**AtMRP1** gene of *Arabidopsis* encodes a glutathione S-conjugate pump: Isolation and functional definition of a plant ATP-binding cassette transporter gene

(anthocyanins/herbicides/heterologous expression/vacuolar membrane/xenobiotic detoxification)

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**ABSTRACT** Because plants produce cytotoxic compounds to which they, themselves, are susceptible and are exposed to exogenous toxins (microbial products, allelochemicals, and agrochemicals), cell survival is contingent on mechanisms for detoxifying these agents. One detoxification mechanism is the glutathione S-transferase-catalyzed glutathionylation of the toxin, or an activated derivative, and transport of the conjugate out of the cytosol. We show here that a transporter responsible for the removal of glutathione S-conjugates from the cytosol, a specific Mg2+-ATPase, is encoded by the *AtMRP1* gene of *Arabidopsis thaliana*. The sequence of *AtMRP1* and the transport capabilities of membranes prepared from yeast cells transformed with plasmid-borne *AtMRP1* demonstrate that this gene encodes an ATP-binding cassette transporter competent in the transport of glutathione S-conjugates of xenobiotics and endogenous substances, including herbicides and anthocyanins.

The recent finding that intact vacuoles and vacuolar membrane vesicles isolated from vascular plants mediate MgATP-dependent accumulation of glutathione S-conjugates (GS-conjugates) in the absence of a transmembrane H+ electrochemical potential difference (1, 2) is seminal in two respects. Not only does it implicate MgATP as a direct energy source for organic solute transport across plant membranes but, because the compounds transported include glutathionylated herbicides (1, 2), isoflavonoid phytoalexins (3), and possibly anthocyanins (4), a mechanism for the vacuolar sequestration of GS-conjugable xenobiotics and endogenous substances appears to have been discovered.

Despite its strategic value for manipulating and investigating toxin compartmentation in plants, neither the protein nor the gene encoding the plant vacuolar GS-conjugate pump have been identified. Significant therefore are three clues as to its possible identity. (i) The resemblance of plant vacuolar GS-conjugate transport to that mediated by the GS-conjugate transporting Mg2+-ATPase, GS-X pump (5), of mammalian cells. In both cases, transport is selective for GS-conjugates and oxidized glutathione (GSSG), but not reduced glutathione (GSH), directly energized by MgATP and potently inhibited by the phosphorylation transition state analog, vanadate (1, 2). (ii) The ability of the human multidrug resistance-associated protein gene (*HmMRP1*), an ATP-binding cassette transport protein (ABC transporter) gene isolated from drug-resistant small cell lung carcinoma cell lines (6), to confer GS-X pump activity, specifically MgATP-energized transport of leukotriene C4 and related GS-conjugates, on transfected cells (7, 8). (iii) The functional and structural equivalence of the *Saccharomyces cerevisiae* (yeast) cadmium factor (*ScYCF1*) gene product, identified according to its ability to confer cadmium resistance (9), to *HmMRP1*, its localization to the vacuolar membrane (10) and the capacity of *HmMRP1* for alleviating the cadmium-hypersensitive phenotype and restoring GS-conjugate transport in membranes prepared from yeast strains deleted for the *YCF1* gene (11, 12). Here we describe the isolation of a gene from *Arabidopsis thaliana* (*AtMRP1*), encoding a protein belonging to the same subclass of ABC transporters as *HmMRP1* and *ScYCF1*, whose heterologous expression in yeast confers MgATP-energized GS-conjugate transport. In so doing, we provide a functional definition of an ABC transporter from vascular plants and identify an element involved in the removal of GS-conjugable compounds from the cytosol in a manner independent of the transmembrane H+ gradient.

**MATERIALS AND METHODS**

**Isolation of *AtMRP1***. On the basis of the functional resemblance between plant vacuolar GS-conjugate transport and that mediated by *ScYCF1* and *HmMRP1*, and the 44.6% sequence identity (63.9% similarity) between *ScYCF1* and *HmMRP1*, degenerate oligonucleotide primers corresponding to the second ATP-binding cassette of *ScYCF1* and *HmMRP1* (positions 1,300–1,321 and 1,474–1,494, respectively)—two of the most *ScYCF1* and *HmMRP1*-specific sequences common to both—were used for the isolation of plant genes likely involved in GS-conjugate transport by PCR amplification of *A. thaliana* genomic DNA. The sequences of the two primers yielding a 0.6-kb *HmMRP1*- and *ScYCF1*-hybridizing amplification product by this approach were: 5'-GARAARGTGGATGTTGGGGTGCAT-3' (*MRP2*) and 5'-TCATCATGTRTIIARICTGIGGCCC3' (*MRP4*), where I = inosine, K = T or G, M = C or A, and R = A or G. *MRP2* corresponds to amino acid residues 1,300–1,310 and 1,321–1,331 of *ScYCF1* and *HmMRP1*, respectively; *MRP4* corresponds to residues 1,466–1,474 and 1,486–1,494 (6, 9). After determining that the 0.6-kb PCR product exhibited greatest similarity to *ScYCF1* and *HmMRP1* plus the putative translation product of an unidentified 1.6-kb *A. thaliana* expressed sequence tag (EST) (*ATTS1246* (13), a mixed probe consist-

**Abbreviations**: ABC transporter, ATP-binding cassette transport protein; GST, glutathione S-transferase; C3G, cyanidin 3-glucoside; DNP-GS, S-(2,4-dinitrophenyl)glutathione; GS-conjugate, glutathione S-conjugate; GSH, glutathione; GSSG, oxidized glutathione; MRP1, multidrug resistance-associated protein; *HmMRP1*, human MRP1; YCF1, yeast cadmium factor protein; *ScYCF1*, *Saccharomyces cerevisiae* (yeast) cadmium factor; EST, expressed sequence tag; BAC, bacterial artificial chromosome; CFTR, cystic fibrosis transmembrane conductance regulator; NBF, nucleotide-binding fold.

Data deposition: The cDNA and genomic sequences reported in this paper have been deposited in the GenBank database (accession nos. AF008124 and AF008125, respectively).

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Heterologous Expression of AtMRP1 in S. cerevisiae yefDelta mutants. For constitutive expression of AtMRP1 in S. cerevisiae a derivative of the yeast- Escherichia coli shuttle vector pYES2 (Invitrogen) was constructed in which the 831-bp XbaI/NorI restriction fragment containing the 3-phosphoglycerate kinase (PGK) gene promoter from plasmid pFL61 (19) was inserted between the SpeI/NorI restriction sites of pYES2, so replacing the galactose-inducible yeast GALI promoter of pYES2 with the constitutive yeast PGK promoter to generate pYES3. AtMRP1 cDNA, from which the 5′ untranslated region had been removed by PCR, was inserted into the multiple cloning site located between the PGK promoter and cytochrome c gene (CYC1) termination sequences of pYES3. After confirming the fidelity of the constructs by sequencing, S. cerevisiae yefDelta strain DTY168 (MATa his6 leu2-3,112, ura3-52 ycf1:hisG) (9) was transformed with pYES3-AtMRP1 or empty vector lacking the AtMRP1 insert (pYES3) by the LiOAc/polyethylene glycol method (20) and selected for uracil prototrophy (21).

Measurement of GS-Conjugate Transport. Cells were grown and vascular membrane-enriched vesicles were purified as described (21). Uptake of S-(2,4-dinitrophenyl)[3H]glutathione (DNP-GS), [3H]GSSG, [3H]cyanidin 3-glucoside-GS (CSG-GS), or [3C]metolachlor-GS was measured in 200-μl reaction volumes containing membrane vesicles (10–20 μg), 3 mM ATP, 3 mM MgSO4, 5 μM gramicidin D, 10 mM creatine phosphate, 16 units/ml creatine kinase, 50 mM KCl, 1 mg/ml BSA, 400 mM sorbitol, and 25 mM Tris-Mes (pH 8.0). Uptake was terminated by the addition of 1 ml of ice-cold wash medium (400 mM sorbitol/3 mM Tris-Mes, pH 8.0) and vacuum filtration of the suspension through prewetted Millipore cellulose nitrate filters (pore size 0.45 μm). The filters were rinsed twice with wash medium and the radioactivity retained was determined by liquid scintillation counting. Nonenergized uptake was estimated by the same procedure except that ATP was omitted from the uptake media.

Measurement of Protein. Protein was estimated by a modification of the Lowry method (22).

Chemicals. DNP-[3H]GSH (17.4 mCi/mmol) was synthesized enzymically from 1-chloro-2,4-dinitrobenzene and [3H]GSH and purified as described (2). [3H]GSSG was synthesized enzymically by incubation of 100 μM [3H]GSSG (44 Ci/mmol) and 100 μM hydrogen peroxide with 10 units of glutathione peroxidase (type III from baker’s yeast) for 4 hr at 25°C in 10 mM phosphate buffer, pH 7.5. [3C]Metolachlor-GS was synthesized by general base catalysis by adding 100 μM GSH and 50 μM [3C]metolachlor (8.3 μCi/mmol) to 25 mM sodium borate buffer, pH 9.0, and incubating overnight at 55°C. C3G-[3H]GS was synthesized enzymically by incubating equimolar C3G (8.8 mM) and [3H]GSH (44 Ci/mmol) at 25°C overnight with the total glutathione S-transferase (GST) fraction from maize shoots. Total maize GSTs were extracted and affinity-chromatographed as described (23) except that 1 μM acivicin was added to the extraction buffer to minimize γ-glutamyl transpeptidase activity. All of the conjugates were purified by reverse-phase FPLC as described (22). All other reagents were of analytical grade and purchased from Fisher, Fluka, Research Organics, or Sigma.

RESULTS

Isolation of AtMRP1. PCR amplification of A. thaliana genomic DNA using oligonucleotide primers corresponding to the second ATP-binding cassette of ScYCF1 and HmMRP1 (Fig. 1) yielded a 0.6-kb product that hybridized with the equivalent PCR products of ScYCF1 and HmMRP1. Sequence analysis disclosed that this 0.6-kb PCR product comprised a 76-bp intron and 510-bp exon, the latter of which shared 60.6% and 66.5% amino acid sequence identity with the corresponding regions of ScYCF1 and HmMRP1 and complete nucleotide sequence identity with the 5′ segment of a 1.6-kb A. thaliana

Determination of Physical Map Position of AtMRP1. The physical map position of the AtMRP1 gene was determined by screening two A. thaliana yeast artificial chromosome (YAC) libraries, CIC and YUP (18), with the full-length AtMRP1 cDNA clone. Thirteen positive YAC clones (CIC12A1, CIC5B11, CIC9B11, CIC11B11, CIC12B11, CIC11E10, CIC12E10, YUP1683, YUP1667, YUP1697, YUP11E12, and YUP3F11) were recovered of which seven (YUP15D4, YUP3F11, YUP11E12, YUP16H7, CIC12A12, CIC12B11, and CIC12E10) yielded PCR product of the length (400 bp) and sequence expected of AtMRP1 after amplification with oligonucleotide primers AtMRP11250S (5′-CAGTGTGCTGATGTTTATAGG-3′) and AtMRP9P1 (5′-CGTGTGCGTATATATATCAGG-3′). From a search of the A. thaliana genome database all seven YAC clones were found to map to a region between SSLP markers nga392 and nga280 on chromosome 1.
sequences were as similar to ScYCF1 and HMMP1 as EST, ATTS1246 (13). Given that their deduced amino acid residues (Fig. 1). The 181-kDa polypeptide exhibited mapping between SSLP markers nga392 and nga280 on chromosome 1 and encompassed a single ORF of 1,622 amino acid residues (Fig. 1). The 181-kDa polypeptide encoded exhibited at least 36% overall sequence identity (55% similarity) to ScYCF1, HMMP1, and another established GS-X pump, the rat canalicular multispecific organic anion transporter, and 29% identity (55% similarity) to the human cystic fibrosis transmembrane conductance regulator (CFTR) (Fig. 3). AtMRP1 possessed two nucleotide-binding folds (NBF1 and NBF2), each containing the Walker A and B motifs and C domain, characteristic of ABC transporters (24), and 12 putative transmembrane spans, whose location was consistent with the topologies inferred for HMMP1 (6) and ScYCF1 (9) (Figs. 1 and 3). In addition, two subclass-specific structures were evident: a putative "regulatory" (R) domain contiguous with the topologies inferred for HMMP1 (6) and ScYCF1 (9), common to the MRP and CFTR subclasses, but which is truncated in the former (117–161 vs. 256 amino acid residues) (Fig. 3); and a 192–223 amino acid residue N-terminal extension, absent from the CFTR subclass but present in all MRP subclass members (Fig. 3).

A dendrogram derived from a PAUP analysis comparing AtMRP1 with other representative members of the ABC transporter superfamily clearly demonstrated that AtMRP1, MRP1, and canalicular multispecific organic anion transporter from various sources, ScYCF1, three other ABC transporters, for which GS-X pump activity has yet to be reported—the rabbit epithelial basolateral conductance regulator (RBE-BCR), Leishmania P-glycoprotein-related molecule (LEPGP1), and yeast (S. cerevisiae) oligomycin resistance protein (ScYOR1)—belong to the same subclass, a subclass within the same cluster as CFTR but remote from the multidrug resistance proteins (MDRs or P-gps), major histocompatibility complex class II-linked peptide transporters (TAPs), Schizosaccharomyces pombe heavy metal tolerance protein (SpHMT1), and S. cerevisiae mating factor export protein (ScSTE6) (data not shown).

![Fig. 2. Northern analysis of expression of AtMRP1 transcript in roots, stems, leaves, and flowers of A. thaliana. Total RNA (10 μg per lane) was extracted, separated, blotted, and hybridized with 32P-labeled, random-primed AtMRP1 as described in Materials and Methods. The 5.3-kb band indicated was the only 32P-labeled band detected.](image)

![Fig. 3. Identity and similarity analysis of putative domains of AtMRP1, HMMP1, and rat canalicular multispecific organic anion transporter (RtCMOAT), rat CFTR-like putative regulator domain (RBE-BCR), and human CFTR (Hm-CFTR). In addition to the overall sequences (X), the regions examined were the N-terminal extension (N), transmembrane segments 1 and 2 (TM1 and TM2, respectively), CFTR-like putative regulatory domain (R), NBF1 and NBF2, and C terminus (C). The compilation was derived from the results of GAP comparisons using the default parameters of the GCG package (version 8.0, University of Wisconsin, Madison, WI). The GenBank/EMBL accession numbers for the proteins shown were: AF008124, AtMRP1; L35237, ScYCF1; J05628, HMMP1; L49379, RtCMOAT; M28668, Hm-CFTR.](image)
**AtMRP1 Catalyzes GS-Conjugate Transport.** To determine if the structural similarity between AtMRP1, ScYCF1, and HmMRP1 signifies a functional equivalence, the capacity of heterologously expressed AtMRP1 for MgATP-energized, uncoupler-insensitive GS-conjugate transport was tested. For this purpose, *S. cerevisiae* ycf1Δ strain DTY168, from which 95% of the coding sequence of the YCF1 gene had been deleted (9) and high-affinity, MgATP-dependent, uncoupler-insensitive vacuolar GS-conjugate transport is abolished (10–12), was transformed with empty expression vector, pYES3, or vector containing the entire ORF of AtMRP1 (pYES3-AtMRP1) under the control of the constitutive yeast phosphoglycerate kinase gene (PGK) promoter. After growth on selective media, vacuolar membrane-enriched vesicles (21) were prepared from these and untransformed DTY168 cells and assayed for GS-conjugate transport. To facilitate direct comparisons of the transport activity of AtMRP1 with those of ScYCF1, HmMRP1, and the endogenous plant vacuolar GS-conjugate pump, the model compound, DNP-GS—the only compound whose transport characteristics have been determined in all three of these systems (1, 2, 7, 8, 10–12)—was used as transport substrate for the initial experiments.

Vector alone exerted negligible effect on DNP-GS uptake, implying that the cDNA insert of pYES3-AtMRP1 was the factor responsible for the increases in transport measured. When assayed at an initial DNP-[3H]GS concentration of 61.3 μM, the rates of MgATP-dependent, uncoupler-insensitive uptake by vacuolar membrane-enriched vesicles purified from untransformed DTY168 cells ("DTY168 cells") and pYES3-transformed DTY168 cells ("DTY168/pYES3 cells") were indistinguishable and more than 3- to 4-fold lower than for the equivalent membrane fraction from pYES3-AtMRP1-transformed DTY168 cells ("DTY168/pYES3-AtMRP1 cells") (Fig. 4A).

The increases in MgATP-dependent DNP-GS uptake exhibited by DTY168/pYES3-AtMRP1 membranes were exclusively attributable to vanadate-sensitive, uncoupler-insensitive transport. Protonophores (carbonylcyanide *p*-trifluoro-
Direct comparisons between the activity of this transporter and heterologously expressed AtMRP1 revealed a basic functional equivalence. Not only did the $K_M$ for inhibition of MgATP-dependent, uncoupler-insensitive DNP-[3H]GS uptake into DTY168/pYES3-AtMRP1 membrane vesicles by vanadate (Fig. 4B) coincide with that for uptake by mung bean vacuolar membrane vesicles (7.5 ± 3.9 μM) (2) but the two processes also exhibited similar concentration dependencies and GS-conjugate preferences. AtMRP1-dependent uptake conformed to Michaelis–Menten kinetics with respect to DNP-GS concentration to yield a $K_M$ (48.7 ± 15.4 μM) (Fig. 5) within the range reported for the equivalent transport function in vacuolar membranes from mung bean (81.3 ± 41.8 μM) (2). The rates of AtMRP1-dependent uptake of DNP-GS, GSSG, and glutathionated derivatives of the herbicide metolachlor (metolachlor-GS) and the anthocyanin C3G-GS fell in the same rank order as for uptake by mung bean vacuolar membrane vesicles. These were C3G-GS > metolachlor-GS > DNP-GS ≥ GSSG (uptake ratio = 2.7:1.5:1.0:0.7) for AtMRP1-dependent uptake by yeast vacuolar membrane-enriched vesicles (Table 2) and C3G-GS (26.43 ± 9.56 nmol/mg per 10 min) > metolachlor-GS (18.45 ± 5.00 nmol/mg per 10 min) > DNP-GS (4.83 ± 0.59 nmol/mg per 10 min) ≥ GSSG (3.85 ± 0.43 nmol/mg per 10 min) (uptake ratio = 5.5:3.9:1.0:0.8) for MgATP-dependent, uncoupler-insensitive uptake by mung bean vacuolar membrane vesicles. In neither membrane preparation was MgATP-dependent, uncoupler-insensitive uptake of the unconjugated precursors of these compounds, DNP, GSH, metolachlor, and C3G, detectable (data not shown).

The overall equivalence of the kinetics of uptake and uptake ratios for different glutathionated derivatives by the membrane preparations from mung bean and pYES3-AtMRP1-transformed yeast, together with the pronounced osmotic dependence of uptake by both preparations (data not shown), indicate that genuine transport into the intravesicular space, rather than ATP-dependent adsorption to membranes containing an abundance of endogenous or heterologous protein, is what was measured in these experiments.

**Table 2.** AtMRP1-dependent uptake of different GS-conjugates

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<thead>
<tr>
<th>GS-conjugate</th>
<th>AtMRP1-dependent uptake, nmol/mg per 10 min</th>
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<tr>
<td>DNP-GS</td>
<td>2.91 ± 0.42</td>
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<tr>
<td>GSSG</td>
<td>2.07 ± 0.71</td>
</tr>
<tr>
<td>Metolachlor-GS</td>
<td>4.44 ± 0.82</td>
</tr>
<tr>
<td>C3G-GS</td>
<td>7.85 ± 0.75</td>
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MgATP-dependent, uncoupler-insensitive uptake by DTY168/pYES3-AtMRP1 and DTY168/pYES3 membrane vesicles was measured and AtMRP1-dependent uptake was calculated as described in the legend to Fig. 5. All of the GS-conjugates were present at a concentration of 61.3 μM. Values shown are means ± SE ($n$ = 3–6).

**DISCUSSION**

The results demonstrating that heterologous expression of AtMRP1 alone is sufficient for the reconstitution of vanadate-inhibitable, MgATP-dependent, uncoupler-insensitive GS-conjugate transport in yeast membranes suggest that a plant GS-conjugate pump has been cloned in its entirety. As such, they provide the first combined molecular and functional identification of an ABC transporter from vascular plants. Several sequence-related ESTs and one MDR-like gene (AT-PGP1) have been isolated from *A. thaliana* (26) and a number of candidate ABC transporter activities, other than vacuolar GS-conjugate uptake, have been reported for plants (27, 28) but no transport function had been unambiguously assigned to a plant ABC transporter gene. The structural characteristics of AtMRP1, its mode of energization, and its membership of the CFTR-related MRP subclass of ABC transporters defines an MgATP-energized organic solute transporter, antedating the evolutionary separation of animals and plants, which, contrary to the prevailing model for energy-dependent transport in plants (29), is not driven by a transmembrane H⁺-electrochemical potential difference. The acute sensitivity of AtMRP1 to vanadate, a specific inhibitor of aspartyl- or glutamyl-phosphate formation, its insensitivity to uncouplers, and the inability of the nonhydrolyzable ATP analog, adenosine 5′-[β,γ-imino]triphosphate to support uptake (Table 1) exclude chemiosmotic coupling and establish that AtMRP1-mediated transport is contingent on hydrolysis of the γ-phosphate of ATP and formation of a phosphoenzyme intermediate.

There is always a possibility, in view of the moderate rates of GS-conjugate transport catalyzed by AtMRP1 after its expression in yeast, that glutathionated compounds are not the sole substrates, or even the principal substrates for this transporter in *vivo*. However, the capacity of AtMRP1 for transport of the glutathionated chloroacetanilide herbicide, metolachlor, and by implication GS-conjugates of other herbicides, such as atrazine and simetryn, for which glutathionation in *vivo* (30) and transport by the endogenous vacuolar GS-conjugate pump in *vivo* (1) are also demonstrable, is consistent with the molecular identification of a transporter capable of removing these and related compounds from the cytosol. Given that some GS-conjugates are themselves toxic and most will end-product inhibit GSTs if their cytosolic concentrations increase sufficiently (29), transport functions such as that of AtMRP1 are critical if the detoxification of herbicides and other xenobiotics is to continue unimpeded. Analogously, its ability to

**Fig. 5.** Concentration dependence of AtMRP1-dependent DNP-GS uptake. AtMRP1-dependent DNP-[3H]GS uptake rates were calculated by subtracting the radioactivity taken up by vacuolar membrane-enriched membrane vesicles prepared from pYES3-transformed DTY168 cells from that taken up by the equivalent membrane fraction from pYES3-AtMRP1-transformed DTY168 cells. The data were fitted to a single Michaelis–Menten function by nonlinear least-squares analysis (25) to yield a $K_M$ and $V_{max}$ of 49.7 ± 15.4 μM and 6.0 ± 1.7 nmol/mg per 10 min, respectively. Values shown are means ± SE ($n$ = 3–6).
transport C3G-GS at rates severalfold higher than DNP-GS, its functional resemblance to the endogenous vacuolar GS-conjugate pump of plants, and the results of analyses of the maize (Zea mays) gene Bronze-2 (Bz2) implicate AtMRP1 in plant cell pigmentation. Although it has been known for some time that the bronze coloration of b2 mutants is due to the accumulation of C3G derivatives in the cytosol—in wild-type plants anthocyanins are accumulated in the vacuole as purple or red derivatives but in b2 mutants they are restricted to the cytosol where they undergo oxidation to brown pigments—the biochemical basis of this lesion was not understood. Incisive, therefore, are experiments showing that Bz2 encodes a GST capable of conjugating C3G with GSH (4). In view of the efficacy of C3G-GS, but not C3G, as a substrate for transport by heterologously expressed AtMRP1 and the endogenous vacuolar GS-conjugate pump, the probable basis of the b2 phenotype follows automatically. Being defective in the glutathionation of anthocyanins, b2 mutants are unable to pump these pigments from the cytosol into the vacuole; a conclusion supported by the ability of vanadate treatment to phenocopy the b2 mutation in wild-type protoplasts (4) and the exquisite sensitivity of AtMRP1-dependent transport to this compound. Against this background it is instructive to note that the final steps in anthocyanin biosynthesis and the initial steps in xenobiotic detoxification require the same types of enzymes: cytochrome P450s, glucosyltransferases, and GSTs (31). When account is taken of this, the facility of cytochrome P450s for conferring the requisite electrophilicity on otherwise unreactive compounds for subsequent conjugation with GSH, the high capacity of the vacuolar GS-conjugate pump for the high affinity transport of glutathionated medicarpin, an isoflavonoid phytoalexin (3), and the amenability of GSSG, a product of peroxide detoxification and protein thiol reduction to transport by AtMRP1, the spectrum of processes that likely converge and depend on this and related transporters is extended beyond herbicide detoxification and cell pigmenta-
tion to include the alleviation of oxidative damage and storage of pathogen-elicted compounds. Thus, the identification of AtMRP1 not only provides fresh insights into the molecular basis of energy-dependent organic solute transport in plants but also offers the prospect of manipulating and investigating many fundamental processes that have hitherto been neglected at the transport level.

In the general context of GS-conjugate pumps, not only in plants but also other organisms, the findings reported here indicate a dichotomy. While AtMRP1 is evidently a structural homolog of yeast YCF1 and mammalian MRP1, its functional capabilities are not exactly equivalent. Unlike ScYCF1, which is active in the transport of both organic GS-conjugates (10, 11) and heavy metals, such as Cd$^{2+}$, after their complexation with GSH (12), and HmMRP1, which is inferred to have a similarly broad substrate range (11), AtMRP1 appears capable of transporting only organic GS-conjugates: AtMRP1 neither confers resistance to nor mediates the GSH-dependent transport of Cd$^{2+}$. As such, the properties of AtMRP1 highlight a basic functional bifurcation within the MRP1/YCF1 subclass of ABC transporters. Given our facility for expressing AtMRP1 in a functionally active state in yeast, the structural basis of these differences, and the important question of which domains of YCF1 and/or MRP1 confer the capacity for Cd$^{2+}$ transport, may now be addressed by the construction and biochemical characterization of AtMRP1-YCF1 and/or At-
MRP1-MRP1 protein chimeras.

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