Complementation of the *Saccharomyces cerevisiae* Plasma Membrane H\(^+\)-ATPase by a Plant H\(^+\)-ATPase Generates a Highly Abundant Fusicoccin Binding Site*

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Accumulating evidence suggests that the H\(^+\)-ATPase of the plant plasma membrane is activated by a direct, reversible interaction with 14-3-3 proteins involving the displacement of the C-terminal autoinhibitory domain of the enzyme. The fungal phytotoxin fusicoccin (FC) appears to stabilize this H\(^+\)-ATPase-14-3-3 complex, thus leading to a persistent activation of the H\(^+\)-ATPase *in vivo*. In this study we show that functional replacement of the *Saccharomyces cerevisiae* H\(^+\)-ATPase genes by a *Nicotiana plumbaginifolia* H\(^+\)-ATPase (pma2) results in the generation of a high affinity fusicoccin binding site that is exceptionally abundant. Acquisition of FC binding capacity is accompanied by a significant increase in the amount of plasma membrane-associated yeast 14-3-3 homologs. The existence of a (plant) PMA2-(yeast)14-3-3 complex was demonstrated using two-dimensional gel systems (native/denaturing). After expression of PMA2 lacking most of its C-terminal region, neither H\(^+\)-ATPase-14-3-3 complex formation nor FC binding activity could be observed. Furthermore, we obtained direct biochemical evidence for a minimal FC binding complex consisting of the C-terminal PMA2 domain and yeast 14-3-3 homologs. Thus we demonstrated unambiguously the relevance of this regulatory ATPase domain for 14-3-3 interaction as well as its requirement for FC binding.

Solute transport across the plasma membrane of plants and fungi is driven by an electrochemical proton gradient, which is generated by the activity of a H\(^+\)-ATPase. The pivotal role of the plasma membrane H\(^+\)-ATPase in nutrient uptake and growth makes it a primary target for regulatory mechanisms (1, 2).

At the C terminus of plant and yeast H\(^+\)-ATPases, an autoinhibitory domain is present that is thought to play a role in regulation of enzyme activity. In plants, the fungal phytotoxin fusicoccin (FC)\(^1\) seems to cause the displacement of this inhibitory domain from the catalytic site, and as a consequence, it induces an activation of the H\(^+\)-ATPase *in vivo* (3, 4).

Accumulating evidence indicates that 14-3-3 proteins are mediators of FC action as well as regulators of plant H\(^+\)-ATPase activity. 1) One component of the high affinity FC-binding protein of the plant plasma membrane was shown to belong to the family of eukaryotic 14-3-3 proteins (5–7). Members of this family are highly conserved hydrophilic proteins that serve diverse regulatory functions by mediating protein-protein interactions as well as regulating protein kinase activities (reviewed in Ref. 8). 2) Recent observations suggest that 14-3-3 dimers interact directly with the H\(^+\)-ATPase, involving the displacement of the C-terminal autoinhibitory domain of the enzyme. The H\(^+\)-ATPase-14-3-3 complex represents an activated state of the enzyme that can be stabilized by FC binding *in vivo* (9–11) or *in vitro* (9). 3) Circumstantial evidence has been presented that indicates a role of the C-terminal ATPase regulator in complex formation with 14-3-3 homologs (9, 10) as well as FC binding capacity of the corresponding 14-3-3s (9). However, direct biochemical evidence for the relevance of the C terminus of the H\(^+\)-ATPase in these processes has been elusive.

We analyzed the regulation of the plant H\(^+\)-ATPase by 14-3-3 homologs after heterologous expression in the yeast *Saccharomyces cerevisiae*. In this species, the H\(^+\)-ATPase is encoded by two genes (PMA1 and PMA2), only one of which (PMA1) is constitutively expressed and essential for growth (12). Yeast transformed with pma2, a H\(^+\)-ATPase isoform from *Nicotiana plumbaginifolia*, was still able to grow when the endogenous H\(^+\)-ATPase genes were deleted (13). On the contrary, the three *Arabidopsis thaliana* H\(^+\)-ATPase genes AHA1, AHA2, and AHA3 were not able to functionally replace the yeast PMA1 unless, as shown for AHA2, the enzyme was activated by deletion of its C terminus (14–16). Apparently, the *N. plumbaginifolia* PMA2 was active in yeast despite of its C-terminal autoinhibitory domain. The yeast *S. cerevisiae* possesses two 14-3-3 isoforms, BMH1 and BMH2 (17, 18), which are candidate activators of the heterologously expressed plant H\(^+\)-ATPase.

To analyze the possible involvement of endogenous 14-3-3 proteins in the regulation of the plant PMA2 expressed in the yeast strain deleted of its own H\(^+\)-ATPase genes, the FC binding activity of this strain was investigated. Analyzing plasma membranes, we observed the presence of a high affinity FC binding site that is exceptionally abundant and the presence of a (plant)PMA2-(yeast)14-3-3 complex. Moreover, we obtained direct biochemical evidence of a PMA2 C terminus-14-3-3 complex representing the minimal complex with FC binding capacity.

EXPERIMENTAL PROCEDURES

Nomenclature—pma2 and PMA2 designate the *N. plumbaginifolia* H\(^+\)-ATPase gene and gene product, respectively. PMA1 and PMA2 and PMA1 and PMA2 designate the *S. cerevisiae* H\(^+\)-ATPase genes and gene products, respectively.

Media and Strains—Yeast cells were grown in rich medium containing 2% (w/v) glucose, 2% (w/v) yeast extract, and 20 mM KH\(_2\)PO\(_4\) adjusted with KOH to pH 6.5.
The YPS14-4 strain (Mata, ade2-101, leu2-3, his3-200, ura3-52, trp1Δ63, lys2-801, pma1Δ-D:His3, pma2Δ-D:TRP1) carries the yeast plasma membrane H^+\text{-ATPase} PMA1 gene under the control of its own promoter on a LEU2 centromeric plasmid (19). The YAKpma2 strain has the same genotype as YPS14-4 except that the plasmid carrying the yeast PMA1 gene is replaced by the 2 μm PMA1::yma2 plasmid, which harbors the N. plumbaginifolia pma2 gene under the control of the yeast PMA1 promoter on a 2 μm LEU2 plasmid (13). In YAKpma2–882stop, the N. plumbaginifolia pma2 gene had a non-sense mutation in the 3'-coding region, resulting in a H^+\text{-ATPase} deleted of its C-terminal 75-amino acid residues.

**Plasma Membrane Preparation and Solubilization of Protein**—Plasma membranes were prepared as described (20) with the modifications mentioned in Morsomme et al. (21).

Solubilization of plasma membrane proteins using dodecyl-β-D-maltoside as detergent was performed in 10 mM MOPS-Bis/Tris, 5 mM EDTA, 0.1 mM dithiothreitol, 0.5 mM ATP, 20% (v/v) glycerol, pH 7.0 (solubilization buffer). In brief, plasma membranes (4 mg ml\(^{-1}\)) were mixed dropwise with an equal volume of solubilization buffer including 20 mg ml\(^{-1}\) dodecyl-β-D-maltoside. After 30 min with continuous stirring at room temperature, unsolubilized material was pelleted at 200,000 \(\times g\) for 25 min (Beckman TL 100). The supernatant was used for further experiments.

**Mild Tryptic Treatment of Plasma Membranes**—Mild proteolysis of plasma membranes was performed to remove the C-terminal autoinhibitory domain of the H^+\text{-ATPase} following the procedure of Palmgren et al. (22). Briefly, plasma membranes (2.5 mg ml\(^{-1}\)) were mixed with an equal volume of trypsin (100 μg ml\(^{-1}\)) at a trypsin to inhibitor ratio of 1:20, w/w) in the buffer used for solubilization buffer containing 20% (v/v) glycerol. After 30 min with continuous stirring at room temperature, unsolubilized material was pelleted at 200,000 \(\times g\) for 25 min (Beckman TL 100). The supernatant was used for further experiments.

**Functional Replacement of the Yeast H^+\text{-ATPase} (N-terminal region)** and yeast 14-3-3 homologs in plasma membranes obtained from the yeast strain YPS14-4 (expressing only the yeast PMA1) as well as the strains YAKpma2 or YAKpma2–882stop (expressing only the plant wild type or C-terminal-truncated PMA2, respectively). Plasma membrane proteins (30 μg) derived either from control (lane −) or FC pretreated yeast cells (3 μM FC in vivo, lane +) were applied to SDS-PAGE (10%) followed by immunoblotting. The lower panel shows FC radioligand binding activity (pmol (mg of protein)\(^{-1}\)) of the corresponding plasma membrane preparation.

**RESULTS**

**Functional Replacement of the Yeast H^+\text{-ATPase} by the Plant PMA2**—**Genes Generates a Highly Abundant FC Binding Site**—Based on sequence information obtained from peptides, one component of the FC-binding protein (FCBP) purified from plant plasma membranes has been identified as a 14-3-3 protein (5–7). It has been proposed that the functional FCBP is represented by a reversible complex between a 14-3-3 dimer and the C-terminal domain of the H^+\text{-ATPase} (9). Formation of the H^+\text{-ATPase} 14-3-3 complex results in the activation of the H^+\text{-ATPase} (9–11). The reason for this activation seems to be a suppression of the autoinhibitory activity of the C-terminal domain of the enzyme (3, 4).

Several plant H^+\text{-ATPases} have been expressed individually in S. cerevisiae and exhibited different capabilities to sustain yeast growth in the absence of the endogenous H^+\text{-ATPase} PMA1 (13–16). The three A. thaliana genes AHA1, AHA2, and AHA3 were not able to complement the yeast PMA1 (14, 15). Interestingly, increasing the activity of AHA2 by deletion of its C-terminal autoinhibitor allowed the truncated enzyme to functionally replace the yeast H^+\text{-ATPase} (16). On the other hand, the N. plumbaginifolia H^+\text{-ATPase} PMA2 sustained yeast growth in the absence of endogenous H^+\text{-ATPase} genes without deletion of its C terminus (Ref. 13, strain YAKpma2), indicating that PMA2 exists in an apparently active state in transformed yeast. This raised the question as to whether yeast 14-3-3 proteins are involved in regulation of the heterologously expressed plant PMA2. Because participation of 14-3-3 proteins may result in FC binding capacity, we analyzed different yeast strains with respect to FC binding sites.

In agreement with earlier studies showing that wild-type yeast is devoid of any FC binding sites (32), no FC binding activity was found in the yeast strain YPS14-4 expressing the yeast PMA1 (Fig. 1, lower panel). In contrast, FC binding activity could clearly be detected in enriched plasma membranes obtained from YAKpma2 (expressing only the N. plumbaginifolia PMA2, Fig. 1, lower panel, Fig. 2).

Because the FCBP was postulated to be a complex between 14-3-3 homologs and the H^+\text{-ATPase} (9), we expected the plant PMA2 to recruit yeast 14-3-3 isoforms to the plasma membrane. As shown by immunodetection (Fig. 1), the amount of plasma membrane-associated 14-3-3 polypeptides is significantly increased in the yeast strain YAKpma2 compared with YPS14-4.

Interestingly, expression of PMA2 lacking most of its C-terminal autoinhibitory region (strain YAKpma2–882stop), which represents a per se activated state of the enzyme, failed to create FC binding sites. In this case accumulation of 14-3-3 proteins at the plasma membrane did not occur (Fig. 1).

Saturaton analysis of \(^{[3]H}\text{FCol}\) binding to plasma membranes derived from YAKpma2 revealed a high affinity site for FC (K\(_{d}\): 2.42 nm; Fig. 2), resembling plant FCBP-s (reviewed in Ref. 35). No low affinity second site could be detected as de-
nor formation of a complex between PMA2 lacking its C-terminal autoinhibitory region (strain YAKpma2-882stop), neither FC binding activity (Fig. 1) nor formation of a complex between 14-3-3 homologs and the C-terminally truncated PMA2 could be observed (Fig. 4).

Mild tryptic treatment of plasma membranes is known to remove in vivo the C terminus of the H\(^+\)-ATPase (22). To investigate the effect of limited proteolysis on YAKpma2 plasma membranes, we analyzed proteolytically released proteins by native PAGE according to size. Mild tryptic treatment of FC-stabilized yeast plasma membranes liberated a complex consisting of the C-terminal fragment of PMA2 and a 14-3-3 dimer (Fig. 5, lane +). Resolution of this native complex under denaturing conditions showed its individual components, namely 14-3-3 proteins and two peptides of approximately 5–8 kDa (Fig. 6, lane +, A, triangles). These peptides were identified as C-terminal H\(^+\)-ATPase fragments by use of antibodies raised against the C-terminal region of PMA2 (Fig. 6, lane +, B). FC treatment was necessary to demonstrate the complex (Fig. 5, lane −, Fig. 6, lane −). In the absence of FC, the individual components of the complex were released separately from the plasma membrane. As a consequence, solely 14-3-3 dimers could be detected in the native gel system, whereas the C-terminal PMA2 peptide passes the front of the gel because of its small size.

14-3-3 dimers themselves that have been released by mild tryptic treatment of YAKpma2 plasma membranes in the absence of FC were unable to bind FC (Fig. 5, lower panel). On the other hand, FC binding activity could clearly be demonstrated in the fraction containing the complex between a 14-3-3 dimer and the C terminus of PMA2 (Fig. 5, lower panel). Thus, the C terminus-14-3-3 complex represents the minimal unit exhibiting FC binding capability.

Effect of FC treatment—It has been established that an in vivo treatment of intact plant tissues with FC leads to an increase in the amount of plasma membrane-associated 14-3-3 homologs (5, 7). Under these conditions, a direct interaction of 14-3-3 homologs with the H\(^+\)-ATPase was demonstrated, indicating that FC stabilizes this complex during plant plasma membrane preparation, resulting in its increased recovery (9–11). In contrast, treatment of YAKpma2 with FC in vitro did not increase the amount of yeast 14-3-3 proteins associated with the plasma membrane (Fig. 1), indicating that the PMA2-14-3-3 complex is unusually stable and persists during yeast plasma membrane preparation, even in the absence of FC. Nevertheless, FC treatment is necessary to demonstrate this complex after native electrophoresis (Fig. 3). Taking into account that FC binding depends on the presence of the H\(^+\)-ATPase-14-3-3 complex (Fig. 5), the data strongly suggest that FC does not promote the interaction between 14-3-3 homologs and the plant PMA2; rather, it stabilizes a preformed complex.

The increase in the amount of the H\(^+\)-ATPase-plant 14-3-3 complex obtained after an in vitro FC treatment of plant tissue is accompanied by activation of the H\(^+\)-ATPase (5, 7). In contrast, FC treatment of YAKpma2 in vitro did not activate PMA2 (Table I), which is in agreement with the unaltered amount of the complex (Fig. 1). Additionally, an in vitro FC treatment had no effect on PMA2 activity (Table I). Taken together, the data suggest that binding of FC to the preformed complex does not activate PMA2; rather, the complex represents an already activated state of the H\(^+\)-ATPase. This idea is supported by the phosphohydrolytic activity of PMA2 in YAKpma2 (Table I); at pH 7.3, its activity is only slightly reduced (20%) compared with the C-terminal-truncated PMA2 (strain YAKpma2-882stop), representing a hyperactive state of PMA2. Hence, the interaction of the C terminus of PMA2 with yeast 14-3-3 homologs seems to resemble, at least partly, the effect of a deletion of the C-terminal autoinhibitor.
Generation of a Fusicoccin Binding Site in S. cerevisiae

FIG. 3. Immunodetection of the H⁺-ATPase (N-terminal region) and yeast 14-3-3 homologs after two-dimensional resolution of solubilized plasma membrane proteins obtained from YAKpma2 (expressing only the plant PMA2). Membrane proteins (30 μg) were solubilized either from control plasma membranes incubated in the absence (control) or presence of FC (3 μM FC in vitro) or from plasma membranes derived from FC pretreated yeast cells (3 μM FC in vivo). Following BN-PAGE, individual lanes of the gel were cut and transferred horizontally on a SDS-PAGE for electrophoresis in the second dimension and subsequent immunoblotting. MW, molecular mass.

DISCUSSION

Interaction of the C-terminal Domain of the Plant H⁺-ATPase with a 14-3-3 Dimer Creates a FC-binding Protein—Although the fusicoccin-binding protein initially has been identified as a 14-3-3 protein (5–7), several observations indicate that plant 14-3-3 homologs themselves are not able to bind fusicoccin. FC binding activity of partially purified plasma membrane-associated 14-3-3 proteins could only be detected when coelution with the H⁺-ATPase was observed (9). In addition, the expression of plant 14-3-3 isoforms in S. cerevisiae did not result in the generation of a FC binding site.

In this study we showed that functional expression of the plant H⁺-ATPase PMA2 in yeast deleted of its own H⁺-ATPases (strain YAKpma2) generated a highly abundant FC binding site in the yeast plasma membrane (Figs. 1 and 2). Acquisition of FC binding activity is due to direct interaction of PMA2 with endogenous 14-3-3 homologs (Fig. 3). The C-terminal-truncated PMA2 failed to interact with 14-3-3 proteins (Fig. 4) as well as to create FC binding sites (Fig. 1). Thus, this H⁺-ATPase domain appears to be essential for both complex formation with 14-3-3 proteins and generation of a functional FCBP. Additionally, in plants, several hints have been obtained suggesting a participation of the H⁺-ATPase C terminus in complex formation with 14-3-3 protein (9, 10). Nevertheless, the suggestions altogether were based on indirect evidence. With respect to YAKpma2, we obtained direct biochemical evidence for a complex consisting of the C terminus of PMA2 and a yeast 14-3-3 dimer (Fig. 5, 6). Notably, this complex represents the minimal complex with FC binding capacity. In contrast, 14-3-3 dimers themselves were unable to bind FC (Fig. 5).

Interestingly, wild-type yeast expresses 14-3-3 isoforms (17, 18) as well as a plasma membrane H⁺-ATPase but is devoid of any FC binding activity (32). The C-terminal regulatory domain of the yeast H⁺-ATPase (PMA1) is not homologous to the corresponding region of the plant enzyme. This indicates that the generation of a FC binding site mainly depends on the ability of the plant H⁺-ATPase C-terminal domain to interact with a 14-3-3 dimer.

Mechanism of FC Action—FC binding is a feature of the plant H⁺-ATPase14-3-3 complex, especially of a complex consisting of the C-terminal enzyme domain and a 14-3-3 dimer, and does not occur on the separated components (Fig. 5). Thus, we can rule out that FC promotes formation of the complex. In YAKpma2, this complex is exceptionally highly abundant. Binding of FC did not activate PMA2 (Table I). On the other hand, FC treatment was required for demonstration of the complex after native electrophoresis (Figs. 3 and 5). Thus, FC exerts its effect by stabilizing the complex between the plant H⁺-ATPase and 14-3-3 proteins.

Physiological Significance of the PMA2:14-3-3 Complex in YAKpma2—In plasma membranes obtained from YAKpma2, we observed a high amount of a complex consisting of the plant PMA2 and yeast 14-3-3 homologs and able to bind FC (Figs. 1–3). In contrast to the situation in plants, in vivo FC treatment of YAKpma2 did not increase the amount of the H⁺-ATPase14-3-3 complex (Fig. 1), indicating that it is extremely stable, even in the absence of FC. The exceptionally high abundance of FC binding sites in YAKpma2 plasma membranes compared with plant plasma membranes (Fig. 2) may thus be explained either by the higher amount of the complex in vivo and/or by the higher stability of the complex during yeast membrane isolation.

Is there any physiological significance for the formation of a plant PMA2:yeast 14-3-3 complex in YAKpma2? PMA2 is capable of functionally replacing the yeast H⁺-ATPase, suggesting an active state of PMA2 despite of the presence of its

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complete C-terminal domain. Obviously, this autoinhibitory region is involved in complex formation with 14-3-3 proteins (Fig. 5). In plants the H⁺-ATPase 14-3-3 complex represents an activated state of the H⁺-ATPase in which the autoinhibitory action of its C-terminal region is suppressed (9–11). Formation of a stable PMA2 14-3-3 complex in yeast might well be the reason why this plant isoform is capable of sustaining yeast growth in the absence of endogenous H⁺-ATPase. We therefore propose that binding of yeast 14-3-3 homologs results in the formation of an activated state of PMA2 in YAKpma2. The lack of activation of PMA2 by FC in YAKpma2 can be explained by supposing that, because of the uncommon stability of the complex, PMA2 is saturated with yeast 14-3-3 homologs and consequently maximal activated. Therefore, in vivo FC treatment could not lead to a higher complex amount and consequently could not result in activation of the PMA2 pool. However, the 14-3-3-PMA2 complex does not represent the most active form of PMA2 since point mutations in various domains of PMA2 were shown to result in increased H⁺-pump activity (21), suggesting that in this case the H⁺-ATPase undergoes a conformational change that results in full enzyme activity.

While this manuscript was in preparation, it was shown that yeast expressing the H⁺-ATPase isoform AHA2 from A. thaliana was able to grow in the absence of yeast PMA1 but only on the condition that FC was added to the growth medium (36). FC binding sites in the yeast expressing AHA2 amounted to 3 pmol mg⁻¹ plasma membrane protein (36), a value significantly lower than that determined for plant plasma membranes by using the same radioligand (Ref. 5, [3H]dihydrofusicoccin: 25–30 pmol mg⁻¹). The exogenous addition of yeast 14-3-3 isoforms in the presence of FC resulted in 5- to 10-fold higher FC binding activity and 2-fold higher ATPase activity of AHA2 (36). In contrast, in the work reported here, a much higher FC binding activity was demonstrated in yeast plasma membranes expressing the N. plumbaginifolia PMA2 (YAKpma2) in the absence of any exogenous 14-3-3 (Fig. 2: 113 pmol mg⁻¹ versus 6 pmol mg⁻¹ determined for plant plasma membranes by using [3H]FCol as radioligand). Moreover, FC had no effect on ATPase activity (Table I). These data suggest that the N. plumbaginifolia PMA2 has a structure allowing a more stable binding of yeast 14-3-3 proteins than the A. thaliana AHA2. This might be an explanation for the capacity of PMA2

| TABLE I |

| H⁺-ATPase activity of plasma membranes derived from the yeast strain YPS14–4 (expressing only the yeast PMA1) as well as the strains YAKpma2 or YAKpma2–882stop (expressing only the plant wild type or C-terminal-truncated PMA2, respectively) |
|-----------------|
|                 |
| Proteins (1 μg) of control plasma membranes incubated in the absence (control) or presence of FC (3 μM FC in vivo) or of plasma membranes derived from FC pretreated yeast cells (3 μM FC in vivo) were analyzed at pH 7.3. Values are given as nanokat (mg of protein)⁻¹. ND, not determined. |
|                  |
| Control         | FC in vivo | FC in vitro |
|-----------------|------------|-------------|
| YPS14–4         | 5.2        | 5.7         | ND           |
| YAKpma2         | 12.03      | 11.51       | 11.89        |
| YAKpma2–882stop | 15.61      | 13.64       | ND           |

FIG. 5. Immunodetection of the C-terminal fragment of PMA2 and of yeast 14-3-3 homologs after BN-PAGE of proteins that were released by mild tryptic treatment of plasma membranes obtained from the yeast strain YAKpma2 (expressing only the plant PMA2). Plasma membrane proteins (20 μg) incubated in the absence (lane —) or presence of FC (3 μM FC in vitro, lanes +) were applied to mild tryptic treatment (5 min). Proteolytically released polypeptides were subjected to BN-PAGE, followed by immunoblotting. The lower panel shows protein-bound FC radioligand (lane +) or binding activity for the FC radioligand (lane —) of the corresponding fractions determined after mild tryptic treatment of plasma membranes that were incubated in the absence (lane —) or presence (lane +) of [3H]FCol. Values are given as percentage of binding as compared with total binding activity of the plasma membrane applied (0 min trypsin exposure). MW, molecular mass.

FIG. 6. Two-dimensional resolution of proteins that were released by mild tryptic treatment of plasma membranes obtained from the yeast strain YAKpma2 (expressing only the plant PMA2). Plasma membrane proteins (20 μg) incubated in the absence (lanes —) or presence of FC (3 μM FC in vitro, lanes +) were applied to mild tryptic treatment (5 min). Proteolytically released polypeptides were subjected to BN-PAGE. Individual lanes of the native gel were cut and transferred horizontally on a SDS-PAGE for electrophoresis in the second dimension, followed by silver staining (A) or immunodetection (B) of the C-terminal fragment of PMA2 as well as yeast 14-3-3 homologs. Relevant areas of the gels are shown. MW, molecular mass.
to allow growth of yeast deleted of its own H\(^{+}\)-ATPase genes in the absence of FC (which stabilizes the AHA2-14-3-3 complex, Ref. 36).

Conclusions—14-3-3 homologs bind directly to the C-terminal autoinhibitory domain of the plant plasma membrane H\(^{+}\)-ATPase, resulting in an activation of the enzyme. The complex consisting of a 14-3-3 dimer and the C terminus of the H\(^{+}\)-ATPase represents the binding site for the fungal phytotoxin fusicoccin and is stabilized by FC treatment.

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REFERENCES

1. Palmgren, M. G. (1991) Physiol. Plant. 83, 314–323
2. Michelet, B., and Boutry, M. (1995) Plant Physiol. 108, 1–6
3. Johansson, F., Sommarin, M., and Larsson, C. (1993) Plant Cell 5, 321–327
4. Lanfermeijer, F. C., and Prins, H. B. A. (1994) Plant Physiol. 104, 1277–1285
5. Korthout, H. A. A. J., and de Boer, A. H. (1994) Plant Physiol. 106, 1497–1501
6. Marra, M., Fullone, M. R., Fogliano, V., Pen, J., Mattei, M., Masi, S., and Aducci, P. (1994) Plant Physiol. 106, 1497–1501
7. Oecking, C., Piotrowski, M., Hagemeyer, J., and Hagemann, K. (1997) Plant J. 12, 441–453
8. Jahn, T., Fuglsang, A. T., Olsson, A., Bruntrup, I. M., Collinge, D. B., Volkman, D., Sommarin, M., Palmgren, M. G., and Larsson, C. (1997) Plant Cell 9, 1805–1814
9. Olivari, C., Meanti, C., De Micheli, M. I., and Rasi-Caldogno, F. (1998) Plant Physiol. 116, 529–537
10. Serrano, R., Kielland-Brandt, M. C., and Fink, G. R. (1986) Nature 319, 689–693
11. de Kerkhove d’Exaerde, A., Supply, P., Dufour, J.-P., Bogaerts, P., Thines, D., Goffeau, A., and Boutry, M. (1995) J. Biol. Chem. 270, 23828–23837
12. Villalba, J. M., Palmgren, M. G., Berberian, G. E., Ferguson, C., and Serrano, R. (1992) J. Biol. Chem. 267, 12341–12349
13. Palmgren, M. G., and Christensen, G. (1994) J. Biol. Chem. 269, 3027–3033
14. Regenberg, B., Villalba, J. M., Lanfermeijer, F. C., and Palmgren, M. G. (1995) Plant Cell 7, 1655–1666
15. van Heusden, G. P. H., Wenzel, T. J., Lagendijk, E. L., Steensma, H. Y., and van den Berg, J. A. (1992) FEBS Lett. 302, 145–150
16. van Heusden, G. P. H., Griffiths, D. J. F., Ford, J. C., Chin-A-Woeng, T. F. C., Schrader P. A. T., Carr, A. M., and Steensma, H. Y. (1995) Eur. J. Biochem. 229, 45–53
17. Supply, P., Wach, A., Thines-Sempoux, D., and Goffeau, A. (1993) J. Biol. Chem. 268, 19744–19752
18. Goffeau, A., and Dufour, J. P. (1988) Methods Enzymol. 157, 528–533
19. Marsonne, P., de Kerkhove D’Exaerde, A., de Meester, S., Thines, D., Goffeau, A., and Boutry, M. (1996) EMBO J. 15, 5513–5526
20. Palmgren, M. G., Larsson, C., and Sommarin, M. (1990) J. Biol. Chem. 265, 13425–13426
21. Feyerabend, M., and Weiler, E. W. (1988) Planta 174, 115–122
22. Bruns, R. F., Lawison-Wendling, K., and Pugsley, T. A. (1983) Anal. Biochem. 132, 74–81
23. Hodges, T. K., and Leonard, R. T. (1974) Methods Enzymol. 32, 292–324
24. Graf, P., and Weiler, E. W. (1989) Physiol. Plant. 75, 469–478
25. Laemmli, U. K. (1970) Nature 227, 680–685
26. Schagger, H., Cramer, W. A., and van Jagow, G. (1994) Anal. Biochem. 217, 220–230
27. Townhin, H., Staelhevin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350–4354
28. Serrano, R., Monk, B. C., Villalba, J. M., Montesinos, C., and Weiler, E. W. (1993) Eur. J. Biochem. 212, 737–744
29. Paredes-Soler, A., Parko, J. M., and Serrano, R. (1990) Plant Physiol. 93, 1654–1658
30. Meyer, C., Waldkötter, K., Sprenger, A., Schlosser, U. G., Luther, M., and Weiler, E. W. (1993) Zeit. Naturforsch. Biosci. 48, 595–602
31. Aducci, P., Marra, M., Fogliano, V., and Fullone, M. R. (1995) J Exp. Bot. 46, 1463–1478
32. Meyer, C., Feyerabend, M., and Weiler, E. W. (1989) Plant Physiol. 89, 692–699
33. Oesch, C., and Weiler, E. W. (1991) Eur. J. Biochem. 199, 685–689
34. Baunsgaard, L., Fuglsang, A. T., Jahn, T., Korthout, H. A. A. J., de Boer, A. H., and Palmgren, M. G. (1998) Plant J. 13, 661–671
35. Scatchard, G. (1949) Ann. N. Y. Acad. Sci. U. S. A. 51, 660–672