A High Content in Lipid-modified Peripheral Proteins and Integral Receptor Kinases Features in the *Arabidopsis* Plasma Membrane Proteome*§

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The proteomics of plasma membrane has brought to date only scarce and partial information on the actual protein repertoire. In this work, the plant plasma membrane proteome of *Arabidopsis thaliana* was investigated. A highly purified plasma membrane fraction was washed by NaCl and Na₂CO₃ salts, and the insoluble fractions were further analyzed by nano-LC-MS/MS. With 446 proteins identified, we hereby describe the largest plasma membrane proteome diversity reported so far. Half of the proteins were predicted to display transmembrane domains and/or to be anchored to the membrane, validating *a posteriori* the pertinence of the approach. A fine analysis highlighted two main specific and novel features. First, the main functional category is represented by a majority of as yet unreported signaling proteins, including 11% receptor-like kinases. Second, 16% of the identified proteins are predicted to be lipid-modified, specifically involving double lipid linkage through N-terminal myristoylation, C-terminal prenylation, or glycosylphosphatidylinositol anchors. Thus, our approach led for the first time to the identification of a large number of peripheral proteins as part of the plasma membrane and allowed the functionality of the plasma membrane in the cell context to be reconsidered. *Molecular & Cellular Proteomics* 6:1980–1996, 2007.

The plasma membrane (PM) has a peculiar status among membrane systems as it is directly in connection with the extracellular environment. Consequently this membrane is an essential element for cell primary functions, such as cellular differentiation or proliferation, and a privileged target for abiotic and biotic factors. To achieve all these functions, a large variety of proteins is necessary, including transport proteins, receptor proteins, and also proteins involved in signaling or cellular traffic. Schematically two types of membrane proteins occur. Integral proteins span the membrane and strongly interact with it usually through at least one hydrophobic transmembrane α-helix or less frequently with a β-barrel structure. Insertion of integral proteins into the membrane often needs the so-called secretion pathway through the endoplasmic reticulum and involves the use of an N-terminal signal peptide. When uncleaved by signal peptidase, the hydrophobic composition of the signal peptide can also participate in membrane binding. Peripheral proteins represent the second class of membrane proteins. They do not have typical hydrophobic domains and interact with the membrane with only one domain. Such an interaction may involve amphipathic helices, hydrophobic loops, and covalent links to lipid anchors, electrostatic interactions with the lipids of the membrane, or even protein-protein interactions.

The investigation of a membrane proteome remains challenging with respect to both direct analyses (e.g. biochemical fractionation and proteomics analyses) and *in silico* approaches. Because of the existence of structural features such as the occurrence of a signal peptide or transmembrane (TM) α-helices, predictive analysis using bioinformatics has been a powerful means to predict membrane proteomes. For instance, 20–30% of the predicted ORFs of a typical animal or plant proteome are usually predicted to display at least one TM helix (1). A combination of various criteria, based on both the amino acid sequence of an ORF including prediction of endoplasmic targeting and the occurrence of TM helices, led to the proposal that as much as 25% of the ORFs of the plant factor; LRR, leucine-rich repeat; RLK, receptor-like kinase; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor; HIR, hypersensitive induced reaction protein; ER, endoplasmic reticulum; CYP, cyclophilin; SEC, Sec protein export pathway; RAB, Ras related in brain.

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1 The abbreviations used are: PM, plasma membrane; GPI, glycosylphosphatidylinositol; TM, transmembrane; TAIR, The Arabidopsis Information Resource; NCBI, National Center for Biotechnology Information; PPDB, Plastid Proteome Database; ARF, ADP-ribosylation.
Arabidopsis thaliana corresponded to potential integral membrane proteins (2). Prediction of the exact membrane or compartment where these proteins are targeted is a hard task as well and often leads to confusing or contradictive interpretations (3). Identification of the peripheral membrane proteins is a much more problematic issue. Whole genome predictions of lipid modification by N-myristoylation, prenylation, or glycosyl-phosphatidylinositol (GPI) anchors have been initiated as an attempt toward the identification of peripheral proteomes (4–7). The ratio of peripheral proteins modified by lipids is assessed to be less than 4%, and the issue of the exact membrane location is not yet solved. Because they do not have specific features in their amino acid sequence, other types of peripheral membrane proteins are difficult to identify through predictive approaches.

To make a decisive contribution to the remaining unanswered issues relative to membrane localization and identification of peripheral proteins, direct approaches involving membrane fractionation and protein characterization by proteomics investigation are needed. Such approaches are fully expected also to confirm the predictions. Due to their diversity of functions, the PM proteins display a wide variety of biochemical properties, and they are associated with lipid domains differing in composition. Consequently a combination of techniques that differentially fractionates proteins according to their physicochemical properties and/or their degree of their integration in the membrane is expected to allow a better overview of the PM proteome protein diversity. Early attempts have led to the identification of less than 300 proteins as part of the PM proteome, i.e., 1 order of magnitude less than the value expected from the aforementioned bioinformatics studies (8–11). More targeted plant plasma membrane analyses provided a more extensive coverage of the plasma membrane proteome including some GPI-anchored and lipid raft proteins (12–16). Collectively a set of about 500 PM proteins, identified via proteomics analyses, has been reported in the literature so far, indicating that a majority of PM proteins are still to be identified through such approaches.

In a previous study, about 100 putative proteins were identified as part of the hydrophobic plasma membrane proteome isolated from Arabidopsis cell suspensions, 95% of which were yet unidentified proteins (9). To increase the PM repertoire, a highly purified plasma membrane fraction was washed by NaCl and Na2CO3 salts; the insoluble fractions were analyzed by nano-LC-MS/MS mass spectrometry. From these two approaches, we identified around 450 proteins, among which more than 45% were predicted to possess transmembrane domains and/or to be anchored to the membrane. Moreover among these 450 proteins, we identified 289 proteins (65%) that had never been reported in other plant PM proteomics investigations. In the present report, we describe the largest plasma membrane proteome reported so far, allowing us to give a deeper insight into the plant PM and its role in the cell.

EXPERIMENTAL PROCEDURES

Cell Culture and Plasma Membrane Purification—The Arabidopsis cell culture condition, the plasma membrane purification procedure, and associated purity assessments were as described in Marmagne et al. (9, 17). Briefly 5-day-old suspension culture cells were collected, and a microsomal fraction was obtained after grinding and a series of differential centrifugations. A PM-enriched fraction was purified from microsomes by two-phase partitioning between polyethylene glycol and dextran (6.4%, w/w). To eliminate polyethylene glycol, a second partitioning was achieved with a 0.7 M KH2PO4 solution. The PM fraction recovered in the saline lower phase was ultracentrifuged (110,000 × g), and the resulting pellet was resuspended in 50 mM MOPS/NaOH (pH 7.8), 1 mM dithiothreitol. This yielded the purified PM fraction that was further analyzed.

Preparation of PM Protein Fractions and Trypsin Digestion—Preparations of NaCl- and Na2CO3-insoluble protein fractions were as described previously (17). PM protein fractions (0.2 mg) were suspended in 0.2 ml (final volume) of 1 M NaCl or 0.1 M Na2CO3. Each mixture was chilled for 15–30 min on ice and centrifuged for 20 min at 15,000 × g. Insoluble proteins were recovered as pellets and resuspended in denaturing gel electrophoresis buffer (8% SDS (v/v), 0.188 M Tris-HCl (pH 6.8), 0.1% bromphenol blue (v/v), 0.16 M dithiothreitol, 40% glycerol (v/v)). One-third of either the NaCl or Na2CO3 PM fraction was resolved by 12% SDS-PAGE. Sample migration was allowed for a length of 3.5 cm according to bromphenol blue stain. After Coomassie Blue staining of each salt fraction (NaCl and Na2CO3, respectively), the gel was cut into 11 discrete bands. In-gel digestion with trypsin (sequencing grade; Promega, Madison, WI) as proteolytic enzyme was carried out as described previously (9) with the following modification. After washing and drying, gel pieces were rehydrated in 100 μl of 7% H2O2 at room temperature for 15 min in the dark. This step led to cysteine oxidation and conversion of the methionine residues into sulfone (18). Gel pieces were then finally extracted with 5% (v/v) formic acid solution.

Mass Spectrometry and Protein Identification—Tryptic peptides were resuspended in 0.5% aqueous trifluoroacetic acid. The samples were injected into a CapLC (Waters) nano-LC system and first pre-concentrated on a 300-μm × 5-mm PepMap C18 precolumn. The peptides were then eluted onto a C18 column (75 μm × 150 mm). The chromatographic separation used a gradient from solution A (2% acetonitrile, 98% water, 0.1% formic acid) to solution B (80% acetonitrile, 20% water, 0.08% formic acid) over 60 min at a flow rate of 200 nl/min. The LC system was directly coupled to a Q-TOF Ultima mass spectrometer (Waters). MS and MS/MS data were acquired and processed automatically using MassLynx 4.0 software. Mascot was used for database searching, and proteins that were identified with at least two peptides both showing a score higher than 40 were automatically validated. The score threshold for automatic validation was fixed at 40 because (i) for both NaCl and Na2CO3 datasets, this value is just validated. The score threshold for automatic validation was fixed at 40 because (i) for both NaCl and Na2CO3 datasets, this value is just above the Mascot score above which there is identity or extensive homology for peptide assignment with a probability of at least 95% as quoted by the Mascot output and (ii) the false positive rate, as described by Peng et al. (19), was estimated to be 0.1 and 0% for the NaCl and the Na2CO3 datasets, respectively. Consequently we estimated that proteins identified by at least two peptides with a score higher than 40 were significantly present in our samples. For proteins identified by only one peptide having a score higher than 40, the peptide sequence was checked manually. Peptides with scores higher than 20 and lower than 40 were systematically checked and/or interpreted manually to confirm or cancel the Mascot suggestion. Such a validation and false positive rate assessment was achieved using a home-made validation software, which allowed protein redundancy to be eliminated on the basis of protein identification by the
same set or a subset of peptides (see Supplemental Table III) from both NaCl and Na$_2$CO$_3$ datasets.

Database searching was carried out using the Mascot 2.0 program (Matrix Science). Two databases were used: a home-made list of well known contaminants (keratins and trypsin; 21 entries) and an updated compilation of the A. thaliana protein database provided by TAIR (nuclear, mitochondrial, and chloroplastic genome; TAIR version 6.0; July 9, 2006; 30,899 entries). The variable modifications allowed were as follows: acetyl (protein), acetyl N terminus, methionine oxidation, methionine sulfone, cysteic acid, false mass assignment + 1, and false mass assignment + 2. Two missed trypsin cleavages were allowed.

Similarly to other plasma membrane proteomes, about 30 ribosomal proteins were identified (60 and 40 S) that likely originated from cytoskeleton-bound polysomes anchored to the plasma membrane via actin filaments (20, 21). A total of 446 proteins could eventually be retrieved.

**Predictive Approaches**—Protein names and/or associated functions were retrieved from NCBI (www.ncbi.nlm.nih.gov/entrez), PPDB (ppdb.tc.cornell.edu/), or TAIR (www.arabidopsis.org). GRAVY, molecular weight, pl, and TargetP (22) annotations were retrieved from PPDB (ppdb.tc.cornell.edu/) (23). Predictions of membrane-spanning regions (i.e. transmembrane domains) were collected from the ARAMEMNON database (2). The HMMTOP program (24) was also used for TM domain predictions. Post-translational modification predictions were performed on line using various dedicated programs. N-terminal myristoylation was first predicted by TermiNator (25) using relaxed criteria. Positive entries were next validated using the updated (December 2006) version of the Arabidopsis database of myristoylated proteins (25) using the software developed by Boisson et al. (5). S-Palmitoylation on vicinal cysteines next to the N terminus of N-myristoylated proteins was predicted with TermiNator (26). C-terminal prenylation was predicted by PrePS (27). Robust predictions of GPI anchors were retrieved from the ARAMEMNON database that complies three prediction programs (big-PI, DGPI, and GPI-SOM).

### RESULTS AND DISCUSSION

**A PM Proteome Derived from Salt Treatment-based Enrichment: Identification of Proteins Actually Located in the Plasma Membrane**

PM enrichment and degree of purity were assessed as described in Marmagne et al. (9) on each new PM preparation by Western blots (data not shown). Considering previous biochemical and immunological tests, chloroplastic and mitochondrial contamination was estimated to be less than 5% each. Salt treatments (including NaCl and Na$_2$CO$_3$) of the membrane fraction are believed to abolish electrostatic interactions with integral membrane proteins or the polar head of lipids (28). Improperly or weakly linked membrane proteins are eliminated by such treatments, leading to an enrichment in genuine membrane proteins and to a lower sample complexity. Sodium chloride and sodium carbonate, which have been proven to be efficient in previous analyses (29, 30), were chosen to increase further our knowledge of the PM proteome.

Analyses of insoluble NaCl and Na$_2$CO$_3$ plasma membrane fractions led to the identification of 446 proteins (356 and 243, respectively) including only 153 common entries (34%). In this set, only 42 entries (9%) have already been identified in the hydrophobic PM proteome resulting from a chloroform/methanol solubilization or an alkaline washing of the PM fraction (9) demonstrating that the different treatments are complementary. To classify the 446 proteins (Supplemental Table I), we compiled (i) information about the proteomics data (number of peptides, etc.), (ii) results of predictions by bioinformatics tools (TM domains, cell localization, etc.), (iii) information (function, localization, etc.) retrieved from different protein databases (TAIR, NCBI, and ExPaSy), and (iv) appropriate literature as indicated in Supplemental Table I.

Table I shows that 286 of 446 proteins had no predicted subcellular targeting. 110 proteins were predicted as secreted proteins, strongly suggesting PM association (Table I). Indeed 85 of these 446 proteins have been experimentally shown to be localized in membranes in particular 61 in PM (Supplemental Table I). Among the 26 proteins targeted to mitochondria, 10 have already been identified in other PM proteomes, and three have been shown to be localized in the PM, suggesting an inaccurate targeting prediction, for at least part of them, unless these proteins have a dual targeting (Table I). For example, the small G-proteins ARFs were predicted to be mitochondrial, although they are known to be involved in the secretory pathway and cytoskeleton organization (31). The
occurrence of a PM receptor for GDP-bound Arf6 in mammal cells (32) is in agreement with a PM localization of ARFs. Likewise prediction of a mitochondrial location for V-ATPase subunits is likely to be wrong because the associated function is usually assigned to the tonoplast and/or the PM (33–35). Wrong predictions were also found among proteins predicted to be chloroplastic (Table I). This appears to be the case of newly identified plasma membrane proteins, such as CDPK7 (36), PHOT2 (37), or PDR8 (38). Such dubious chloroplastic predictions may involve at least five proteins that have been found in previous PM proteomes (Table I and Supplemental Table I). In this context, it is noteworthy that abundant mitochondrial and chloroplastic membrane proteins, such as the ATP synthase subunits (39) and the triose phosphate translocator (40), respectively, were not identified in the present PM proteome. On the other hand, the PM fraction was also slightly contaminated by proteins from other organelles or from the cytosol as evidenced by the presence of histones and ribosomal proteins.

Finally Table I shows that only 157 proteins have already been identified in other plasma membrane proteome studies (Supplemental Table I), consolidating their PM localization and further validating the present proteome. We concluded that our proteome displayed proteins that are known or are very likely to be located in the PM.

**A PM Proteome Predicted to Include Both Integral and Lipid-modified Peripheral Proteins**

According to the ARAMEMNON database, 136 proteins (30%) of the present proteome can be considered as integral as they are predicted to possess at least one TM domain (Fig. 1A). Other programs, including HMMTOP or TMHMM to a lesser extent, predicted a larger amount of proteins to display at least one TM domain (Supplemental Table I). For example, receptors LRR-receptor-like kinase (RLK) (At3g58690 and At5g22050) and Lys-M-RLK (At2g17120 and At1g21880), which are known to span the membrane, were not predicted to have any TM domain by ARAMEMNON, whereas HMMTOP predicted at least one TM domain for each of these four proteins.

Other sets of proteins need to be taken into account in a PM proteome if they are co- or post-translationally modified by lipid moieties (Supplemental Table I). In this respect, 10 proteins with one TM domain were predicted to display such a modification either with a GPI anchor (one protein) or via N-myristoylation (nine proteins; see Supplemental Table I). In addition, 71 proteins without predicted TM domains were also predicted to be lipid-anchored. This included 27 GPI anchors, 24 N-myristoylations, 19 C-prenylations, and one predicted with both myristoylation and prenylation (Supplemental Table I). This set of putatively lipid-modified proteins (16% of the proteome) must be considered as peripheral proteins as they (i) are not expected to be embedded in the PM unlike integral proteins, (ii) do not use the SEC pathway for membrane targeting, and (iii) usually do not have TM domains. GPI anchors are firmly bound to the membrane through double acylation involving both myristate and palmitate attachment linked via a phosphatidylinositol group to the C-terminal side of the target protein. To make membrane binding of prenylated or myristoylated proteins tighter, another binding motif is required. Such additional binding site includes S-palmitoylation, polar contacts to the lipids of the membranes via polybasic stretch(es), or protein-protein interaction domains (41, 42). Together with N-myristoylation, palmitoylation significantly increases the hydrophobicity of the targeted protein. Among N-myristoylated proteins, 13 (i.e. 54%) were predicted to display an additional S-palmitoylation site at their N terminus (Supplemental Table I). This ratio corresponds to a much increased value compared with 30%, the average percentage characterizing the complete N-myristoylated proteome (5). In contrast, none of the N-myristoylated proteins displayed a polybasic motif unlike in the myristoylated proteome. These data are fully in keeping with the surface modifications induced by salt treatments that are expected to inhibit the effects associated with electrostatic binding modes. This suggests that among N-myristoylated proteins salt treatment had led to loss of those using a polybasic stretch (such as the so-called MARCKS [myristoylated alanine-rich C-kinase sub-
Specific Features in Arabidopsis Plasma Membrane Proteome

TABLE II

Major proteins associated with the plasma membrane

| AGI accession no. | Protein name | Protein family | Function | TM | Criterion I score (NaCl) | Criterion II score (Na2CO3) | Criterion III occurrence |
|------------------|--------------|----------------|----------|----|--------------------------|-----------------------------|-------------------------|
| Class I | At4g30190 | AHA2 | P-type H+-ATPase | Transport | 10 | 1079.00 | 886.40 | 5 |
| At2g18960 | AHA1 | P-type H+-ATPase | Transport | 10 | 845.28 | 946.13 | 5 |
| At4g12420 | SKU5 | Multicopper oxidase/pectinesterase | Cell structure | 0 | 825.32 | 694.87 | 9 |
| At3g02880 | None | RLK/Pelle, LRR-III | Cell signaling | 1 | 501.51 | 343.57 | 5 |
| At4g01290 | HIR | Hypersensitive induced reaction protein | Cell signaling | 0 | 434.62 | 389.99 | 4 |
| Class II | At4g26690 | GPDL2 | Glycerophosphoryl diester phosphodiesterase | Metabolism | 0 | 428.85 | 313.97 | 5 |
| Class III | At5g62740 | HIR | Hypersensitive induced response protein | Cell signaling | 0 | 346.50 | 468.58 | 4 |
| At1g78900 | V-ATPase A | V-type H+-ATPase | Transport | 0 | 419.46 | 441.21 | 5 |
| At4g20830 | None | Reticulin oxidase-like protein | Metabolism | 0 | 97.11 | 419.81 | 4 |
| At1g69840 | HIR | Hypersensitive induced reaction protein | Cell signaling | 0 | 411.45 | 413.69 | 3 |
| At4g12730 | FLA2 | Fasciclin-like arabinogalactan protein | Cell structure | 0 | 60.93 | 381.54 | 4 |
| Class IV | At2g38940 | PT2 | Phosphate transporter | Transport | 11 | 717.80 | 909.18 | 4 |
| At3g04120 | G3PDH C | Glyceraldehyde-3-phosphate dehydrogenase | Metabolism | 0 | 538.95 | 515.78 | 1 |
| At2g01820 | CYC18 | RLK/Pelle, LRR-IX subfamily, cyclin | Cell signaling | 1 | 474.01 | 726.87 | 0 |
| At4g31840 | None | Early nodulin-like protein | Cell structure | 0 | 464.95 | 444.54 | 4 |

As a whole, 219 proteins (49%) were predicted to have either at least a TM domain or to be associated with the membrane by a lipid anchor (Fig. 1A). Among the remaining proteins, a number of them have already been identified in the plasma membrane. For instance, phospholipases PLC2 (At3g08510) (43) and PLD (At4g35790) (44), Developmentally Regulated Plasma Membrane Polypeptide proteins (At4g20260) (45), and annexin (At1g35720) (46) are known to be associated with the PM through protein-protein or lipid-protein interactions.

Differential Effects Are Associated with Either Salt Treatment for Retrieving Lipid-modified Protein

The Na2CO3 treatment led to the identification of a higher content of very hydrophobic proteins compared with the NaCl treatment. Indeed from the Na2CO3 and the NaCl treatments, 164 proteins of 356 (46%) and 138 proteins of 243 (39%), respectively, were predicted to have at least one TM domain (Fig. 1A). If the number of transmembrane segments is considered as a reliable probe of protein hydrophobicity, the Na2CO3 treatment appears therefore more appropriate compared with the NaCl treatment for retrieving integral plasma membrane proteins. It is also likely to more quickly remove soluble proteins that are weakly bound to the plasma membrane, such as peripheral proteins. Nevertheless protein fractionation by the two techniques had almost the same efficiency for extracting proteins displaying lipid modifications (65 with NaCl versus 63 with Na2CO3), although both treatments did not lead to the extraction of the same protein categories. Predicted N-myristoylated proteins were preferentially retrieved from the NaCl fraction (92%; Fig. 1B). In contrast, the Na2CO3 pellet contained all the predicted GPI-anchored proteins (27 proteins), among which only 19 were identified in the NaCl pellet (Fig. 1B). Finally the two procedures were equivalent for fishing out putative prenylated proteins (Fig. 1B). Together these data indicate how powerful salt treatments could be when dealing with the retrieval of specific types of putatively lipid-modified protein. For instance, NaCl should be preferred over Na2CO3 for N-myristoylation, and Na2CO3 should be preferred for GPI anchors.

Although the topic of the present work was not related to quantitative proteomics, it is tempting to determine the most abundant proteins associated with the PM by taking into account three criteria: the best scores after either NaCl (criterion I) or Na2CO3 (criterion II) washing and the highest number of identifications in other PM proteomes (criterion III). Table II shows 15 proteins displaying at least two of these
three criteria. Besides pumps, transporters, and receptors, it is surprising to find proteins involved in metabolism without any TM domains, suggesting strong interactions with the PM likely due to functional complexes. Among the last four proteins, only PT2 and G3PDH C were found in our studies and also in another proteomics work (see Criterion III occurrence column).

A Functional Proteome Fully in Keeping with Functions Expected from the Plasma Membrane

The functional classification of the 446 putative PM proteins is shown in Fig. 2. Based on both sequence homologies with known proteins and identification of predicted conserved domains, only 14% of the identified proteins were classified as unknown. This value is significantly lower than those reported previously and demonstrates the recent progress in Arabidopsis genome annotation. The proteins with predicted functions have been divided into seven functional classes: cell traffic, transport, cell structure, protein maturation, protein turnover, cell signaling, and metabolism. An eighth class contains two proteins involved in DNA structure that are likely to correspond to contaminants. Among the 446 identified proteins, 157 have already been identified in other PM proteomes, many of which belong to functional classes that are representative of well established PM functions (Supplemental Table I). This is illustrated by the identification of several members of the ATPase superfamily in the transport class (pump functional subclass; Supplemental Table I), such as P-type H+-ATPases and Ca++-ATPases (47, 48) and several V-type H+-ATPase subunits (49). Proteins from other large families, such as P-type H+-ATPases (47, 48) and several V-type H+-ATPase subunits (49), are also frequently found (Supplemental Table I). Among newly identified PM proteins, a similar proportion (110 of 289, i.e. 38%) has been classified also in the cell signaling category and is represented by the same subclasses except kinases (Table III and Fig. 3). A detailed analysis pointed out that detoxification and GTP binding classes are mainly represented by new proteins (83 and 96%, respectively; Table IV) and therefore constitute, with the phosphatases, three new subclasses specific to this proteome (Table IV). The identification of a series of enzymes in the detoxification group (Table III and Supplemental Table I), such as peroxiredoxins PRXII and PRXIII (At1g65980 and At1g60740) (66); glutathione transferases ERD13, GSTU5, GSTU19, and GSTF9 (At2g30870, At2g29450, At1g78380, and At2g30860) (67), three thioredoxins-h (At5g42980, TRXh3; and At3g08710, TRXh9) (68); two glutathione transferases ERD13, GSTU5, GSTU19, and GSTF9 (At2g30870, At2g29450, At1g78380, and At2g30860) (67), three thioredoxins-h (At5g42980, TRXh3; and At3g08710, TRXh9) (68); two glutathione dehydrogenases, DHAR1 and DHAR2 (At1g19570 and At1g75270) (69); or the glutathione peroxidases ATGPX6 and GPX5 (At4g11600 and At3g63080) (70), suggests a role of PM proteins in oxidative stress protection. At3g08710 and At3g63080 are predicted to be N-myristoylated proteins, which further ascertains their functional role at the PM.

New PM Proteins Further Highlight the Unique Role of PM in Cell Signaling

In addition to the 157 aforementioned proteins assigned to plasma membrane from previous proteomics studies, the remaining proteins (289) were newly identified in a PM proteome. A functional classification of these new PM proteins and their main physicochemical properties are reported in Table III. In this subset, a striking feature is that 39 proteins were predicted as lipid-anchored proteins, among which 18 (of 25) would be N-myristoylated and 18 (of 20) would be prenylated proteins. Thus, the present proteome was enriched in proteins likely involved in transient interactions with the PM, suggesting possible roles in signaling processes (see below). Indeed an outstanding characteristic of the proteins identified in the present PM proteome is that 38% belong to the signaling class with the more representative ones classified as detoxification enzymes, GTP-binding proteins, kinases, or receptors. Stress-response proteins such as "hypersensitive induced reaction" proteins (HfR) and remorins (64, 65) were also frequently found (Supplemental Table I). Among newly identified PM proteins, a similar proportion (110 of 289, i.e. 38%) has been classified also in the cell signaling category and is represented by the same subclasses except kinases (Table III and Fig. 3). A detailed analysis pointed out that detoxification and GTP binding classes are mainly represented by new proteins (83 and 96%, respectively; Table IV) and therefore constitute, with the phosphatases, three new subclasses specific to this proteome (Table IV). The identification of a series of enzymes in the detoxification group (Table III and Supplemental Table I), such as peroxiredoxins PRXII and PRXIII (At1g65980 and At1g60740) (66); glutathione transferases ERD13, GSTU5, GSTU19, and GSTF9 (At2g30870, At2g29450, At1g78380, and At2g30860) (67), three thioredoxins-h (At5g42980, TRXh3; and At3g08710, TRXh9) (68); two glutathione dehydrogenases, DHAR1 and DHAR2 (At1g19570 and At1g75270) (69); or the glutathione peroxidases ATGPX6 and GPX5 (At4g11600 and At3g63080) (70), suggests a role of PM proteins in oxidative stress protection. At3g08710 and At3g63080 are predicted to be N-myristoylated proteins, which further ascertains their functional role at the PM.
### Table III

The main characteristics of the 289 new proteins identified in the plasma membrane proteome

LOC, protein localization according to TargetP (22). S, secreted; C, chloroplast; M, mitochondria; —, not predicted. TM, predicted number of transmembrane domains \( \alpha \)-helices or \( \beta \)-sheets when mentioned (2). The theoretical molecular mass in kDa and theoretical pl are given. AGI, Arabidopsis Gene Index; ABC, ATP-binding cassette; SNAP, synaptosome-associated protein; VAMP, vesicle-associated membrane protein; v-SNARE, vesicle SNARE; ACC, 1-aminocyclopropane-1-carboxylic; SERK, somatic embryogenesis receptor kinase.

| AGI accession no. | Protein name | Protein family | LOC | TM | Molecular mass | pl |
|-------------------|--------------|----------------|-----|----|----------------|----|
| **Transport**     |              |                |     |    |                |    |
| Channel           |              |                |     |    |                |    |
| At5g15090         | POR2         | Outer mitochondrial membrane protein | —   | 14β| 29.19          | 7.84|
| Pump              |              |                |     |    |                |    |
| At3g57330         | ACA11        | Ca\(^{2+}\)-ATPase | C   | 10 | 111.87         | 5.97|
| At4g02620         | VATF         | V-type H\(^+\)-ATPase | —   | 0  | 14.25          | 6.08|
| At1g75630         | AVAP4        | V-type H\(^+\)-ATPase | S   | 3  | 16.67          | 8.62|
| **Transporter**   |              |                |     |    |                |    |
| At1g67940         | NAP3         | ABC transporter | —   | 0  | 28.66          | 5.34|
| At3g47960         | POT          | Proton-dependent oligopeptide transport | —   | 12 | 67.32          | 8.94|
| At3g54700         | PHT1-7       | Phosphate transporter | S   | 7  | 47.27          | 8.21|
| At3g08580         | AAC1         | Mitochondrial ADP, ATP carrier | —   | 3  | 41.45          | 9.84|
| **Cell structure**|              |                |     |    |                |    |
| Cell wall component|             |                |     |    |                |    |
| At5g60490         | FLA12        | Fasciclin family | S   | 0  | 26.35          | 5.02|
| At2g02100         | LCR69/PDF2.2 | Cysteine-rich protein | S   | 0  | 8.52           | 9.37|
| At5g53870         | PUP2         | Early noduline-like protein | S   | 0  | 38.37          | 9.20|
| At4g31840         | None         | Early noduline-like protein | S   | 0  | 18.95          | 8.99|
| Cytoskeleton      |              |                |     |    |                |    |
| At1g64740         | TUA1         | Tubulin         | —   | 0  | 49.77          | 4.92|
| At2g37620         | ACT3         | Actin           | —   | 0  | 41.77          | 5.31|
| At4g29350         | PRO2/PFN2    | Profilin        | —   | 0  | 13.99          | 4.91|
| At5g10470         | TH65         | Kinesin-related protein/microtubule motor | C   | 0  | 140.95         | 5.87|
| At2g19760         | PRO1/PFN1    | Profilin        | —   | 0  | 14.26          | 4.70|
| At4g20890         | TUB9         | Tubulin         | —   | 0  | 49.63          | 4.69|
| At5g62690         | TUB2         | Tubulin         | —   | 0  | 50.70          | 4.70|
| At5g9880          | ADF3         | Actin-depolymerizing factor | —   | 0  | 15.91          | 5.94|
| **Cell traffic**  |              |                |     |    |                |    |
| Intracellular     |              |                |     |    |                |    |
| At2g14120         | ADL2b        | Dynamin-like protein | C   | 0  | 86.59          | 5.95|
| At3g56450         | α-SNAP1      | SNAP            | M   | 0  | 43.85          | 7.87|
| At1g08560         | SYP11/KOLLE  | Syntaxin-related protein | —   | 1  | 35.25          | 5.82|
| At1g08820         | VAP 27.2     | VAMP/synaptobrevin-associated protein | —   | 1  | 43.24          | 6.53|
| At3g58170         | BS14a/BET11  | SNARE           | —   | 1  | 13.96          | 6.30|
| At4g32150         | VAMP711      | v-SNARE synaptobrevin | S   | 1  | 25.02          | 9.25|
| At4g23460         | None         | \( \beta \)-Adaptin | —   | 0  | 99.03          | 4.91|
| At4g08520         | None         | Clathrin adaptor complex small chain | —   | 0  | 19.86          | 4.59|
| At4g21450         | None         | VAMP/synaptobrevin-associated protein | —   | 0  | 32.94          | 9.39|
| At1g04750         | VAMP721      | v-SNARE synaptobrevin | S   | 1  | 24.75          | 0.00|
| At1g11900         | Sec22        | 25.3-kDa vesicle transport protein | —   | 1  | 25.32          | 9.07|
| At2g45140         | VAP27.1      | VAMP/synaptobrevin-associated protein | —   | 1  | 26.43          | 8.97|
| At3g60600         | VAP27.1      | VAMP/synaptobrevin-associated protein | —   | 1  | 28.45          | 8.59|
| At4g14600         | None         | Bet1-like protein | M   | 1  | 15.37          | 0.00|
| **Secretion**     |              |                |     |    |                |    |
| At3g10380         | Sec8         | Exocyst complex component | —   | 0  | 116.53         | 5.61|
| At1g29310         | Sec          | Transport protein | M   | 10 | 52.14          | 9.12|
| At2g01470         | Sec12-2      | Sec12-like protein | —   | 1  | 42.77          | 5.47|
| At3g52190         | PHF1/Sec12-1 | Sec12-like protein | C   | 1  | 43.74          | 7.03|
| At1g06800         | PATL4        | Transporter, SEC14-like protein | —   | 0  | 61.15          | 4.90|
| At1g71820         | Sec6         | Exocyst complex component | —   | 0  | 85.62          | 4.80|
| At1g76850         | Sec5         | Exocyst complex component | —   | 0  | 121.83         | 5.57|
| At4g02350         | Sec15-like   | Exocyst complex component | —   | 0  | 86.48          | 5.99|
| At5g12370         | Sec10        | Exocyst complex component | —   | 0  | 89.64          | 5.23|
| At1g07000         | EXO70B2      | Exocyst complex component | —   | 0  | 67.67          | 5.08|
| At5g03540         | EXO70A1      | Exocyst complex component | —   | 0  | 72.25          | 8.16|
| AGI accession no. | Protein name | Protein family | LOC | TM | Molecular mass | pI |
|------------------|--------------|---------------|-----|----|----------------|----|
| At3g51670        | PATL6        | Transporter, SEC14-like protein | —   | 0  | 46.48         | 8.41 |
| At3g48570        | SEC61γ       | Transport protein | —   | 1  | 7.68          | 9.86 |
| At1g56330        | AtSARA1b     | ADP-ribosylation factor | S   | 0  | 21.97         | 6.52 |

**Metabolism**

**Cell wall synthesis**
- At4g30270: MERI-5, Endotransglycosylase
- At2g30490: C4H, Cytochrome P450 family
- At1g12640: None, Acyltransferase
- At1g63000: None, Nucleotide-rhamnose synthase/epimerase-reductase
- At1g67120: ADH1, Alcohol dehydrogenase

**Energetic metabolism**
- At1g09780: None, Phosphoglycerate mutase
- At1g23190: PGM 1, Cytoplasmic phosphoglucosumutase
- At1g27450: PGM 2, Cytoplasmic phosphoglucosumutase
- At2g31390: SCRK1, Putative fructokinase
- At2g38740: None, β-Phosphoglucomutase
- At3g03250: UDPGP1, UTP-glucose-1-phosphate uridylyltransferase
- At3g52930: None, Fructose-bisphosphate aldolase
- At5g11670: None, Malate dehydrogenase
- At4g17260: LDH, l-Lactate dehydrogenase
- At5g16980: P2, NADP-dependent oxidoreductase
- At5g17310: UDPGP 1, UTP-glucose-1-phosphate uridylyltransferase
- At5g33300: MDHC2, Cytoplasmic malate dehydrogenase
- At2g42910: PRS4, Ribose-phosphate pyrophosphokinase
- At1g65930: None, Isocitrate dehydrogenase
- At2g36460: None, Fructose-bisphosphate aldolase
- At3g55440: CTIMC, Cytoplasmic triose-phosphate isomerase
- At5g35600: ATB5-A, Cytochrome b5

**Secondary metabolism**
- At1g33990: None, Hydrolase, α/β fold family protein
- At3g57030: None, Strictosidine synthase family protein
- At3g03330: None, Oxidoreductase
- At3g10700: None, Nucleotidytransferase
- At1g74020: SS2, Strictosidine synthase
- At3g03780: MS2, Methionine synthase
- At3g61220: None, Oxidoreductase
- At4g38800: None, Expressed protein
- At5g48230: None, Acetyl-CoA C-acetyltransferase
- At1g60690: None, Oxidoreductase
- At1g62360: None, ACC oxidase
- At3g14990: None, 4-Methyl-5(β-hydroxyethyl)-thiazole protein
- At3g26000: None, Dienelactone hydrolase
- At4g13930: SHM4, Serine hydroxymethyltransferase
- At4g55830: ACO1, Acylate hydratase
- At3g17820: GLN1;3/GSKB6, Glutamate-ammonia ligase
- At5g19550: ASP2, Aspartate aminotransferase
- At5g37600: GLN1;1, Glutamate-ammonia ligase

**Cell signaling**

**Calcium binding**
- At5g47100: CBL9, Calcineurin B-like protein
| AGI accession no. | Protein name | Protein family | LOC | TM | Molecular mass | pI   |
|------------------|--------------|----------------|-----|----|----------------|------|
| At2g32450        | None         | Calcium-binding EF-hand family protein | --- | 0  | 90.17          | 6.32 |
| At2g27030        | CAMS/TCH1    | Calmodulin     | --- | 0  | 16.81          | 4.11 |
| At4g28600        | NPG2         | Calmodulin-binding protein | --- | 0  | 82.68          | 7.60 |
| At5g07300        | BONZAI 2     | Calcium-dependent membrane-binding | --- | 0  | 63.99          | 5.05 |
| At1g12310        | CML13        | Calmodulin-like | --- | 0  | 16.51          | 4.78 |
| Detoxification   | At3g08710    | TRX-H2/ATH9    |    |    | 15.32          | 5.12 |
| At5g66110        | atf6-like    | Metal ion transporter | --- | 0  | 16.64          | 9.65 |
| At1g19570        | DHAR1        | Glutathione dehydrogenase | --- | 0  | 23.63          | 5.56 |
| At1g45145        | TRX-H-5      | Thiorcalcine |    |    | 11.31          | 5.19 |
| At1g65980        | PRXII-B      | Peroxiredoxin | --- | 0  | 17.42          | 5.17 |
| At1g75270        | DHAR2        | Glutathione dehydrogenase | --- | 0  | 23.39          | 5.79 |
| At1g77510        | PDIL1-2      | Thiorcalcine | --- | 0  | 36.33          | 4.90 |
| At1g78580        | GSTU19       | Glutathione-transferase | --- | 0  | 25.63          | 5.80 |
| At2g16060        | AHB1/GBL1    | Non-symbiotic hemoglobin | --- | 0  | 18.02          | 8.46 |
| At2g30860        | GSTF9        | Glutathione transferase | --- | 0  | 24.13          | 6.17 |
| At5g40370        | None         | Glutaredoxin S | S   | 0  | 11.75          | 6.71 |
| At1g60740        | PRXII-D/TPX2 | Peroxiredoxin | --- | 0  | 17.46          | 5.33 |
| At2g29450        | GSTU5/103-1A | Glutathione S-transferase | --- | 0  | 25.98          | 5.44 |
| At2g30870        | ERD13        | Glutathione transferase | S   | 1  | 24.21          | 5.49 |
| At1g07890        | APX1         | l-Ascorbate peroxidase | --- | 0  | 27.54          | 5.72 |
| At1g75280        | None         | Isoglucobrin redoxase | --- | 0  | 33.72          | 5.66 |
| At5g42980        | TRX-H-3      | Thiorcalcine | --- | 0  | 13.10          | 5.06 |
| At4g11600        | ATGPX6       | Glutathione peroxidase | C   | 0  | 25.57          | 9.38 |
| At4g25100        | FSD1         | Superoxide dismutase | --- | 0  | 23.78          | 6.06 |
| At4g27270        | None         | Quinone reductase | --- | 0  | 21.78          | 6.08 |
| GTP binding      | At1g70490    | ARFA1d        | ADP-riboseylation factor | M | 0  | 20.58          | 0.00 |
| At2g24765        | ARF3         | ADP-riboseylation factor | --- | 0  | 20.23          | 5.24 |
| At1g43890        | RABC1        | Small GTP-binding Rab | S   | 0  | 23.52          | 5.69 |
| At1g28550        | RABA1i       | Small GTP-binding Rab | --- | 0  | 24.26          | 5.64 |
| At1g43900        | RABG3e       | Small GTP-binding Rab | M   | 0  | 22.97          | 4.96 |
| At1g73640        | RABA6a       | Small GTP-binding Rab | --- | 0  | 26.02          | 5.08 |
| At2g46600        | RABH1b       | Small GTP-binding Rab | --- | 0  | 23.12          | 7.67 |
| At5g20010        | RAN1         | Small GTP-binding Ran | --- | 0  | 25.26          | 6.39 |
| At1g07410        | RABA2b       | Small GTP-binding Rab | --- | 0  | 23.67          | 6.44 |
| At3g11730        | RABD1        | Small GTP-binding Rab | S   | 0  | 22.71          | 5.15 |
| At3g63420        | AGG1         | Heterotrimeric G protein | --- | 0  | 10.94          | 4.49 |
| At4g02060        | SAR1c/Sar2   | ADP-riboseylation factor | S   | 0  | 22.02          | 6.97 |
| At2g41330        | RABA5c       | Small GTP-binding Rab | --- | 0  | 23.97          | 4.98 |
| At5g45750        | RABA1c       | Small GTP-binding Rab | --- | 0  | 23.86          | 5.60 |
| At1g09630        | RABA2a       | Small GTP-binding Rab | --- | 0  | 24.09          | 6.20 |
| At4g17170        | RABB1c       | Small GTP-binding Rab | --- | 0  | 23.15          | 6.96 |
| At4g19640        | RABF2b       | Small GTP-binding Rab | S   | 0  | 21.86          | 0.00 |
| At3g49870        | ARLA1c       | ADP-riboseylation factor | S   | 0  | 20.39          | 0.00 |
| At3g46060        | RABE1c       | Small GTP-binding Rab | --- | 0  | 22.73          | 8.65 |
| At3g18820        | RABG3f       | Small GTP-binding Rab | M   | 0  | 23.09          | 4.98 |
| At3g07410        | RABASb       | Small GTP-binding Rab | --- | 0  | 24.31          | 4.74 |
| At4g17530        | RABD2c       | Small GTP-binding Rab | S   | 0  | 22.30          | 5.27 |
| Kinase activity  | At5g59010    | None           | Kinase (TKL; PTKL-I; PTKL-II) | --- | 0  | 54.82          | 5.74 |
| At1g07870        | RCK7         | Protein kinase | --- | 0  | 46.73          | 8.45 |
| At5g56460        | None         | Kinase (PSK; RLPK; PK1) | --- | 0  | 45.84          | 7.14 |
| At5g55200        | DSK1-like    | Protein kinase family protein | --- | 0  | 75.30          | 7.04 |
| At3g15890        | None         | Kinase (PSK; RLPK; Pto-like) | --- | 0  | 40.93          | 5.36 |
| At3g59350        | None         | Kinase (PSK; RLPK; PK1) | --- | 0  | 45.63          | 8.69 |
| At3g09820        | ADK1         | Adenosine kinase | --- | 0  | 37.81          | 5.29 |
| At5g03300        | ADK2         | Adenosine kinase | --- | 0  | 37.82          | 5.14 |
| At1g06700        | None         | Putative kinase interactor | --- | 0  | 39.79          | 7.14 |
| At2g02800        | APK2b        | Protein kinase (APK2b) | C   | 0  | 46.26          | 9.67 |
| AGI accession no. | Protein name       | Protein family          | LOC | TM | Molecular mass | pI  |
|-------------------|--------------------|-------------------------|-----|----|----------------|-----|
| Phosphatase activity | PP2C               | Protein phosphatase     | 0   | 30.83 | 8.80           |     |
|                   | BSL2               | Serine/threonine-protein phosphatase | 0 | 108.51 | 5.47           |     |
|                   | PP2A3              | Serine/threonine-protein phosphatase, regulatory | 0 | 65.47 | 4.94           |     |
|                   | PP2A1/RCN1         | Serine/threonine-protein phosphatase, regulatory | 0 | 65.45 | 4.94           |     |
|                   | PP2C               | Protein phosphatase     | 0   | 30.96 | 7.13           |     |
|                   | PP2A1              | Dual specificity protein phosphatase | 0 | 17.30 | 5.76           |     |
|                   | PP2A2              | Serine/threonine-protein phosphatase, catalytic | 0 | 35.49 | 4.72           |     |
|                   | SAC1c/SAC7         | SAC1-like protein       | 3   | 68.19 | 8.39           |     |
|                   | PP2A1              | Protein phosphatase     | 0   | 45.75 | 6.23           |     |
|                   | VTC4               | myo-inositol monophosphatase | 0 | 21.90 | 6.09           |     |
|                   | LPP3               | Phosphatidic phosphatase | 6   | 40.75 | 6.23           |     |
| Protein interaction | CC-NBS-LRR        | Disease resistance protein | 1 | 101.61 | 5.68           |     |
|                   | None               | Disease resistance-like protein | S | 29.97 | 5.20           |     |
|                   | AIR9               | Leucine-rich repeat family protein | 0 | 133.60 | 6.00           |     |
|                   | PIRLS              | Leucine-rich repeat family protein | 0 | 64.60 | 5.52           |     |
| Protein regulation | MSBP1/MP1          | Membrane steroid-binding protein | S | 24.39 | 5.39           |     |
|                   | NTF2B              | Nuclear transport factor | 0   | 27.75 | 5.20           |     |
|                   | SPDSYN2            | Spermidine synthase 2   | 0   | 37.12 | 0.00           |     |
|                   | grf7               | 14-3-3-like protein GF14 | 0 | 17.30 | 5.76           |     |
|                   | CDC48a             | Cell division control protein | 0 | 89.34 | 5.13           |     |
|                   | PKCI               | Protein kinase C inhibitor | S | 15.99 | 6.52           |     |
|                   | grf3               | 14-3-3-like protein GF14 | 0 | 28.30 | 4.75           |     |
|                   | GTF9               | 14-3-3-like protein GF14 | 0 | 29.50 | 4.86           |     |
|                   | GRF5               | 14-3-3-like protein GF14 | 0 | 30.16 | 4.73           |     |
|                   | NPH3               | Phototrophic responsive NPH3 family protein | 0 | 64.36 | 6.92           |     |
| Signal reception  | None               | RLK/Pelle, extensin subfamily | S | 43.92 | 5.79           |     |
|                   | SERK-like          | Protein kinase          | M   | 35.28 | 5.19           |     |
|                   | SERF1              | RLK/Pelle, LRR-VII-2 subfamily | S | 112.97 | 6.47           |     |
|                   | None               | RLK/Pelle, LRR-V subfamily/STRUBBELIG family | S | 84.77 | 6.71           |     |
|                   | None               | RLK/Pelle, CRLK1L-1 subfamily | S | 91.41 | 5.91           |     |
|                   | None               | RLK/Pelle, LRR-III subfamily | 1 | 108.72 | 5.91           |     |
|                   | None               | RLK/Pelle, S-domain-2b subfamily | S | 91.24 | 5.91           |     |
|                   | SERK-like          | RLK/Pelle               | 0   | 39.84 | 5.71           |     |
|                   | PHOT2              | Non-phototropic hypocotyl 1-like protein | 0 | 102.41 | 7.05           |     |
|                   | SERK1              | RLK/Pelle, LRR-II subfamily | S | 68.98 | 0.00           |     |
|                   | None               | RLK/Pelle, LRR-III subfamily | S | 73.49 | 6.25           |     |
|                   | None               | RLK/Pelle, LRR-Villa subfamily | S | 123.59 | 5.99           |     |
|                   | None               | RLK/Pelle, LRR-III subfamily | S | 114.64 | 6.32           |     |
|                   | None               | RLK/Pelle, PERK subfamily | C | 72.34 | 6.94           |     |
|                   | None               | RLK/Pelle, S-domain-2b subfamily | S | 89.68 | 6.08           |     |
|                   | None               | RLK/Pelle, LRR-Xa subfamily | S | 69.10 | 7.02           |     |
| Signal reception  | None               | RLK/Pelle, extensin subfamily | 2 | 79.00 | 6.91           |     |
|                   | SRF3               | RLK/Pelle, LRR-V subfamily/STRUBBELIG | 2 | 84.67 | 5.74           |     |
|                   | None               | RLK/Pelle, LRR-IX subfamily | S | 132.82 | 6.58           |     |
|                   | CRLK1L-1 subfamily | CRLK1L-1 subfamily | S | 120.91 | 5.99           |     |
|                   | None               | CRLK1L-1 subfamily | S | 91.26 | 6.13           |     |
|                   | None               | CRLK1L-1 subfamily | S | 128.74 | 5.53           |     |
|                   | None               | CRLK1L-1 subfamily | S | 93.24 | 5.70           |     |
|                   | None               | CRLK1L-1 subfamily | S | 68.72 | 5.36           |     |
| Protein turnover   | None               | Caspase                 | 0   | 45.46 | 0.00           |     |

**Specific Features in Arabidopsis Plasma Membrane Proteome**
| AGI accession no. | Protein name | Protein family | LOC | TM | Molecular mass | pI |
|------------------|--------------|----------------|-----|----|----------------|----|
| **Ubiquitin-proteasome pathway** | | | | | | |
| At4g02075 | PITCHOUN 1 | RING protein/ubiquitin-protein ligase | — | 1 | 25.30 | 5.50 |
| At1g31340 | RUB1/UBQ1 | NEDD8-like protein precursor/ubiquitin | — | 0 | 17.39 | 0.00 |
| At1g76390 | None | ARM repeat protein/ubiquitin-protein ligase | — | 0 | 89.05 | 5.24 |
| At2g22125 | None | ARM repeat protein | — | 0 | 230.56 | 5.21 |
| At3g60820 | PBF1 | Threonine endopeptidase | — | 0 | 24.63 | 6.95 |
| At1g16890 | None | Ubiquitin-conjugating enzyme E2 | — | 0 | 17.21 | 6.74 |
| At1g20780 | None | ARM repeat protein/ubiquitin-protein ligase | — | 0 | 88.32 | 5.76 |
| At1g64230 | UBC9A | Ubiquitin-conjugating enzyme | — | 0 | 16.50 | 7.22 |
| At2g02560 | CAND1 | SCF complex | — | 0 | 134.81 | 5.77 |
| At1g22510 | None | RING protein/ubiquitin-protein ligase | C | 2 | 20.77 | 8.08 |

**Protein maturation**

**Protein folding**

| AGI accession no. | Protein name | Protein family | LOC | TM | Molecular mass | pI |
|------------------|--------------|----------------|-----|----|----------------|----|
| At3g55920 | CYP21-2 | Peptidyl-prolyl cis-trans isomerase | S | 0 | 24.49 | 6.52 |
| At2g24280 | STICHEL/STI DNA polymerase III / subunit | C | 0 | 135.22 | 9.06 |
| At2g21130 | ROC6/CYP2 Peptidyl-prolyl cis-trans isomerase | — | 0 | 18.45 | 8.32 |
| At4g2450 | None | p23 co-chaperone | — | 0 | 25.44 | 4.44 |
| At4g24190 | Hsp90-7/SHD Heat shock protein | S | 0 | 94.15 | 4.94 |
| At5g56010 | HSP81-3 (90-3) Heat shock protein | S | 0 | 80.00 | 4.95 |
| At1g79920 | None | Heat shock protein | — | 0 | 81.73 | 8.76 |
| At2g38730 | CYP22-1 Peptidyl-prolyl cis-trans isomerase | — | 0 | 21.48 | 8.47 |
| At5g02490 | Hsc70.2 Heat shock protein | — | 0 | 71.34 | 5.03 |
| At5g52640 | HSP81-1 Heat shock protein | — | 0 | 81.13 | 4.95 |
| At3g09440 | Hsc70.3 Heat shock protein | — | 0 | 71.10 | 4.97 |
| At4g34870 | ROC5/CYP1 Peptidyl-prolyl cis-trans isomerase | — | 0 | 18.37 | 8.90 |

**Protein modification**

| AGI accession no. | Protein name | Protein family | LOC | TM | Molecular mass | pI |
|------------------|--------------|----------------|-----|----|----------------|----|
| At4g34640 | SQS1 | Squalene synthase 1 | — | 1 | 47.11 | 6.18 |
| At3g15710 | None | Putative signal peptide subunit | — | 3 | 19.83 | 5.95 |
| At4g33090 | APM1 | Aminopeptidase | — | 0 | 98.12 | 5.34 |
| At1g63770 | None | M1 aminopeptidase | C | 0 | 103.40 | 6.09 |

**Translation**

| AGI accession no. | Protein name | Protein family | LOC | TM | Molecular mass | pI |
|------------------|--------------|----------------|-----|----|----------------|----|
| At1g54270 | EIF4A-2 ATP-dependent helicase | — | 0 | 46.73 | 5.45 |
| At1g56070 | EF-2/LOS1 Elongation factor | — | 0 | 93.83 | 5.89 |

**DNA structure**

| AGI accession no. | Protein name | Protein family | LOC | TM | Molecular mass | pI |
|------------------|--------------|----------------|-----|----|----------------|----|
| At4g13940 | HOG1 | Adenosylhomocysteinase | — | 0 | 53.34 | 5.66 |
| At1g07660 | H4 | Histone | — | 0 | 11.40 | 11.48 |

**Unknown**

| AGI accession no. | Protein name | Protein family | LOC | TM | Molecular mass | pI |
|------------------|--------------|----------------|-----|----|----------------|----|
| At3g08600 | None | Expressed protein | M | 1 | 34.71 | 9.58 |
| At3g01520 | None | Unknown protein | — | 0 | 19.56 | 5.61 |
| At1g05960 | None | Expressed protein | M | 0 | 16.88 | 5.54 |
| At5g25265 | None | Expressed protein | S | 0 | 40.65 | 8.67 |
| At1g29790 | None | Unknown protein | — | 0 | 41.93 | 9.47 |
| At3g60950 | None | Unknown protein | M | 0 | 66.72 | 7.10 |
| At2g46150 | None | Expressed protein | C | 1 | 24.08 | 9.78 |
| At5g20900 | None | Expressed protein | — | 0 | 12.43 | 9.34 |
| At5g02000 | None | Expressed protein | S | 1 | 23.39 | 9.28 |
| At2g46170 | RTNLb5 Reticulon family protein | — | 3 | 28.67 | 7.16 |
| At1g44960 | None | Expressed protein | S | 4 | 28.20 | 9.60 |
| At2g25270 | None | Expressed protein | S | 5 | 59.87 | 6.18 |
| At1g15270 | None | Expressed protein | — | 0 | 6.97 | 9.95 |
| At1g47550 | None | Expressed protein | — | 0 | 100.00 | 5.57 |
| At2g01540 | None | Unknown protein | — | 0 | 20.07 | 6.42 |
| At2g17705 | None | Expressed protein | — | 0 | 14.36 | 8.67 |
| At2g22660 | None | Unknown protein | — | 0 | 66.44 | 5.98 |
| At2g44060 | LEA Late embryogenesis abundant protein | — | 0 | 36.01 | 4.69 |
| At2g46140 | LEA type-2 Desiccation-related protein, related | — | 0 | 17.84 | 4.53 |
| At3g16640 | TCTP Transcriptionally controlled tumor protein | — | 0 | 18.90 | 4.52 |
New protein kinases were also identified in the PM proteome (Table III and Supplemental Table I), several of them featuring putative N-terminal myristoylation (CRK4, At5g24430; CPK7, At5g12480; and CPK13, At3g51850). Membrane targeting of CPK7 has been demonstrated (36). In this major category of proteins potentially modified by both N-myristoylation and S-palmitoylation (25, 71), the lipidic modification is essential for plasma membrane targeting. This was clearly demonstrated for CPK1 and CPK2 in Arabidopsis (36, 72). Another plant CPK1 from ice plant was shown to undergo a reversible modification in subcellular localization from the plasma membrane to the nucleus, endoplasmic reticulum, and actin microfilaments in response to reduction in humidity (73). In relation to bacterial defense responses, among new non-myristoylated kinases, the kinase Pto (At3g15890) and two Pto kinase interactors 1 (Pti1; At3g59350 and At1g06700) were identified in the PM proteome. Homologous genes in tomato are involved in a phosphorylation cascade initiated by recognition of the bacterial protein AvrPto at the cell surface (74), bringing further evidence for a role of these proteins at the PM.

A large number of small GTP-binding proteins newly identified in the present proteome correspond to RAB proteins (Table III, Fig. 3, and Supplemental Table I). These proteins are active in their GTP-bound form, which is membrane-associated following post-translational lipid modifications, i.e. prenylation, in A. thaliana. Half of them belong to the RABA subfamily, which might play a role in signaling pathways leading to the delivery of new cell wall components to the plasma membrane. The large number of RABA members in that subfamily would reflect the great diversity of cell wall material to be delivered to the plasma membrane, such as hemicellulose, integral cell wall proteins, or cellulose synthase complex. The two other RAB subfamilies well represented (Table III), RABF and RABG, would be involved in the regulation of endocytic trafficking pathway (31).

One of the most interesting classes of proteins found in this

| AGI accession no. | Protein name | Protein family | LOC | TM | Molecular mass | pl |
|------------------|--------------|----------------|-----|----|----------------|----|
| At3g17210        | None         | Putative Pop3 protein | —   | 0  | 12.18          | 5.42 |
| At4g27450        | None         | Unknown protein   | —   | 0  | 27.60          | 5.56 |
| At5g02240        | None         | Expressed protein | —   | 0  | 27.09          | 6.20 |
| At5g47210        | None         | Putative protein  | —   | 0  | 37.98          | 8.75 |
| At5g47710        | None         | Unknown protein   | —   | 0  | 18.32          | 6.74 |
| At4g24990        | MUB3/GP4     | Membrane-anchored ubiquitin-fold protein | —   | 0  | 12.83          | 9.17 |
| At1g10590        | None         | Expressed protein | —   | 0  | 15.44          | 6.60 |
| At1g23140        | None         | Expressed protein | M   | 0  | 18.56          | 4.48 |
| At1g30070        | None         | Expressed protein | —   | 0  | 24.80          | 8.78 |
| At2g01080        | None         | Expressed protein | —   | 0  | 24.75          | 9.18 |
| At2g33470        | None         | Glycolipid transfer protein (GLTP) | —   | 0  | 22.73          | 6.95 |
| At3g22850        | None         | Expressed protein | —   | 0  | 27.09          | 5.85 |
| At5g11680        | None         | Expressed protein | —   | 0  | 23.09          | 6.35 |
| At5g19140        | None         | Expressed protein | C   | 0  | 25.00          | 5.62 |
| At1g78355        | None         | Expressed protein | —   | 1  | 17.09          | 5.93 |
| At3g49720        | None         | Expressed protein | M   | 1  | 28.51          | 9.36 |
| At5g58640        | None         | Selenoprotein-related | S   | 1  | 24.98          | 9.27 |
| At4g11220        | RTNLB2/BT12  | Reticulon family protein | —   | 3  | 30.26          | 8.61 |
| At3g03270        | None         | Unknown protein   | —   | 0  | 22.59          | 5.53 |
| At3g17020        | None         | Unknown protein   | —   | 0  | 17.76          | 6.41 |
| At5g49830        | None         | Expressed protein | —   | 0  | 82.70          | 5.21 |
| At2g01410        | None         | Hypothetical protein | S   | 1  | 42.99          | 5.42 |
| At4g23720        | None         | Hypothetical protein | S   | 1  | 35.17          | 8.55 |
| At1g18180        | None         | Unknown protein   | S   | 6  | 33.62          | 9.22 |
| At5g39730        | AIG2 protein-like | Avirulence induced gene protein | —   | 0  | 20.00          | 5.01 |
| At2g30930        | None         | Hypothetical protein | C   | 0  | 16.93          | 4.92 |
| At2g37970        | None         | Unknown protein   | —   | 0  | 24.91          | 8.77 |
| At1g73650        | None         | Unknown protein   | S   | 6  | 32.94          | 9.14 |

![Cell signaling](https://www.mcponline.org)
PM proteome (Table III, Fig. 3, and Supplemental Table I) was the one of receptors especially because of their very large number. We focused on RLKs (signal reception subclass), which play important roles in many plant signal transduction pathways. RLKs are secreted proteins with part of the protein anchored within the plasma membrane as suggested by the occurrence of a single transmembrane domain, a cytoplasmic C-terminal kinase domain, and an extracellular N-terminal domain. This large family is represented by more than 600 genes in the Arabidopsis genome (75). To date 23 RLK proteins have been identified in former proteomes (10, 11, 16), and it is noteworthy that never more than 11 RLKs had been reported in the same proteome (11). In the present proteome, 49 RLKs were identified, including 26 new members, representative of different subfamilies, such as extensin, LRR, or CrRLK. Among the new RLKs, we identified the somatic embryogenesis receptor-like kinase, AtSERK1 (At1g71830), a well-characterized plasma membrane receptor (76) involved in the acquisition of embryogenic competence and in male sporogenesis (77, 78). Moreover a refined analysis of the list showed that the proteome contained the 14-3-3v (GRF7, At3g02520) and the CDC48A (At3g09840), two proteins participating with the co-receptor BRI1-associated receptor kinase 1, also in the present proteome (BAK1, At4g33430), in the SERK1 protein complex (79). Several other RLK proteins well known for their roles in defense responses against pathogen attacks are also present; this is the case for the flagellin-sensing 2 (FLS2, At5g46330) receptor that mediates innate response to bacterial pathogens (80) and the nuclear shuttle protein-interacting kinase (NIK1) involved in antiviral defense response (81). The protein AtPepR1 (At1g73080) is also present; this new RLK has been shown to amplify defense gene signaling for the innate immune response against pathogens (82). Proteins RKL1 (At1g48480) and RLK902 (At3g17840), involved in stress response (83, 84), were also identified. Identification of various subunits of functional PM complexes in the present proteome is further validation of the pertinence of our approach.

**Newly Identified Proteins Further Exemplify the Tight Interactions Occurring between the PM and Other Cell Compartments**

Analysis of the 289 proteins newly identified in the PM pointed out functional classes that were poorly or never observed in proteomics studies published so far (Tables III and IV, Fig. 3, and Supplemental Table I). These new proteins define different functional subclasses and to some extent assign new functions that emphasize the tight interaction occurring between plasma membrane and other cellular compartments.

**PM and Endomembranes—**Proteins participating in the sequential steps of endocellular trafficking and secretion were identified: (i) subunits Sec5, Sec6, Sec8, and Sec10 of the exocyst complex involved in polarized exocytosis at the PM (85), (ii) several SNARE proteins, such as VAMP721 and SYP111, involved in specific vesicle fusion with the PM, (iii) dynamin-like proteins (ADL2b and ADL3) that might be in-
volved in clathrin-mediated vesicle trafficking together with some of the new proteins also identified in the present proteome, such as clathrin small chain (At4g08520), β-adaptin (At4g23460), and clathrin heavy chain (At3g11130), and (iv) other proteins such as Sec12-2 and SARAI1B involved in vesicle formation from the ER to the Golgi compartments (54, 86). These data illustrate the interplay between PM and the various endomembrane systems of the cell.

**PM and Cytoskeleton**—Newly identified proteins belonging to large families, TH65 from kinesins and two profilins (PRO1 and PRO2), known to regulate cytoskeleton dynamics and play specific roles in cell elongation and cell division, were also identified. The presence of this protein category is another example of the close interactions between PM and cytoskeleton (87, 88).

**PM and Cytosol/Cell Wall**—Two cytosolic glutamine synthases, GLN1;1 and GLN1;3 (89), were identified in the PM proteome. This suggests that enzymes could be transiently targeted to the PM for facilitating interactions with PM proteins. GLN1;1 was demonstrated to interact with CRK3, a myristoylated protein kinase (71, 90). Likewise the cell wall glycin-rich protein GRP2 might interact with a PM wall associated kinase as it was shown in the case of GRP3 (91). A last feature to emphasize is the identification of metabolism enzymes, several participating in cell wall composition or in the biosynthesis of cell wall components such as cinnamyl alcohol dehydrogenase or nucleotide-rhamnose synthase/epimerase-reductase (3,5-epimerase) (92–94). Several of them, including the strictosidine synthase and the endotransglycosylase MERI-5, have been identified in the cell wall proteome (95) showing the faint barrier between PM and cell wall proteins. Functionally the presence in the same compartment of these enzymes and RABA G-proteins (see above) suggests that these processes are regulated at least partly at the plasma membrane level.

**Emergence of New Protein Classes Confirms the Role of PM in Protein Processing and Turnover**

The rest of the identified proteins (~25%) define functional classes (Fig. 3 and Table IV) that were as yet never associated with a PM. Interestingly the proteins belonging to the class “protein turnover” is composed almost exclusively (12 of 13) of proteins that were never identified in previous PM proteomics studies. These proteins are mainly soluble proteins, but their biological role may involve transient interaction with the PM. This was suggested in the caspase cascade, which could be activated from the cell surface (96). In agreement with this hypothesis, in mammals, signaling protein oligomerization transduction structures (SPOTS complex), which are known to be essential for Fas apoptosis signaling, are formed at the plasma membrane (97). As to the armadillo repeat-containing proteins, it was also shown in tobacco that the armadillo protein NtPUB4 interacts with the receptor like-kinase CHRK1 at the plasma membrane (98). Thus, the presence in the plasma membrane proteome of proteins involved in protein degradation processes is not unexpected because the apoptotic signaling pathway can be induced by many extracellular stimuli.

The PM proteome analysis revealed a second new class of PM proteins, the “protein maturation” class (Fig. 3 and Table IV). Among these proteins, cytosolic forms of chaperones (several Hsp proteins) and cyclophilins (several CYP proteins) were found. The interaction between proteins from these families and PM proteins have already been reported, Hsp90 with AtFKBP42 (99) and CYP20.1 with RCN1, a PP2A (100). We also identified several peptidases, such as APM1, an aminopeptidase bound to the PM (101).

**Concluding Remarks**

With a few exceptions, the 446 proteins identified in the course of this study represent the largest proteome for a plant plasma membrane reported so far because of the combination of two extraction procedures. Compared with a previous study based on CHCl3/MeOH extraction and NaOH washing (9), the PM repertoire was widened with respect to the physicochemical properties of the identified proteins and the functions associated with these proteins; some of them have never been pointed out in previous proteomics analyses of PM. Interestingly part of the plant PM proteome overlaps the proteome of lipid rafts (16). Lipid rafts are known as PM microdomains that have specific roles in stress response and signaling and are enriched in lipid-modified proteins. In the present PM proteome, we identified a large number of proteins displaying putative lipid modifications, suggesting that the PM proteome is enriched in peripheral proteins transiently interacting with the PM. Whether all or only parts of the putatively lipid-modified proteins of the PM proteome belong to lipid rafts is still unknown. In particular, predicted prenylated proteins were included in the new set of PM proteins. To our knowledge, such proteins had never been observed in former studies including lipid rafts.

Functional analysis of the newly identified proteins highlights three other remarkable characteristics of the present proteome. First, close interaction with other cell compartments, mainly cytosol, cytoskeleton, cell wall, and endomembrane systems, highlights the central role of PM. Proteins from the two subclasses, cell wall component and cell wall synthesis, might be extrinsic due to their location at the interface between the cell wall and the PM and have a function that depends on the two compartments. In other respects, evidence for functional interactions with the endomembranes is the identification of proteins involved in the intracellular cell traffic (Sec proteins, dynamins, and SNAREs) and GTP-binding proteins (RABs etc.) suggesting a close connection between the PM and the ER/Golgi apparatus. Second, the high enrichment in signaling proteins, in particular in receptor-like...
kinases, confirms the unique role of PM in signal perception and transduction processes. As already pointed out, several sets of proteins participating in functional complexes at the PM level were identified in the present PM proteome. Third, new functional classes were identified, further supporting previous evidence for a role of PM in protein processing, i.e., protein degradation via the ubiquitin-proteasome pathway and protein maturation. Altogether our data exemplify that the combined proteomics approach on a target membrane, the plasma membrane herein, is a powerful tool for retrieving specific types of putatively lipid-modified proteins and may also bring new insights toward a functional proteome.

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