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CAX1, an H^+/Ca^{2+} antiporter from *Arabidopsis*

(*Arabidopsis thaliana*/calcium transport/vacuole/membrane protein/*Saccharomyces cerevisiae*)

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ABSTRACT **Reestablishment of the resting state after stimulus-coupled elevations of cytosolic-free Ca^{2+} requires the rapid removal of Ca^{2+} from the cytosol of plant cells. Here we describe the isolation of two genes, *CAX1* and *CAX2*, from *Arabidopsis thaliana* that suppress a mutant of *Saccharomyces cerevisiae* that has a defect in vacuolar Ca^{2+} accumulation. Both genes encode polypeptides showing sequence similarities to microbial H^+/Ca^{2+} antiporters. Experiments on vacuolar membrane-enriched vesicles isolated from yeast expressing *CAX1* or *CAX2* demonstrate that these genes encode high efficiency and low efficiency H^+/Ca^{2+} exchangers, respectively. The properties of the *CAX1* gene product indicate that it is the high capacity transporter responsible for maintaining low cytosolic-free Ca^{2+} concentrations in plant cells by catalyzing pH gradient-energized vacuolar Ca^{2+} accumulation.**

Calcium plays a central role in signal transduction by plant cells (1, 2). Plant responses to red light (3), gravity (4) touch, cold shock, and fungal elicitors (5) are accompanied by transient elevations of cytosolic-free Ca^{2+} ($[Ca^{2+}]_{free}$). In animals, Ca^{2+} is primarily mobilized from the endoplasmic reticulum via inositol 1,4,5-trisphosphate-gated and cADPR-gated channels (6, 7), but in plants the same types of channel mediate release from the vacuole (8–10). Since vacuolar $[Ca^{2+}]_{free}$ is in the millimolar range, whereas cytosolic $[Ca^{2+}]_{free}$ is 0.1 μM or less and rises to 0.5-1 μM during signal transduction (1, 2), restoration of the resting state requires Ca^{2+} transport into the vacuole against a transmembrane concentration difference of at least 10^4.

The plant vacuolar membrane is known to possess high capacity H^+/Ca^{2+} exchange activity (11–13) but all attempts to identify the transporter(s) responsible have failed. Although vacuolar H^+/Ca^{2+} antiport activity has been solubilized, partially purified, and reconstituted from *Avena sativa* (14), the number and identities of the polypeptides directly contributing to activity have not been determined because of insufficient purification.

The recent characterization of yeast mutants defective in Ca^{2+} transport has afforded a means of screening for plant H^+/Ca^{2+} exchanger genes. Yeast *vcx1 pmc1* mutants deleted for the vacuolar H^+/Ca^{2+} antiporter (*VCX1*) and vacuolar P-type Ca^{2+}-ATPase (*PMC1*) genes are hypersensitive to Ca^{2+} in the growth medium (15, 16). Functional cloning of these plant genes should now be possible by the transformation of a *Saccharomyces cerevisiae vcx1 pmc1* strain with an *Arabidopsis thaliana* cDNA library and selection for suppression of Ca^{2+} hypersensitivity.

Here, we describe the isolation and characterization of two *A. thaliana* genes, designated *CAX1* and *CAX2* (calcium exchangers 1 and 2), that suppress Ca^{2+} hypersensitivity in yeast *vcx1 pmc1* mutants. Both genes encode polypeptides possessing structural similarities to the H^+/Ca^{2+} exchangers from microbial sources. *In vitro* measurements demonstrate that expression of *CAX1* and *CAX2* results in high and low efficiency transmembrane pH difference (ΔpH)-dependent Ca^{2+} uptake, respectively. On the basis of these findings, we conclude that CAX1p and CAX2p belong to the same family of membrane proteins as the microbial antiporters. The activity associated with CAX1p is indistinguishable from the H^+/Ca^{2+} antiport activity identified previously on the vacuolar membrane of plant cells.

MATERIALS AND METHODS

Yeast Strains and Plant Materials. Yeast strains K605 *MAT*a (*pmc1*::*TRP1*), K607 *MAT*a (*cnb1*::*LEU2 pmc1*::*TRP1*) and K665 *MAT*a (*vcx1*::*hisG pmc1*::*TRP1*) were used (15, 16). All strains were otherwise isogenic and harbored the following additional mutations: *ade2-1 can1-100 his3-11, 15 leu2-3, 112 trp1-1*, and *ura3-1*. *A. thaliana* ecotype Columbia was the source of DNA and RNA for the hybridization analyses.

Yeast Transformation and Selection. To identify plant genes that were able to suppress Ca^{2+} hypersensitivity, *S. cerevisiae vcx1 pmc1* strain K665 was transformed with an *Arabidopsis* cDNA library constructed in the episomal yeast-*Escherichia coli* shuttle vector pFL61 (17) and stable Ura^+ transformants were selected by plating on synthetic complete-Ura medium (18). Of these, $\approx 6 \times 10^5$ colonies were replicated to yeast extract/peptone/dextrose medium (1% Difco extract/2% Bacto-peptone/2% dextrose, pH 5.5) containing 200 mM $CaCl_2$ (15) to identify Ca^{2+}-tolerant transformants. Two of the independent clones identified by this procedure, pFL61-*CAX1* and pFL61-*CAX2*, were characterized further. To establish that the restoration of growth on high Ca^{2+} was attributable to the *Arabidopsis* cDNA inserts, pFL61-*CAX1* and pFL61-*CAX2* were reselected in liquid medium and plasmid DNA was isolated and introduced into several mutant strains defective in vacuolar Ca^{2+} sequestration. In all cases, growth on 5-fluoroorotic acid (19) cured the positive transformants of the newly acquired plasmid and restored Ca^{2+} hypersensitivity.

DNA and RNA Manipulations. Standard DNA techniques were used (20). RNA was purified and analyzed as described (21). Degenerate PCR was performed using tomato genomic DNA as template. The sequences of the *CAX* primers were 5′-GCTTTTATCAGTRYCATCTTGCTTCC-3′, where R = A or G and Y = C or T, and 5′-GTCCATCTKTTGMCCCATCCAWCCAAT-3′, where K = T or G, M = C or A, and W = A or T. Amplification was for 50 cycles using the following thermal profile: 94°C for 1 min, 55°C for 1.5 min, 72°C for 1 min, followed by a final 5 min of extension at 72°C. The PCR product was isolated, cloned into pGEM-T vector (Promega) and sequenced.

Abbreviations: *CAX1* and *CAX2*, cDNAs (genes) encoding *Arabidopsis* Ca^{2+} exchangers 1 and 2, respectively; *chaA*, gene encoding *Escherichia coli* Ca/H^+ exchanger; *PMC1*, gene encoding *Saccharomyces cerevisiae* vacuolar Ca^{2+}-ATPase; V-ATPase, vacuolar H^+-translocating ATPase; *VCX1*, gene encoding *Saccharomyces cerevisiae* vacuolar calcium exchanger; ΔpH, transmembrane pH difference; EST, expressed sequence tag.

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Preparation of Membrane Vesicles. Vacuolar membrane-enriched vesicles were prepared as described (22). Stationary phase cultures (200 ml) of pFL61-*CAX1*-, pFL61-*CAX2*-, or pFL61-transformed K665 cells were diluted into 1 liter volumes of fresh AHC medium [0.17% (wt/vol) ammonium sulfate/1% (wt/vol) acid-hydrolyzed casein/0.002% (wt/vol) adennine hemisulfate/2% (wt/vol) glucose/50 μM Tris·Mes, pH 5.5] supplemented with 2% (wt/vol) glucose, grown for 20 h at 30°C to an OD_{600} of $\approx$1.4, and collected by centrifugation. After resuspension in 10 mM dithiothreitol and 100 mM Tris·HCl (pH 9.4) and incubation for 20 min at 37°C with gentle shaking, the cells were pelleted, resuspended in 100 ml yeast extract/peptone medium [1% (wt/vol) yeast extract/2% (wt/vol) Bacto-peptone] containing 0.7 M sorbitol, 5 mM dithiothreitol, and 100 mM Tris·HCl (pH 7.5), and converted to spheroplasts by the addition of 50 mg of Zymolyase 20T (ICN). The suspension was incubated for 60–90 min at 30°C with gentle shaking, pelleted by centrifugation, and resuspended in 50 ml ice-cold homogenization medium [10% (wt/vol) glycerol/1.5% (wt/vol) polyvinylpyrrolidone/5 mM Tris·EGTA/2 mg/ml bovine serum albumin/50 mM Tris·ascorbate, pH 7.6] containing 1 mM phenylmethylsulfonyl fluoride and 1 μg/ml leupeptin. After lysis by homogenization, the crude lysate was cleared of cell debris and unbroken cells by centrifugation at 4000 × *g* for 5 min. The pellet was resuspended in another 50 ml of homogenization medium, homogenized again, and recentrifuged. The supernatants from both low speed centrifugations were pooled and centrifuged at 100,000 × *g* for 35 min. The pellet was resuspended in suspension medium (1.1 M glycerol/2 mM dithiothreitol/2 mg/ml bovine serum albumin/5 mM Tris·HCl, pH 7.6) containing 1 mM phenylmethylsulfonyl fluoride and 1 μg/ml leupeptin, layered onto a discontinuous 10% (wt/wt):28% sucrose density gradient, and centrifuged at 100,000 × *g* for 2 h. Vacuolar membrane-enriched vesicles were withdrawn from the 10–28% interface, diluted more than 250-fold with suspension medium (without bovine serum albumin), and pelleted by centrifugation at 100,000 × *g* for 35 min.

Measurement of $^{45}Ca^{2+}$ Uptake. For the measurements of the time dependence of Ca^{2+} uptake, membrane vesicles (35–40 μg/ml) were incubated in buffer (0.6 ml) containing 1 mM 1,3-bis[tris(hydroxymethyl)ethylamino]propane (BTP)-ATP, 50 mM KCl, 0.1 mM sodium vanadate, 0.2 mM sodium azide, 0.4 M glycerol, and 5 mM BTP-Mes (pH 8.0). Vacuolar H^+-translocating ATPase (V-ATPase)-catalyzed H^+-translocation was initiated by the addition of 1 mM $MgSO_4$ and the vesicles were allowed to reach steady state with respect to ΔpH for 5 min at 25°C before the addition of $^{45}Ca^{2+}$ (10 μM; 33 Ci/mol calcium; 1 Ci = 37 GBq). At the times indicated, aliquots (70 μl) of the incubation medium were removed and filtered through premoistened 0.22 μm pore-size cellulose acetate GS type filters (Millipore). After three successive 1 ml washes with ice-cold wash buffer (0.4 M glycerol/5 mM BTP-Mes, pH 8.0) containing 1 mM $CaCl_2$, the filters were air-dried and radioactivity was determined by liquid scintillation counting. The concentration dependence of uptake was measured under initial rate conditions with single samples taken 30 s after the addition of $^{45}Ca^{2+}$. Uncoupler-sensitive (ΔpH-dependent) uptake was estimated as the difference between parallel samples with and without the addition of carbonylcyanide *p*-(trifluoromethoxy)phenylhydrazone (FCCP) (5 μM). The conditions were otherwise as for the measurements of the time dependence of uptake.

All glycerol stock solutions were subjected to cation-exchange with Dowex 50W (H^+ form) before use to reduce contaminating Ca^{2+} to less than 0.1 μM (13).

Protein Assays. Membrane protein was determined by a modification of the Bradford method (23) using bovine serum albumin as standard.

RESULTS

Isolation of *CAX1* and *CAX2* cDNAs. *S. cerevisiae* strain K665 deleted for the high affinity Ca^{2+}-ATPase and low affinity Ca^{2+}/H^+ antiporter (*PMC1* and *VCX1*) is intolerant of high Ca^{2+} in the growth medium (15, 16) (Fig. 1). To clone *Arabidopsis* H^+/Ca^{2+} exchangers, we transformed this *vcx1 pmc1* strain with an *A. thaliana* cDNA library and selected for uracil prototrophy and suppression of Ca^{2+} hypersensitivity. Two plasmids isolated from this screen, pFL61-*CAX1* and pFL61-*CAX2*, restore growth on high Ca^{2+} in a manner indistinguishable from pFL61-*VCX1* cells (Fig. 1).

The lengths of the inserts of pFL61-*CAX1* and pFL61-*CAX2* and the lengths of their respective mature heterologous and endogenous transcripts coincide, indicating that both cDNAs are near full length. Northern blot analyses detected a single band of $\approx$1.6 kb after hybridization of random-primed *CAX1* or *CAX2* cDNA with total RNA extracted from either pFL61-*CAX1*- or pFL61-*CAX2*-transformed K665 cells or from several tissues (roots, rosette leaves, stems, flowers, and siliques) of 5-week-old *Arabidopsis* plants (data not shown). Likewise, Southern blot analyses using either *CAX1* or *CAX2* cDNA as probes revealed strongly hybridizing sequences in the *Arabidopsis* genome, confirming that both clones are derived from *Arabidopsis* genes rather than nonplant contaminants of the cDNA library.

Sequence Characteristics. Sequence analysis established that the open reading frames of *CAX1* and *CAX2* encode closely related (43% identical, 62% similar) polypeptides of 459 and 399 amino acids, respectively, belonging to the same family of membrane proteins as microbial H^+/Ca^{2+} antiporters (Fig. 2*A*). First, a BLAST search of CAX1p and CAX2p against GenBank data base release 90 (27) revealed 33% identity (53% similarity) and 38% identity (53% similarity) to VCX1p, and 18% identity (46% similarity) and 21% identity (44% similarity) to the *E. coli* Ca^{2+}/H^+ antiporter, chaA (26) (Fig. 2*A*). Second, CAX1p, CAX2p, VCX1, and chaA appear to have analogous domain organizations. Computer-assisted hydropathy analyses generated profiles with an equivalent spacing and alternation of hydrophilic with hydrophobic domains, consistent with an intrinsic membrane protein that spans the membrane 11 times (Fig. 2*B*). All four transporters contain a central hydrophilic motif, rich in acidic amino acid residues, that bisects the polypeptide into two groups of five or six transmembrane spans (Fig. 2 *A* and *B*).

The BLAST search also disclosed a partial cDNA [expressed sequence tag (EST)] sequence from *Oryza sativa* encoding a polypeptide showing 48% and 69% identity to CAX1p and CAX2p, respectively, over a span of 124 residues (Fig. 2*A*). Similarly, degenerate PCR using tomato genomic DNA as

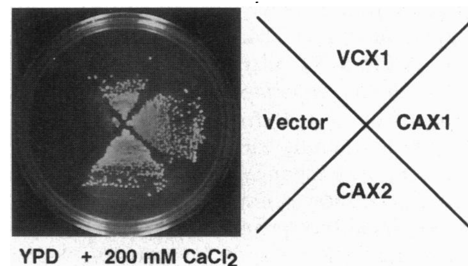

FIG. 1. Suppression of the Ca^{2+} hypersensitivity of the *S. cerevisiae vcx1 pmc1* mutant by *Arabidopsis CAX1* and *CAX2*. *S. cerevisiae vcx1 pmc1* strain K665 was transformed with control vector, pFL61 (Vector), vector containing the entire reading frame of *VCX1*, pFL61-*VCX1* (VCX1), vector containing the entire reading frame of *Arabidopsis CAX1*, pFL61-*CAX1* (CAX1), or vector containing the entire reading frame of *CAX2*, pFL61-*CAX2* (CAX2). The cultures were streaked on yeast extract/peptone/dextrose (YPD) plates supplemented with 200 mM $CaCl_2$.

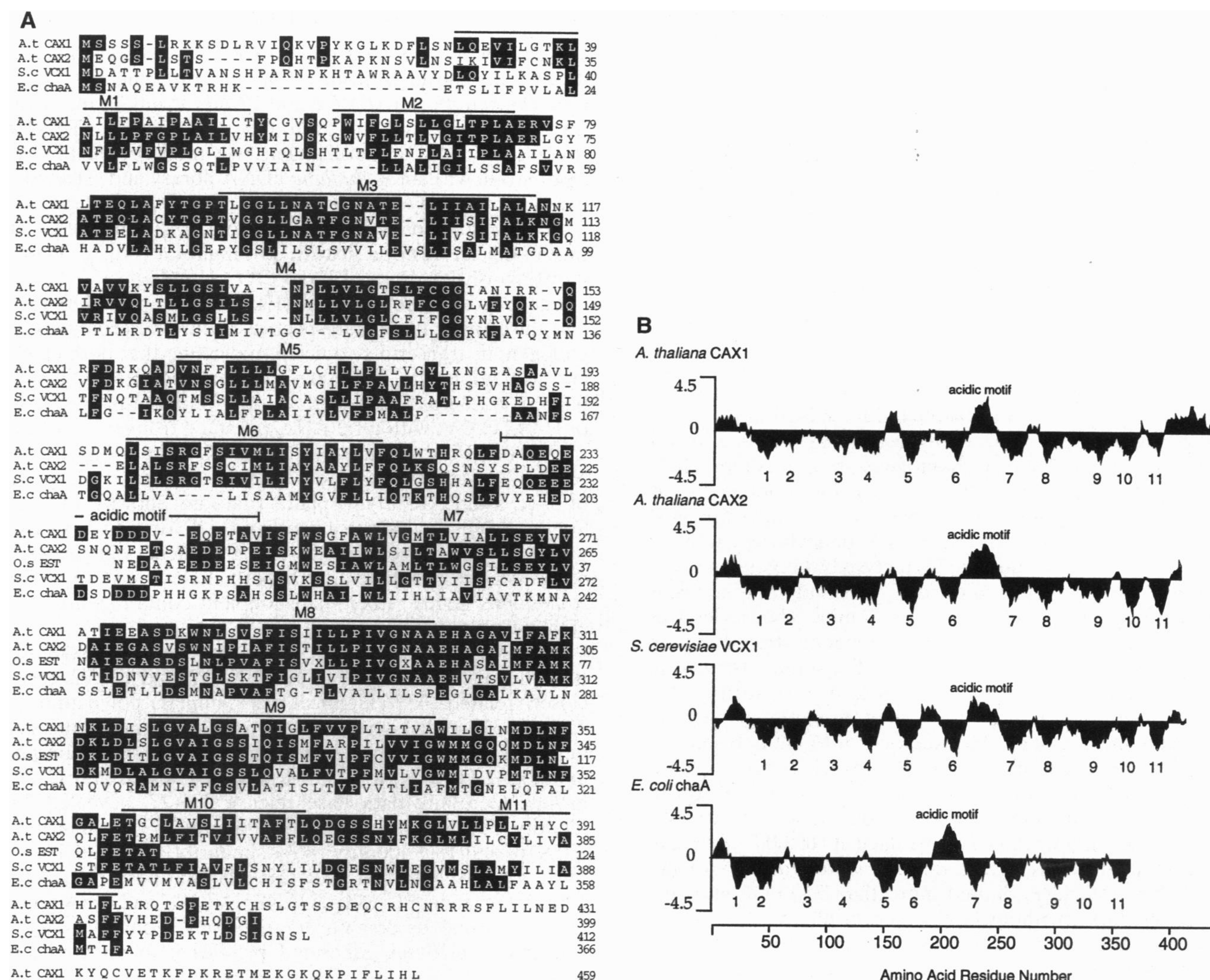

FIG. 2. (*A*) Alignment of deduced amino acid sequences of polypeptides encoded by *A. thaliana* (A.t) *CAX1* and *CAX2*, a partial cDNA sequence from *O. sativa* (O.s EST) of unknown function, *S. cerevisiae* (S.c) *VCX1*, and *E. coli* (E.c) *chaA*. Sequences were aligned by the CLUSTAL method (24) using the MEGALIGN program (DNAstar, Madison, WI). X, ambiguous amino acids in the partial cDNA from rice. Identical residues are shown in white on a black background. Gaps introduced to maximize the alignments are denoted by hyphens. The 11 putative transmembrane spans (M1–M11) predicted for all three transporters and the central hydrophilic motif rich in acidic residues are overlined. (*B*) Hydropathy plots of *Arabidopsis* CAX1p and CAX2p, *S. cerevisiae* VCX1p, and *E. coli* chaA. Putative transmembrane spans are numbered. Hydropathy was computed and putative transmembrane spans were predicted according to Kyte and Doolittle (25) over a running window of 15 amino acid residues. The accession numbers for yeast *VCX1* (16), *Arabidopsis CAX1* and *CAX2*, *E. coli chaA* (26), and the rice EST are U36603, U57411, U57412, P31801, and D15647, respectively.

template yielded an amplification product that was 85% identical to *CAX1* (data not shown).

ΔpH-Dependent Ca^{2+} Transport. The resemblance of CAX1p and CAX2p to VCX1p and chaA and the finding that suppression of the *S. cerevisiae vcx1 pmc1* double mutant by high-copy expression of *CAX1* or *CAX2* increased the nonexchangeable (predominantly vacuolar) Ca^{2+} pool several-fold without effecting the exchangeable (predominantly cytoplasmic) pool (data not shown) implies that CAX1p and CAX2p are *Arabidopsis* VCX1p equivalents. Presumably, *CAX1* and *CAX2* remedy the *vcx1* mutation by restoring vacuolar H^+/Ca^{2+} antiport. To test this proposal directly, vacuolar membrane-enriched vesicles were purified from pFL61, pFL61-*CAX1*, and pFL61-*CAX2* transformed K665 cells and their capacity for ΔpH-dependent Ca^{2+} uptake was examined.

Provision of MgATP and establishment of a steady state ΔpH by the V-ATPase associated with the vacuolar membrane before the addition of $^{45}Ca^{2+}$ resulted in appreciable uptake by membrane vesicles from the *CAX1* transformants (Fig. 3). The initial rate and extent of $^{45}Ca^{2+}$ uptake by vesicles from pFL61-*CAX1* transformed K665 cells were 7 nmol/mg protein per min and 10 nmol/mg protein, respectively (Fig. 3*A*). The corresponding values for the equivalent membrane fraction from pFL61-*CAX2* transformed cells were 1.2 nmol/mg protein per min and 4.2 nmol/mg protein (Fig. 3*B*). In both cases, inclusion of the V-ATPase inhibitor, bafilomycin A_1, or the protonophore, FCCP, in the uptake medium decreased $^{45}Ca^{2+}$ uptake to a level similar to that seen in the absence of MgATP (Fig. 3 *A* and *B*). The low rates and extents of uptake (0.3 nmol/mg protein per min and 1 nmol/mg protein) obtained with membranes from K665 cells transformed with control vector, lacking *CAX1* or *CAX2*, were inhibited by neither bafilomycin A_1 nor FCCP (Fig. 3*C*). These results, together with the immediate release of $^{45}Ca^{2+}$ seen on the addition of the Ca^{2+} ionophore, A23187, to pFL61-*CAX1* (Fig. 3*A*) or pFL61-*CAX2* vesicles (Fig. 3*B*) versus the small increase in $^{45}Ca^{2+}$ uptake seen with pFL61 vesicles (Fig. 3*C*), demonstrate that ΔpH-dependent, *CAX*-dependent uptake is concentrative, whereas ΔpH-independent, *CAX*-independent uptake is equilibrative.

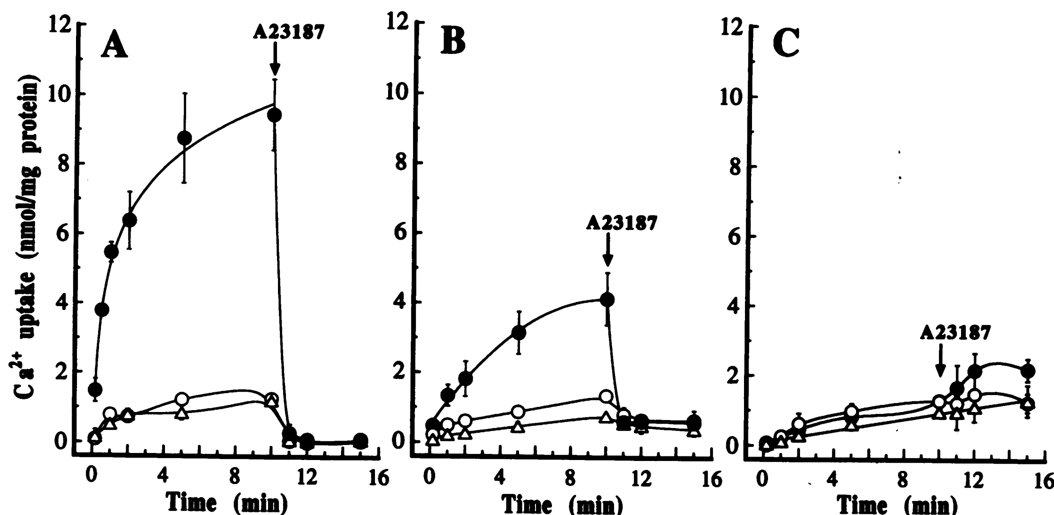

FIG. 3. Time-course of $^{45}Ca^{2+}$ uptake into vacuolar membrane-enriched vesicles prepared from *S. cerevisiae* strain K665 after transformation with pFL61-*CAX1* (*A*), pFL61-*CAX2* (*B*), or pFL61 (*C*). Uptake in the absence of inhibitors and protonophores (●); uptake in the presence of 5 μM FCCP (○); uptake in the presence of 500 nM bafilomycin A_1 (△). The Ca^{2+} ionophore, A23187 (5 μM), was added at the times indicated. $^{45}Ca^{2+}$ was added at a concentration of 10 μM throughout.

Regardless of whether antiport was assayed as V-ATPase-dependent (bafilomycin A_1-sensitive) $^{45}Ca^{2+}$ uptake (data not shown) or ΔpH-dependent (FCCP-sensitive) uptake (Fig. 4), the initial rate for vacuolar membrane-enriched vesicles purified from pFL61-*CAX1* transformants increased as a simple hyperbolic (Michaelian) function of Ca^{2+} concentration to yield K_m and V_{max} values of 13.1 ± 6.1 μM and 12.4 nmol/mg protein per min, respectively. The corresponding kinetic parameters for membranes from pFL61-*CAX2* cells were > 100 μM and < 4 nmol/mg protein per min. By contrast, FCCP-sensitive uptake by the equivalent membrane fraction from

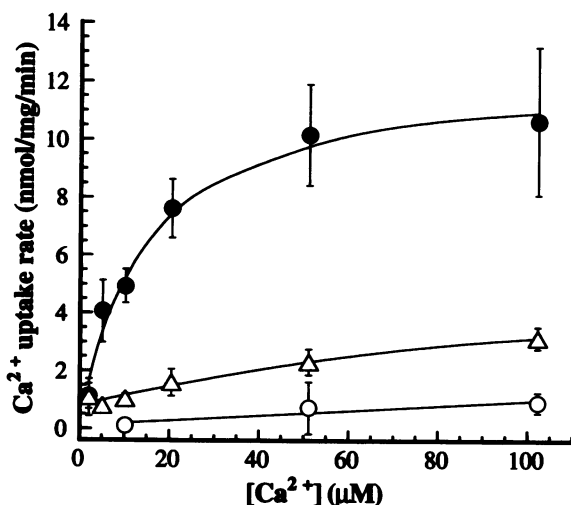

FIG. 4. Kinetics of uncoupler-sensitive Ca^{2+} uptake into vacuolar membrane-enriched vesicles prepared from *S. cerevisiae* strain K665 after transformation with pFL61-*CAX1* (●), pFL61-*CAX2* (△), or pFL61 (○). Uptake was measured under initial rate conditions with single samples taken 30 s after the addition of $^{45}Ca^{2+}$. Uncoupler-sensitive (ΔpH-dependent) uptake was estimated as the difference between parallel samples with and without the addition of FCCP (5 μM). The conditions were otherwise as described in Fig. 3. A nonlinear least squares algorithm (ULTRAFIT package, BioSoft, Ferguson, MO; ref. 28) was used to provide a least squares fit to the Michaelis-Menten equation to yield K_m and V_{max} values of 13.1 ± 6.1 μM and 12.3 ± 1.8 nmol/mg protein per min (±SD), respectively, for uptake by the membranes from pFL61-*CAX1* transformants. Data points are means ± SD for 4–7 sets of measurements on two independent membrane preparations.

K665 pFL61 cells increased as a linear function of Ca^{2+} concentration and was more than 25- and 5-fold slower than *CAX1*- and *CAX2*-dependent uptake, respectively, throughout the concentration range examined.

DISCUSSION

Maintenance of cytosolic $[Ca^{2+}]_{free}$ in plants has been attributed in part to the low-affinity, high-capacity H^+/Ca^{2+} exchanger on the vacuolar membrane (11–14, 29); however, no previous molecular identification has been reported for this biochemical activity. Identification of the polypeptide(s) responsible for such transport activity is inherently difficult given its low abundance, the lack of known type-specific affinity probes and the need for cumbersome *in vitro* reconstitutions for ascription of activity. Therefore, an independent genetic approach was adopted to identify the H^+/Ca^{2+} transport activity. Specifically, a plant cDNA library was screened for clones capable of suppressing the Ca^{2+} hypersensitivity of *S. cerevisiae* strains defective in vacuolar Ca^{2+} transport.

Both of the *Arabidopsis* cDNA clones isolated by this approach, *CAX1* and *CAX2*, satisfy the requirements of ΔpH-energized divalent cation antiporters. At the structural level, CAX1p and CAX2p contain 11 putative transmembrane spans and an intervening acidic hydrophilic motif, typical of the two previously characterized H^+/Ca^{2+} antiporters, VCX1p and chaA, from microbial sources. At the functional level, CAX1p and CAX2p catalyze ΔpH-dependent Ca^{2+} transport. High copy expression of *CAX1* or *CAX2* suppresses a yeast mutant defective in vacuolar Ca^{2+} sequestration and *in vitro* measurements of uptake by vacuolar membrane vesicles from pFL61-*CAX1* and pFL61-*CAX2* transformed K665 cells demonstrate a strict dependence of Ca^{2+} accumulation on an outwardly directed H^+ gradient.

The kinetics of CAX1p- and CAX2p-mediated transport by yeast vacuolar membranes imply different roles for these transporters in the intact plant. CAX1p activity closely approximates that described for the vacuolar H^+/Ca^{2+} activities from a broad range of plant sources with respect to $K_{m(Ca)}$ and susceptibility to inhibition by lanthanum and ruthenium red (data not shown) (11–14). The high $K_{m(Ca)}$ of CAX2p, on the other hand, suggests that it does not directly participate in cytosolic Ca^{2+} transport; preliminary ion selectivity experiments indicate that CAX2p is a high-affinity, high-capacity H^+/heavy metal cation antiporter (data not shown). Signifi-

cantly, neither CAX1p nor CAX2p has the properties of the plant plasma membrane H^+/Ca^{2+} antiporter that, unlike either protein, transports Mg^{2+} as well as Ca^{2+} (30, 31). Thus, on the basis of their transport activities in yeast, CAX1p is provisionally identified as the high capacity vacuolar H^+/Ca^{2+} antiporter responsible for the transport of cytosolic Ca^{2+}, whereas CAX2p is assigned a role in the transport of heavy metals by plants.

CAX1p and CAX2p appear to function in the yeast vacuolar membrane, suggesting that they fold properly and contain the information required for H^+-coupled Ca^{2+} transport. CAX2p is able to support growth of the mutant even though it has weak H^+/Ca^{2+} antiport activity, presumably because it is overexpressed. Although our data suggest vacuolar localization of these proteins in yeast, their localization *in planta* has not been addressed. However, the availability of the yeast assay system should permit histochemical detection of both gene products in plants by the development of functional tagged versions. By the same token, the availability of the yeast suppression screen for these transporters, and their apparent ubiquity in both dicots and monocots, as judged by the strong homologies between *CAX1* and the tomato PCR product and rice EST, will provide a means for probing the structural basis of CAX-dependent ion homeostasis in plants.

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