestingly, a remarkable increase in FC-dependent 14-3-3 binding occurred when the PMA2 peptide consisting of the C-terminal 50 residues was extended by two additional amino acids, yielding GST+52aa (Fig. 2). No further increase was found by lengthening the C-terminal peptide up to 98 residues (Fig. 2, GST+52aa, corresponding to aa 950–956, through GST+58aa, corresponding to aa 859–956).

Interestingly, the PMA2 region in which truncations strongly modulate the interaction (the C-terminal 35–52 amino acids, corresponding to aa 905–922) overlaps with both the autoinhibitory region II identified in AHA2 (4) and the autoinhibitory section comprising residues 895–915 in PMA2 (Ref. 5; see Fig. 1). Furthermore, this region has a high probability of forming an amphipathic a-helix, suggesting that this predicted secondary structure may be important with respect to FC-dependent 14-3-3 binding.

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**Defining the 14-3-3 Binding Site within the Plant H\(^+\)-ATPase**
Defining the 14-3-3 Binding Site within the Plant H^+-ATPase

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Effect of Site-directed Mutations on the FC-dependent 14-3-3 Site?

The data reported so far provide evidence for the contribution of a second region within the C-terminal domain of the plasma membrane H^+-ATPase to the generation of a FC binding site; in addition to the extreme C-terminal end (Refs. 16 and 17; YTV, see Fig. 1), a region that is involved in autoinhibitory action of the C terminus according to Dambly and Boutry (PMA2; Ref. 5) as well as Axelsen et al. (AHA2; Ref. 4). Gray boxes indicate regions of importance for binding of FC and 14-3-3 proteins (see Fig. 2 and Ref. 17).

Fig. 1. Summary of constructs employed to identify domains within the C terminus of the N. plumbaginifolia H^+-ATPase PMA2 that contribute to FC-dependent binding of 14-3-3 proteins. The indicated amino acids of PMA2 (numbers in parentheses) indicate position in the PMA2 sequence) were expressed as GST fusion peptides in E. coli and subsequently analyzed by means of the 14-3-3 protein overlay assay in the presence of FC. Black bars indicate strong FC-dependent 14-3-3 binding; gray bars, weak FC-dependent 14-3-3 binding; white bars, no 14-3-3 binding in the presence of FC (see Fig. 2). Analysis of the construct GST + 66aa-3 is reported by Svennelid (1983). Below, the C-terminal end of the PMA2 and AHA2 sequence is shown. Boxes indicate regions of importance for the autoinhibitory action of the C terminus according to Dambly and Boutry (PMA2; Ref. 5) as well as Axelsen et al. (AHA2; Ref. 4). Gray boxes indicate regions of importance for binding of FC and 14-3-3 proteins (see Fig. 2 and Ref. 17).

Fig. 2. Modulation of FC-dependent binding of 14-3-3 proteins by the length of the C-terminal peptide of the N. plumbaginifolia H^+-ATPase PMA2 expressed as GST fusion in E. coli. A, immunodetection of GST in extracts of E. coli expressing C-terminal PMA2 peptides as fusion with GST; lane 14 shows GST; lanes 1–13, the various C-terminal PMA2 peptides expressed as fusion with GST (see Fig. 1). B, 14-3-3 protein overlay corresponding to the gel shown in A; the overlay was performed in the presence of 1.5 μM FC.

Is the Autoinhibitory Section Part of the 14-3-3 Binding Site?

The data reported so far provide evidence for the contribution of a second region within the C-terminal domain of the plasma membrane H^+-ATPase to the generation of a FC binding site; in addition to the extreme C-terminal end (Refs. 16 and 17; YTV, see Fig. 1), a region that is involved in autoinhibition and which possibly forms a helix appears to be essential (Figs. 2 and 3). Since the FC binding site is composed of both the C-terminus of the H^+-ATPase and 14-3-3 proteins (8–10), the question arises whether the PMA2 regions contributing to the FC binding site are identical to those constituting the 14-3-3 binding site in vivo. Although the contribution of the three C-terminal H^+-ATPase residues YTV to the 14-3-3 binding site has been shown unambiguously (YpTV, Refs. 16–18), nothing is known with respect to the second region. Thus, we investigated the effect of the mutations E922A, A917P, E916A, E909A, or A917P was significantly reduced compared with the wild-type (GST + 66aa, Fig. 3). The data indicate that the amino acids Glu-922, Glu-916, Glu-909, and Ala-917 are of particular importance for binding of FC and 14-3-3 to PMA2. We chose Glu-922 and Ala-917 for a more detailed analysis. The negatively charged residue Glu-922 was changed into aspartic acid, asparagine, or lysine (E922D, E922N, E922K, respectively) whereas valine, lysine and glycine were individually substituted for Ala-917 (A917V, A917K, A917G). As indicated by the overlay assay, the charge in position 922 is an important prerequisite for 14-3-3 binding in the presence of FC. In particular, a change from a negatively charged amino acid (Glu, Asp) to an uncharged (Asn) or positively charged (Lys) residue is accompanied by a gradual decrease in interaction (Fig. 3). With respect to position 917, the properties of nonpolar amino acids seem to be important; substitution of the alanine by lysine (A917K) results in a drastic decrease in FC-dependent 14-3-3 binding, whereas the nonpolar residue valine (A917V) shows no effect (Fig. 3). However, changing Ala-917 into proline or glycine reduced the interaction significantly, although these two amino acids are likewise nonpolar. Notably, both of them are known to modify the structure of helices, indicating that structural integrity or charge distribution of an amphipathic α-helix may be an important prerequisite for the generation of a FC binding site.

Is the Autoinhibitory Section Part of the 14-3-3 Binding Site?

The data reported so far provide evidence for the contribution of a second region within the C-terminal domain of the plasma membrane H^+-ATPase to the generation of a FC binding site; in addition to the extreme C-terminal end (Refs. 16 and 17; YTV, see Fig. 1), a region that is involved in autoinhibition and which possibly forms a helix appears to be essential (Figs. 2 and 3). Since the FC binding site is composed of both the C-terminus of the H^+-ATPase and 14-3-3 proteins (8–10), the question arises whether the PMA2 regions contributing to the FC binding site are identical to those constituting the 14-3-3 binding site in vivo. Although the contribution of the three C-terminal H^+-ATPase residues YTV to the 14-3-3 binding site has been shown unambiguously (YpTV, Refs. 16–18), nothing is known with respect to the second region. Thus, we investigated the effect of the mutations E922A, A917P, E916A,
E915A, as well as E909A in the *N. plumbaginifolia* gene *pma2* after heterologous expression in the *S. cerevisiae* strain YAK2. The two endogenous H^+/H11001-ATPase genes are deleted from YAK2, and its survival on galactose medium is made possible by the presence of the yeast H^+/H11001-ATPase gene *pma1* under the control of a galactose-dependent promoter. In contrast, the plant H^+/H11001-ATPase *PMA2* gene is constitutively expressed (23) and as a consequence, plant H^+/H11001-ATPases can be analyzed in this strain for their ability to functionally replace the yeast enzyme on glucose medium.

The wild-type plant PMA2 is able to sustain yeast growth provided that the external pH was kept above pH 5.0 (5, 23, Fig. 4). Since the heterologously expressed PMA2 forms a complex with yeast 14-3-3 proteins (9, 18) and 14-3-3 proteins have been shown to activate the plant H^+/H11001-ATPase (18) it was suggested that the ability of the plant enzyme to form such a complex explains why it is able to functionally replace the endogenous H^+/H11001-ATPase. Furthermore, in yeast this interaction is dependent on the phosphorylation of the C-terminal threonine residue of the plant H^+/H11001-ATPase (YpTV), i.e. the domain that already has been identified as 14-3-3 binding site (16–18).

Hence, the analysis of the mutants mentioned above may provide evidence for the physiological relevance of the second region with respect to 14-3-3 binding *in vivo* (independent of FC). As shown in Fig. 4, YAK2 cells expressing the PMA2 mutants were still able to grow on glucose medium. This was expected since the ability of the plant enzyme to sustain yeast growth is mainly dependent on the phosphorylation of the penultimate threonine residue (YpTV, Refs. 16–18). However, the following PMA2 mutants conferred a significant reduction of yeast growth compared with the wild-type both at pH 5.5 and pH 6.5: PMA2-E922A, PMA2-A917P, PMA2-E916A, as well as PMA2-E915A. Thus, only the growth of yeast cells expressing the PMA2-E915A mutant was unaffected. Notably, there is a correlation between the growth phenotype of transgenic yeast (Fig. 4) and the FC-dependent 14-3-3 binding to the corresponding mutants (Fig. 3). The amount of the mutated PMA2 in yeast plasma membranes was not reduced compared with the wild-type PMA2, indicating that there is no correlation between expression level and the growth phenotype of transgenic yeast cells (Fig. 5, A and B). Hence the question arises whether the impaired capability of the PMA2 mutants to allow yeast growth in the absence of the endogenous H^+/H11001-ATPase is the result of a decreased phosphorylation of the penultimate threonine *in vivo* (YpTV). As a consequence, binding of yeast 14-3-3 homologs would not occur, resulting in a less active plant H^+/H11001-ATPase. Since binding of 14-3-3 proteins in the absence of FC is a direct measure of the amount of phosphorylated H^+/H11001-ATPase (17), we performed an overlay assay by applying the yeast plasma membranes. As shown in Fig. 5C, *in vitro* 14-3-3 binding to the
PMA2 mutants in the absence of FC was unchanged compared with the wild-type, indicating that a modified phosphorylation could not be the reason for the growth phenotype of transgenic yeast. Furthermore, the amount of plasma membrane-associated yeast 14-3-3 homologs was not reduced (Fig. 6). Nevertheless, by treating the membranes with high salt/low detergent, we observed a significant difference regarding the strength of association. In plasma membranes of yeast expressing the wild-type PMA2 or the PMA2-E915A mutant binding of 14-3-3 proteins (Fig. 3) was diminished, whereas the corresponding supernatant (middle panel, containing proteins that were released from the plasma membrane) as well as the corresponding pellet (right panel, containing proteins that were tightly bound to the plasma membrane) were electrophoretically separated and blotted onto nitrocellulose. Immuno detection of the plant H⁺-ATPase was done with the upper part of the nitrocellulose, whereas the lower part was used for immunodetection of yeast 14-3-3 proteins.

FIG. 6. High salt/low detergent treatment of plasma membrane proteins of yeast cells expressing wild-type (WT) and mutant PMA2. Purified plasma membranes (5 μg, left panel) prepared from YAK2 transformed with PMA2 or the mutants PMA2-E922A, PMA2-A917P, PMA2-E916A, PMA2-E915A, and PMA2-E909A were treated with high salt/low detergent (see “Experimental Procedures”) and subsequently centrifuged. The supernatant (middle panel, containing proteins that were released from the plasma membrane) as well as the corresponding pellet (right panel, containing proteins that were tightly bound to the plasma membrane) were electrophoretically separated and blotted onto nitrocellulose.

The identification of an additional region within the C terminus of the H⁺-ATPase that contributes to the FC binding site is in agreement with data obtained by Fuglsang et al. (16). FC is unable to promote binding of 14-3-3 protein to an unphosphorylated short peptide of the C terminus of the H⁺-ATPase (aa 933–948), whereas this is not a problem with a longer peptide (aa 851–948). Consequently, it was suggested by the authors that the binding site for FC might be more complex than just the three C-terminal amino acids (16). Furthermore, the PMA2 peptide, which was purified in a complex with FC and yeast 14-3-3 homologs, consists of the C-terminal 54 residues (18). This complex was observed after trypsin treatment. Since four additional trypsin sites are present in the PMA2 peptide, binding of FC and 14-3-3 proteins appears to prevent access of the protease. This result confirms our data showing that at least the 52 C-terminal residues of PMA2 are required for efficient binding of FC and 14-3-3 (Fig. 2).

Because the FC “receptor” is known as 14-3-3-C terminus (H⁺-ATPase) complex (8–10), the region identified to be essential for FC-dependent binding of 14-3-3 homologs may contribute to FC-independent (phosphorylation-dependent) 14-3-3 binding, too. In order to test this hypothesis, we expressed the PMA2 mutants (E922A, A917P, E916A, E915A, E909A) in the yeast strain YAK2. interestingly, the PMA2 region identified to be essential for FC-dependent binding, too. In order to test this hypothesis, we expressed the PMA2 mutants (E922A, A917P, E916A, E915A, E909A) in the yeast strain YAK2. The wild-type PMA2 is able to functionally replace the endogenous H⁺-ATPase. A significant portion of the 14-3-3 protein associated with the plasma membrane of the corresponding transgenic yeast cells is attached to the C terminus of the plant H⁺-ATPase. The complex represents an activated state of the plant H⁺-ATPase. Furthermore, it is characterized by an extreme stability (9, 18); thus, dissociation during high salt/low detergent treatment does not occur (18, Fig. 6). The same applies to plasma membranes derived from yeast cells expressing the PMA2-E915A mutant (Fig. 6). Remarkably, this mutant conferred a growth phenotype comparable to the wild-type plant H⁺-ATPase (Fig. 4) and, in addition, showed no difference in FC-dependent binding of 14-3-3 proteins compared with the wild type (Fig. 3). In contrast, yeast expressing the PMA2 mutants (E922A, A917P, E916A, E909A) each mutation resulting in a drastic decrease in FC-dependent binding of 14-3-3 proteins, Fig. 3) grew only slowly on glucose medium, suggesting these mutant enzymes to be less active than the wild-type PMA2 (Fig. 4). However, the modified growth phenotype was due neither to changes in the expression level of the plant H⁺-ATPase (Fig. 5B) nor to changes in the amount of phosphorylated plant H⁺-ATPase (Fig. 5C). Rather, there was a clear correlation between the reduced growth of transgenic yeast and the reduced strength of 14-3-3 binding to the plasma membrane; most of the 14-3-3 protein was released from plasma membranes of the corresponding yeast cells by high salt/low detergent treatment (Fig. 6).

Interestingly, the PMA2 region identified to be essential for 14-3-3 binding is known to be involved in autoinhibition (5).
Thus, binding of activatory 14-3-3 proteins is not restricted to a PMA2 region that is uninvolved in autoregulation. Rather, a specific autoinhibitory section contributes to the 14-3-3 binding site in vivo. Hence, the PMA2 region presumably interacting with the 14-3-3 dimer seems to be spread over a large part of the C-terminal domain (see Fig. 1). Alternatively, the plant plasma membrane H\textsuperscript{+}-ATPase could harbor two separate and distinct binding sites for regulatory 14-3-3 proteins, both of which are situated in the C terminus and each of which is able to bind within the amphipathic groove of a 14-3-3 monomer. However, a complete structural analysis of the complex is required in order to distinguish between these possibilities as well as to analyze the formation of an amphipathic α-helix within the region comprising the PMA2 residues 905–922.

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Binding of Regulatory 14-3-3 Proteins to the C Terminus of the Plant Plasma Membrane H⁺-ATPase Involves Part of Its Autoinhibitory Region

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