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Identification of cDNA clones encoding valosin-containing protein and other plant plasma membrane-associated proteins by a general immunoscreening strategy

[anti-(plasma membrane) serum/differential screening/phospholipase C/clathrin heavy chain/*S*-adenosylmethionine:-Δ^{24}-sterol-*C*-methyltransferase]

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ABSTRACT **An approach was developed for the isolation and characterization of soybean plasma membrane-associated proteins by immunoscreening of a cDNA expression library. An antiserum was raised against purified plasma membrane vesicles. In a differential screening of ≈500,000 plaque-forming units with the anti-(plasma membrane) serum and DNA probes derived from highly abundant clones isolated in a preliminary screening, 261 clones were selected from ≈1,200 antiserum-positive plaques. These clones were classified into 40 groups by hybridization analysis and 5′- and 3′-terminal sequencing. By searching nucleic acid and protein sequence data bases, 11 groups of cDNAs were identified, among which valosin-containing protein (VCP), clathrin heavy chain, phospholipase C, and *S*-adenosylmethionine:Δ^{24}-sterol-*C*-methyltransferase have not to date been cloned from plants. The remaining 29 groups did not match any current data base entries and may, therefore, represent additional or yet uncharacterized genes. A full-length cDNA encoding the soybean VCP was sequenced. The high level of amino acid identity with vertebrate VCP and yeast CDC48 protein indicates that the soybean protein is a plant homolog of vertebrate VCP and yeast CDC48 protein.**

The plant plasma membrane delimits the boundary between the cytosol and cell wall and has important physiological functions in cell wall synthesis, active transport of ions and metabolites, hormone action and response, plant–microbe interactions, and stress responses. Many of these functions are done by membrane proteins that are anchored in or associated with the lipid bilayer. Only a few plant membrane proteins have been purified, including H^+-ATPase, Ca^{2+}-ATPase, auxin-stimulated NADPH oxidase, sucrose transporter, and fusicoccin-binding protein complex (1). Similarly, only a few genes encoding plasma membrane proteins have been isolated, including H^+-ATPase, sugar transporter, nitrate transporter, K^+ channel, and water channel (2–7).

The individual protein constituents of the plasma membrane are not readily extracted and purified because of their high degree of hydrophobicity. Although several new detergents are now available, solubilization of membrane proteins without changing their activities is still a major problem for the characterization of membrane proteins. Here we report an alternative approach for characterization of plasma membrane-associated proteins. The technique consists of three steps: (*i*) raising an antiserum against purified plasma membranes, (*ii*) screening a cDNA expression library using the anti-(plasma membrane) serum, and (*iii*) identifying the proteins encoded by the cDNA clones by partial sequence analysis and data base search for homology (8). Using this approach, we have isolated 40 groups of cDNA clones that encode putative plasma membrane-associated proteins from a soybean hypocotyl cDNA expression library. Eleven genes or gene families were identified.§ The possible applications and limitations of this approach are discussed.

MATERIALS AND METHODS

Plant Material. Seeds of soybean (*Glycine max* [L.] Merr.) cultivars Harosoy 63 and Williams 82 were sown in moist vermiculite, and etiolated seedlings were grown for 7 days in the dark under the controlled conditions described earlier (9).

Preparation of Plasma Membranes. Hypocotyls of etiolated seedlings (cv. Harosoy 63) were homogenized at 4°C in extraction buffer containing 0.33 M sucrose, 50 mM Mops (pH 7.5), 5 mM EDTA, 0.2% (wt/vol) bovine serum albumin, 0.2% (wt/vol) casein enzymatic hydrolysate (C-0626), 5 mM ascorbic acid, 1 mM dithiothreitol, and 0.6% (wt/vol) polyvinylpolypyrrolidone. The homogenate was passed through nylon cloth (240 μm), phenylmethylsulfonyl fluoride was added to a final concentration of 1 mM, and the homogenate was centrifuged at 10,000 × *g* for 10 min. The supernatant was collected and centrifuged at 50,000 × *g* for 30 min to obtain the microsomal pellet. Plasma membrane vesicles were prepared from the crude microsomal pellet by partitioning in an aqueous polymer two-phase system as described by Larsson *et al.* (10). Protein concentrations were estimated by using the DC protein assay kit (Bio-Rad) with bovine serum albumin as standard.

Production of Anti-(Plasma Membrane) Serum. Purified plasma membrane vesicles were washed twice by suspending in 10 mM potassium phosphate buffer (pH 7.8) and centrifuged at 100,000 × *g* for 30 min to remove entrapped proteins. Plasma membranes equivalent to 2.6 mg of protein were emulsified in an equal volume of Freund's complete adjuvant and injected into a rabbit. A second injection of 2.6 mg of plasma membrane protein was administered 4 weeks after the first injection. The antiserum was prepared from the blood withdrawn 2 weeks after the second injection.

Gel Electrophoresis and Immunoblotting. Proteins were separated electrophoretically on SDS/polyacrylamide gels (11). For immunoblotting analysis, proteins were transferred to nitrocellulose membranes, which were blocked with 1% bovine serum albumin in Tris-buffered saline, pH 7.2, and probed with the anti-(plasma membrane) serum at various dilutions.

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Abbreviations: VCP, valosin-containing protein; hsp, heat shock protein; CDPK, calcium-dependent protein kinase; sVCP, soybean VCP; pfu, plaque-forming units.

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§The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U20213 and T41432–T41519).

Construction of cDNA Library. Total RNA was prepared from etiolated hypocotyls of soybean cv. Williams 82. Poly(A)$^+$ mRNA was purified by using a PolyATract mRNA isolation kit (Promega). cDNA was synthesized using an *Xho* I/oligo(dT)-linker/primer and cloned unidirectionally into the *Eco*RI and *Xho* I sites of the λ phage vector Uni-ZAP (Stratagene).

Screening Procedures. *Escherichia coli* strain XL1-Blue was used as the host for library plating. Preliminary screening of the cDNA library was conducted on a small scale using only anti-(plasma membrane) serum. A total of 450,000 plaque-forming units (pfu) were then differentially screened by using the antiserum and DNA probes derived from the abundant clones isolated in the preliminary screening. This differential screening procedure reduced the repeated isolation of the abundant sequences in the cDNA library.

The cDNA library was plated at a low density of 10,000 pfu per 120-mm plate. Two sets of duplicate filters were prepared for differential screening. One set of filters was probed by using the anti-(plasma membrane) serum preabsorbed with *E. coli*/phage lysate, BNN97 (Stratagene). The other set was hybridized with DNA probes labeled with [α-^{32}P]dATP using the Prime-a-Gene system (Promega). For differential selection, autoradiographs were aligned with immunoblots, and the clones revealed by the antiserum but not the DNA probes were picked for further analysis.

DNA Sequencing. Plasmids (pBluescript-SK) containing inserts were excised *in vivo* from recombinant phage and introduced into the *E. coli* SOLR strain (Stratagene) according to the manufacturer's instruction. Plasmid DNA was isolated by the modified alkaline lysis method (12) and purified by PEG precipitation. Single-run partial sequencing of the 5′ and 3′ terminals was done by the dideoxynucleotide chain-termination method. The nucleotide sequences were obtained by electrophoresis on an automated DNA sequencer (Applied Biosystems, model 373A).

Computer Analysis of Nucleotide and Protein Sequences. Partial DNA sequences were examined for homology in the GenBank nucleic acid data base. The sequences without GenBank matches were compared with the National Center for Biotechnology Information (NCBI; Bethesda, MD) non-redundant protein data base. The nucleic acid and protein data base searches were conducted on the NCBI's GnInfo network BLAST (13). Comparison of cDNAs among themselves was done by using the PC/GENE program (IntelliGenetics).

RESULTS

Immunoreactivity of Anti-(Plasma Membrane) Serum. The purified plasma membrane vesicles from etiolated soybean hypocotyls accounted for 2.5% of the parent microsomal fractions on the basis of their protein contents. SDS/PAGE analysis of the vesicles showed that the electrophoretic protein pattern of the plasma membranes isolated from etiolated hypocotyls (Fig. 1*A*) resembled that from roots reported earlier (14). Because of differential abundance and antigenic activities of individual plasma membrane proteins, the pattern of immunoreactivity of the antiserum on an immunoblot was not identical to the SDS/PAGE protein pattern (Fig. 1*B*), and more bands were visible when higher concentrations of the antiserum were used.

The relative immunoreactivity of the anti-(plasma membrane) serum to soluble and plasma membrane proteins was compared by SDS/PAGE immunoblot analysis. Bands present in both soluble and plasma membrane fractions presumably reflected multiple locations of some proteins. The antiserum against plasma membrane proteins reacted with some soluble proteins, but differences in band patterns between the two fractions were clearly apparent (Fig. 2).

Isolation of cDNA Clones. In an initial library screening, 50,000 pfu were plated and screened with anti-(plasma membrane) serum at a dilution of 1:5000. Twenty-one clones were randomly selected from 126 antiserum-positive clones. Of these, 17 were identified as 70-kDa heat shock protein (70-kDa hsp) sequences based on partial DNA sequencing and hybridization analysis. The 70-kDa hsp sequences were thereafter used as a DNA probe for differential screening to reduce re-isolation of 70-kDa hsp clones. In a preliminary differential screening, 78,000 pfu were screened with both the antiserum and a 70-kDa hsp DNA probe. Two hundred and seven positive clones were obtained by immunoscreening, 118 of which did not hybridize to the

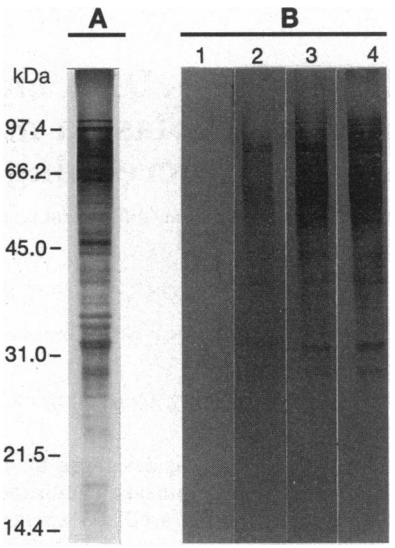

FIG. 1. Protein gel blot analysis showing immunoreactivities of anti-(plasma membrane) serum to soybean plasma membrane proteins at different dilutions of antibody. Plasma membrane vesicles equivalent to 40 μg of protein were loaded in each lane. (*A*) SDS/PAGE analysis. The gel was silver-stained. (*B*) Immunoblot analysis. Dilutions of antiserum used were 1:10,000 (lane 2), 1:1000 (lane 3), and 1:500 (lane 4). Lane 1 was treated with preimmune serum.

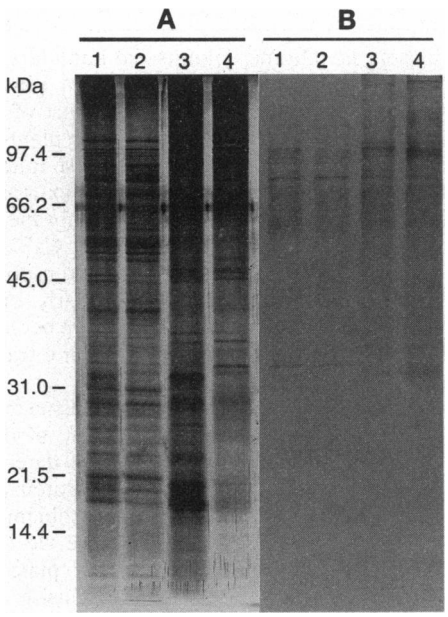

FIG. 2. Protein gel blot analysis showing immunoreactivities of anti-(plasma membrane) serum to different cellular fractions. (*A*) SDS/PAGE analysis. Total cellular protein (lane 1), soluble proteins (lane 2), microsomal fraction (lane 3), and plasma membrane fraction (lane 4) were prepared from etiolated hypocotyls of soybean, and 40 μg of protein was loaded in each lane. The gel was silver-stained. (*B*) Immunoblot analysis of the same cellular fractions as in *A*. The antiserum was diluted 1:5000.

Table 1. Isolation of clones encoding putative plasma membrane proteins by screening a cDNA expression library with anti-(plasma membrane) serum

	Screen number		
	1 (preliminary)	2	3
pfu screened	78,000	200,000	250,000
Antiserum dilution	1:5000	1:5000	1:1000
Positive clones, no.	207	480	691
Groups isolated, no.	13	10	17
Groups identified, no.	7	0	4

70-kDa hsp probe. At least one-third of these clones were identified as calcium-dependent protein kinase (CDPK). Further differential screening used CDPK and 70-kDa hsp sequences, together with a lipoxygenase isolated in the initial screening, to further reduce re-isolation of abundant clones on subsequent large-scale screening.

A total of 450,000 pfu were then screened with the antiserum and the above DNA probes in two separate experiments. The first screening was of 200,000 pfu at an antiserum dilution of 1:5000. Out of 480 antiserum-positive plaques, 74 did not hybridize to the mixed DNA probes. Of these, 51 clones were purified and further analyzed by hybridization and sequencing. The second screening was of 250,000 pfu at an antiserum dilution of 1:1000, and 691 positive plaques were obtained. Of these, 186 did not hybridize with the DNA probes. These plaques were picked, and 164 were purified. The purified clones were analyzed by hybridization using the same DNA probes as above. Seventy clones did not hybridize to the mixed DNA probes and were further analyzed by partial DNA sequencing.

Sequencing and Classification of cDNA Clones. Single-run sequencing was done to generate sequence tags. The length of the sequence tags ranged from 250 to 350 bases. The error frequency (base deletion and insertion) was ≈0.15%, and average ambiguous base calling was 5.53%, as estimated by comparison of the partial sequences of 70-kDa hsp, CDPK, and lipoxygenase clones with corresponding sequences in data bases.

5′-Terminal sequences were generated first and compared against each other. The sequence tags with >90% overlap on a minimum stretch of 50 bases were considered to be overlapping. Independent clones determined on the basis of 5′-terminal sequence were then sequenced from their 3′ ends. The cDNA clones were classified into 40 groups (Table 1). Of these, 13 were isolated in the preliminary screening, most of which represented relatively abundant sequences in the library. Fifteen groups were represented by a single clone. Five genes in the 70-kDa hsp class, at least four CDPK-encoding genes, and three lipoxygenase genes were identified, based on the sequences of overlapping regions. Eighty-eight sequence tags were submitted to the GenBank data base with accession nos. T41432–T41519.

Characterization of cDNA Clones. A homology search of the sequence tags against nucleic acid and protein data bases identified 11 out of the 40 groups of cDNA clones (Table 2). A significant match between two sequences was indicated when the PAM120 score was >100. Among these sequences, annexin, phospholipase C, thioredoxin, and isomers of CDPK and lipoxygenase have previously been reported to be associated with the plasma membrane in higher plants or animals (15–20). The clathrin heavy chain is associated with coated pits on plasma membranes and with coated vesicles in the cytosol. Isomers of 70-kDa hsp are involved in decoating processes (21). The yeast gene *ERG6* encodes a membrane-bound *S*-adenosylmethionine:Δ^{24}-sterol-*C*-methyltransferase (22, 23). The remaining 29 groups of cDNAs showed no matches with any entry in data bases, indicating that they may represent additional, previously uncharacterized genes.

The entire insert of clone Spm 429, which showed homology to vertebrate valosin-containing protein (VCP) at the terminal regions, was sequenced. The open reading frame encoded a protein of 807 amino acids with calculated molecular mass of 89,769 Da. DNA sequence upstream of the ATG start codon contained two in-frame stop codons. The deduced amino acid sequence of the encoded protein contains an internal duplication of ≈200 amino acids. Each of the repeat sequences harbors a putative nucleotide-binding site of the motif A type (aa 249–256; 522–529) and a consensus sequence (aa 349–365; 625–644) for the AAA-protein family (ATPases associated with diverse cellular activities, ref. 24). Sequence comparisons with members of the VCP group of the AAA-protein family showed that the soybean protein has 77.5% and 67.9% amino acid identity to pig VCP (25) and *Saccharomyces cerevisiae* cell-cycle protein CDC48 (26), respectively (Fig. 3). At the nucleotide level, the soybean cDNA is 70% identical to pig VCP in the coding region. Given the evolutionary distances between soybean and pig or yeast, the soybean protein (named soybean VCP, sVCP) probably has similar, if not identical, functions to vertebrate VCP and yeast CDC48 protein.

The AAA-protein family also includes yeast PAS1, SEC18, and AFG2; mammalian *N*-ethylmaleimide-sensitive fusion protein, Tat binding protein 1, and 26S protease subunit 4; and *E. coli* ftsh protein. These proteins have one or two copies of the conserved domain and display diverse cellular functions in

Table 2. cDNA clones encoding proteins that cross-react with anti-(plasma membrane) serum and show sequence similarities to known genes

Clone	Description*	Length compared, aa	Identity,† %	GenBank accession no.
Spm 173	Annexin (P08133)	74	41	T41436
Spm 307	Clathrin heavy chain (P11442)	92	67	T41441
Spm 192	Ca^{2+}-dependent protein kinase (P28582)	107	69	T41437
Spm 8	70-kDa hsp (S14950)	100	92	T41484
Spm 84	Lipoxygenase (S18612)	113	70	T41483
Spm 537	Phospholipase C III (P10895)	74	43	T41472
Spm 483	Pyruvate kinase (Z29492)	92	54	T41469
Spm 15	Thioredoxin (S16590)	65	41	T41434
Spm 16	Vegetative storage protein (M37530)	84	100	T41435
Spm 429	VCP (Q01853)	43	76	T41463
Spm 482	Yeast gene *ERG6* (X54249)	47	57	T41468

*A description of the best data match is given together with data base accession no. of the match in parentheses. Identification is based on both 3′- and 5′-end sequences. Results from one end are presented. aa, Amino acids.

†Percentage represents amino acid identity of length compared.

membrane fusion, peroxisome assembly, 26S proteasome function or regulation of human immunodeficiency virus gene expression (refs. 24, 26–30 and the references contained

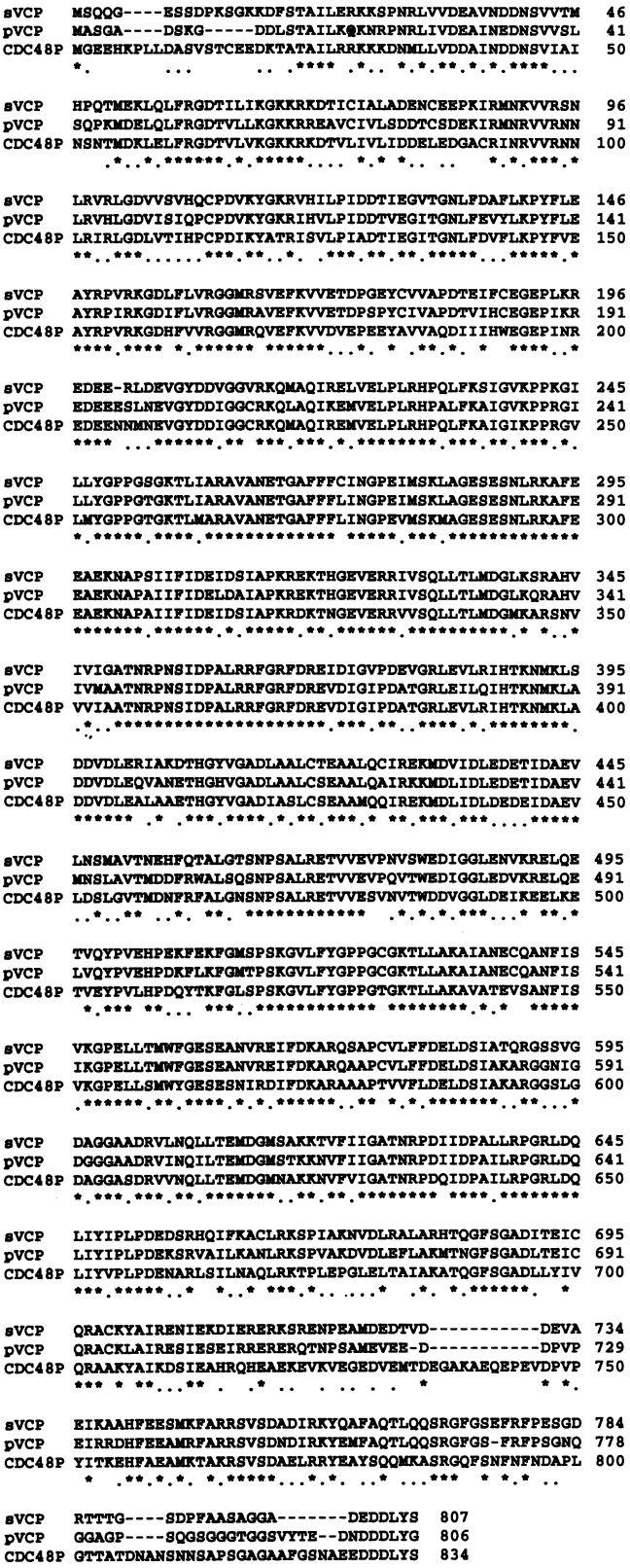

FIG. 3. Alignment of the deduced amino acid sequence of soybean VCP (sVCP) with those of pig VCP (pVCP) and *S. cerevisiae* CDC48 protein. * and • indicate identical and similar amino acids, respectively. The nucleotide sequence data of the sVCP cDNA have been deposited in the GenBank data base under accession no. U20213.

therein). Sequence comparisons between sVCP and these proteins are summarized in Table 3.

Search for Specific cDNA Clones. To verify whether the above screening approach is generally applicable for isolation of cDNAs that encode plasma membrane proteins, including integral membrane proteins, cDNA clones encoding two known integral plasma membrane proteins, H^+-ATPase and sugar transporter, were isolated from the soybean library by using *Arabidopsis* H^+-ATPase (2) and alfalfa sugar transporter (M. J. Harrison, personal communication) cDNAs as probes. About 5×10^5 pfu of the expression library were screened on duplicated filters using these cDNA clones and the anti-(plasma membrane) serum. Around 280 positive clones were obtained from hybridization to H^+-ATPase and sugar transporter cDNAs that showed similar expression levels (based on clone frequency in library) in the etiolated soybean hypocotyls. However, none of these positive clones showed cross-reactivity to the anti-(plasma membrane) serum.

DISCUSSION

We describe a strategy for isolation and identification of cDNA clones that encode putative plasma membrane-associated proteins, based on screening an expression library using an antiserum against purified plasma membrane vesicles. Application of the strategy to soybean has resulted in the isolation of 40 groups of cDNAs from an etiolated hypocotyl cDNA expression library. Of these groups, 11 genes or gene families have been identified by partial DNA sequencing and data base homology searches. By using a similar strategy, genes encoding a plastid envelope Ca^{2+}-ATPase-like protein (31), two plasma membrane proteins, nitrilase and water channel, and a putative plasma membrane-associated aminopeptidase were isolated from *Arabidopsis thaliana* (7, 32, 33). Detailed analyses of the anti-(plasma membrane) serum and antiserum positive clones, however, were not conducted.

In one *A. thaliana* random cDNA sequencing project, 1152 randomly selected cDNA clones were partially sequenced, and 895 clones were identified as unique cDNAs (34). In the present project, ≈1200 anti-(plasma membrane) serum-positive cDNA clones were characterized by differential screening, hybridization analysis, and partial DNA sequencing and then classified into 40 groups. Although the 895 cDNA classes in the *Arabidopsis* random sequencing project are likely to be an overestimate (as indicated by the authors), the present approach clearly presents a more stringent selection strategy (i.e., nonrandom). In the *Arabidopsis* project, 210 cDNA groups (23%) were identified by partial sequencing and data base comparison from the 895 unique cDNAs. Of these identified cDNAs, 59 (28%) encoded ribosomal proteins or translation-related proteins, and at least 44 (21%) encoded

Table 3. Sequence comparison between sVCP and other members of the AAA-protein family

Protein	Source*	Amino acid identity to sVCP, %	
		Overall	Conserved region†
AGF2	*S. cerevisiae* (780)	41	51 (210–707)
NSF	Chinese hamster (744)	27	45 (210–423)
SEC18	*S. cerevisiae* (757)	27	42 (210–423)
PAS1	*S. cerevisiae* (1043)	30	50 (450–665)
TBP1	Human (404)	34	42 (460–705)
26SPS4	Human (440)	34	48 (207–430)
ftsh	*E. coli* (644)	28	44 (210–436)

NSF, mammalian *N*-ethylmaleimide-sensitive fusion protein; TBP1, Tat binding protein 1; 26SPS4, 26S protease subunit 4.
*Amino acid length of the protein is given in parentheses.
†Number in parentheses indicates compared region of sVCP amino acid sequence.

plastidial or mitochondrial proteins, nuclear proteins, vacuolar proteins, seed storage proteins, tubulin, or extension. Many of the identified clones encoded enzymes involved in glycolysis, the tricarboxylic acid cycle, and secondary metabolism; thus the majority of the 210 identified *Arabidopsis* clones encoded nonplasma membrane proteins. In contrast, 8 out of the 11 identified clones obtained in the present work putatively encode plasma membrane-associated proteins.

Of the 40 groups of cDNAs isolated in this study, 11 genes or gene families (27.5%) were identified. Among the 11 genes, 8 encode proteins that have been localized to the plasma membrane or plasma membrane-associated structures in higher plants or mammals. Annexin is a Ca^{2+}-dependent phospholipid-binding protein and is known to be plasma membrane-associated in mammals and higher plants (15, 35, 36). Isomers of CDPK and lipoxygenase are plasma membrane-bound proteins in higher plants (16, 19, 20). Phospholipase C is a key enzyme in the transduction of agonist-dependent signals across the plasma membrane in animal cells and has also been identified in plasma membranes from plants (37). The soybean phospholipase C isolated in this study has been localized to plasma membranes (J.S., R.A.G., and M.K.B., unpublished results). The soybean thioredoxin had high similarity to mammalian thioredoxins, which have multiple subcellular locations including the plasma membrane (17, 18, 38), and was shown to have a putative transmembrane domain using the PC/GENE program RAOARGOS (data not shown). A transmembrane domain has previously been reported in a bacterial thioredoxin that is localized on the cytoplasmic membrane (39). Clathrin heavy chain is the major component of coated-pit proteins. A member of the 70-kDa hsp family has ATPase activity and is involved in the decoating process for clathrin-coated structures (21). Seventy-kilodalton hsp is a well-known chaperone (40) and may, therefore, also be involved in protein translocation across the plasma membrane and protein folding and assembly on the plasma membrane.

The high level of amino acid identity between soybean clone Spm 429 and VCPs indicates that sVCP may be a functional equivalent of the vertebrate VCP and yeast cell cycle CDC48 protein. The vertebrate VCP is a clathrin-binding protein (27) and has been found to form an oligomeric ring-shaped particle in the cytosol and display Mg^{2+}-ATPase activity (41). This feature is shared by the mammalian *N*-ethylmaleimide-sensitive fusion protein, which is a component of the vesicle fusion apparatus (29, 30). The yeast CDC48 protein may play a role in vesicle attachment during spindle-pole proliferation in cell division (26), and sVCP may, therefore, be involved in membrane transport of clathrin-coated pits and vesicles.

A major limitation to the present approach is that the anti-(plasma membrane) serum may not recognize all antigens present in the plasma membrane, as shown here specifically for two integral proteins, H^+-ATPase and sugar transporter. Most cDNAs with known functions obtained through this approach encode peripheral plasma membrane proteins. There may be limitations in raising antisera against integral membrane proteins. Alternatively, it is also possible that the integral membrane proteins are not correctly folded in the present *E. coli*-based expression system so that they are not recognized by the anti-(plasma membrane) serum. Use of eukaryotic expression systems may eliminate such problems.

In summary, the present approach is likely to provide an alternative method of characterizing plasma membrane proteins that are not readily extracted and purified. The isolation of cDNA clones encoding VCP, clathrin heavy chain, annexin, and phospholipase C may facilitate the dissection of basic biological processes—such as exocytosis, endocytosis, intracellular vesicle trafficking, cell cycling, and plasma membrane signal transduction in plants.

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1. Larsson, C., Møller, I. M. & Widell, S. (1990) in *The Plant Plasma Membrane*, eds. Larsson, C. & Møller, I. M. (Springer, Berlin), pp. 1–15.
2. Harper, J. F., Surowy, T. K. & Sussman, M. R. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 1234–1238.
3. Sussman, M. R. (1994) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **45,** 211–234.
4. Sentenac, H., Bonneaud, N., Minet, M., Lacroute, F., Salmon, J.-M., Gaymard, F. & Grignon, C. (1992) *Science* **256,** 663–665.
5. Sauer, N., Friedländer, K. & Graml-Wicke, U. (1990) *EMBO J.* **9,** 3045–3050.
6. Tsay, Y., Schroeder, J. I., Feldman, K. A. & Crawford, N. M. (1993) *Cell* **72,** 705–713.
7. Kammerloher, W., Fischer, U., Piechottka, G. P. & Schaffner, A. R. (1994) *Plant J.* **6,** 187–199.
8. Adams, M. D., Kelley, J. M., Gocayne, J. D., Dubnick, M., Polymeropoulos, M. H., Xiao, H., Merril, C. R., Wu, A., Olde, B., Moreno, R. F., Kerlavage, A. R., McCombie, W. R. & Venter, J. C. (1991) *Science* **252,** 1651–1656.
9. Ward, E. W. B., Lazarovits, G., Unwin, C. H. & Buzzell, R. I. (1979) *Phytopathology* **69,** 951–955.
10. Larsson, C., Widell, S. & Kjellbom, P. (1987) *Methods Enzymol.* **148,** 558–568.
11. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
12. Feliciello, I. & Chinali, G. (1993) *Anal. Biochem.* **212,** 394–401.
13. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. (1990) *J. Mol. Biol.* **215,** 403–410.
14. Grimes, H. D. & Breidenbach, R. W. (1987) *Plant Physiol.* **85,** 1048–1054.
15. Blackbourn, H. D., Barker, P. J., Huskisson, N. S. & Battey, N. H. (1992) *Plant Physiol.* **99,** 864–871.
16. Droillard, M.-J., Rouet-Mayer, M.-A., Bureau, J.-M. & Lauriére, C. (1993) *Plant Physiol.* **103,** 1211–1219.
17. Holmgren, A. & Luthman, M. (1978) *Biochemistry* **17,** 4071–4077.
18. Dean, M., Martin, H. & Sansom, P. A. (1994) *Biochem. J.* **304,** 861–867.
19. Schaller, G. E., Harmon, A. C. & Sussman, R. (1992) *Biochemistry* **31,** 1721–1727.
20. Verhey, S. D., Gaiser, J. C. & Lomax, T. L. (1993) *Plant Physiol.* **103,** 413–419.
21. Kirsch, T. & Beevers, L. (1993) *Plant Physiol.* **103,** 205–212.
22. Gaber, R. F., Copple, D. M., Kennedy, B. K., Vidal, M. & Bard, M. (1989) *Mol. Cell. Biol.* **9,** 3447–3456.
23. Zinser, E., Paltauf, F. & Daum, G. (1993) *J. Bacteriol.* **175,** 2853–2858.
24. Kunau, W.-H., Beyer, A., Goette, K., Marzioch, M., Saidowsky, J., Skaletz-Rorowski, A. & Wiebel, F. F. (1993) *Biochimie* **75,** 209–224.
25. Koller, K. J. & Brownstein, M. J. (1987) *Nature (London)* **325,** 542–545.
26. Fröhlich, K.-U., Fries, H.-W., Rüdiger, M., Erdmann, R., Botstein, D. & Mecke, D. (1991) *J. Cell Biol.* **114,** 443–453.
27. Pleasure, I. T., Black, M. M. & Keen, J. H. (1993) *Nature (London)* **365,** 459–462.
28. Eakle, K. A., Bernstein, M. & Emr, S. D. (1988) *Mol. Cell. Biol.* **8,** 4098–4109.
29. Wilson, D. W., Wilcox, C. A., Flynn, G. C., Chen, E., Kuang, W.-J., Henzel, W. J., Block, M. R., Ullrich, A. & Rothman, J. E. (1989) *Nature (London)* **339,** 355–359.
30. Sollner, T., Whiteheart, S. W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P. & Rothman, J. E. (1993) *Nature (London)* **362,** 318–324.
31. Huang, L., Berkelman, T., Franklin, A. E. & Hoffman, N. E. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 10066–10070.
32. Bartling, D. & Weiler, E. W. (1992) *Eur. J. Biochem.* **205,** 425–431.
33. Bartling, D., Seedorf, M., Mithöfer, A. & Weiler, E. W. (1992) *Eur. J. Biochem.* **205,** 417–424.
34. Höfte, H., Desprez, T., Amselem, J., Chiapello, H., Caboche, M., *et al.* (1993) *Plant J.* **4,** 1051–1061.
35. Clark, G. B., Dauwalder, M. & Roux, S. J. (1992) *Planta* **187,** 1–9.
36. Chung, C. Y. & Erickson, H. P. (1994) *J. Cell Biol.* **126,** 539–548.
37. Melin, P., Pical, C., Jergil, B. & Sommarin, M. (1992) *Biochim. Biophys. Acta* **1123,** 163–169.
38. Marcus, F., Chamberlain, S. H., Chu, C., Masiarz, F. R., Shin, S., Yee, B. C. & Buchanan, B. B. (1991) *Arch. Biochem. Biophys.* **287,** 195–198.
39. Loferer, H., Bott, M. & Hennecke, H. (1993) *EMBO J.* **12,** 3373–3383.
40. Gething, M.-J. & Sambrook, J. (1992) *Nature (London)* **355,** 33–45.
41. Peters, J.-M., Harris, J. R., Lustig, A., Muller, S., Engel, A., Volker, S. & Franke, W. W. (1992) *J. Mol. Biol.* **223,** 557–571.