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Review

Plant ABC transporters

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Abstract

The ATP binding cassette (ABC) superfamily is a large, ubiquitous and diverse group of proteins, most of which mediate transport across biological membranes. ABC transporters have been shown to function not only as ATP-dependent pumps, but also as ion channels and channel regulators. Whilst members of this gene family have been extensively characterised in mammalian and microbial systems, the study of plant ABC transporters is a relatively new field of investigation. Sequences of over 20 plant ABC proteins have been published and include homologues of P-glycoprotein, MRP, PDR5 and organellar transporters. At present, functions have been assigned to a small proportion of these genes and only the MRP subclass has been extensively characterised. This review aims to summarise literature relevant to the study of plant ABC transporters, to review methods of cloning, to discuss the utility of yeast and mammalian systems as models and to speculate on possible roles of uncharacterised ABC transporters in plants.

Keywords: ABC transporter; P-glycoprotein; Multidrug resistance associated protein; PDR-5; Glutathione conjugate pump; Plant

1. Overview

The ATP binding cassette (ABC) superfamily is a large and diverse group of proteins, whose members mediate a wide range of transport functions. Most ABC proteins are primary pumps, which use the energy of ATP hydrolysis to drive transport, but some also modulate the activity of heterologous channels, or have intrinsic channel activity. A few have non-transport functions [1]. Over 100 ABC proteins have been identified to date, in taxa ranging from bacteria to humans: completion of the yeast and *Escherichia coli* genome sequences has revealed 29 and 79 ABC proteins, respectively [2,3], and the catalogue of representatives from multicellular eukaryotes continues to grow with the progress of targeted research and of sequencing projects. Substrates assigned to members of this large family of transporters include compounds as varied as peptides, sugars, lipids, heavy metal chelates, polysaccharides, alkaloids, steroids, inorganic acids and glutathione conjugates [1,5]. This impressive list reflects not only the number and diversity of these transporters, but also their

Abbreviations: ABC, ATP binding cassette; CFTR, cystic fibrosis transmembrane conductance regulator; cMOAT, canalicular multispecific organic anion transporter; DPC, diphenylamine-2-carboxylic acid; GST, glutathione *S*-transferase; GS-X, glutathione conjugate; K-ATP, ATP-sensitive K^+ channel; KCO, potassium channel opener; MDR, multidrug resistance; MDR-P-gp, P-glycoprotein which mediates multidrug resistance; MRP, multidrug resistance associated protein; NBF, nucleotide binding fold; NTP, nucleotide triphosphate (ATP, CTP, GTP or TTP); P-gp, P-glycoprotein; SUR, sulphonylurea receptor; TMD, transmembrane domain

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A

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atpgp1 LSVPAGKTIA LVGSSGSGK. STVVSLIERF YDPNSGQVLL DGQDLKTLKL
humdr1 LKVQSGQTVA LVGNSGCGK. STTVQLMQRL YDPTEGMVSV DGQDIRTINV
atmrp1 LDIPLGSLVA VVGSTGEGKT SLISAMLGEL PARSDATVTL RGS.......
humrp1 FSIPEGALVA VVGQVGCGKL SLLSALLAEM .DKVEGHVAI KGS.......
hucftr FKIERGQLLA VAGSTGAGKT SLLMMIMGEL .EPSEGKIKH SGR.......
                     Walker A

atpgp1 RWLRQQIGLV SQEPALFATS IKENILLGRP DADQVEIEEA ARVANAHSFI
humdr1 RFLREIIGVV SQEPVLFATT IAENIRYGRE NVTMDEIEKA VKEANAYDFI
atmrp1 ......VAYV PQVSWIFNAT VRDNILFG.A PFDQEKYERV IDVTALQHDL
humrp1 ......VAYV PQQAWIQNDS LRENILFG.C QLEEPYYRSV IQACALLPDL
hucftr ......ISFC SQFSWIMPGT IKENIIFG.V SYDEYRYRSV IKACQLEEDI

atpgp1 IKLPDGFDTQ VGERGLQLSG GQKQRIAIAR AMLKNPAILL LDEATSALDS
humdr1 MKLPHKFDTL VGERGAQLSG GQKQRIAIAR ALVRNPKILL LDEATSALDT
atmrp1 ELLPGGDLTE IGERGVNISG GQKQRVSMAR AVYSNSDVCI LDEPLSALDA
humrp1 EILPSGDRTE IGEKGVNLSG GQKQRVSLAR AVYSNADIYL FDDPLSAVDA
hucftr SKFAEKDNIV LGEGGITLSG GQRARISLAR AVYKDADLYL LDSPFGYLDV
                                          Walker B

atpgp1 ESEKLV...Q EALDRFMIGR TTLIIAHRLS TIRKADLVAV LQQGSVSEIG 391-586
humdr1 ESEAVV...Q VALDKARKGR TTIVIAHRLS TVRNADVIAG FDDGVIVEKG 415-610
atmrp1 HVGQQVFEKC I..KRELGQT TRVLVTNQLH FLSQVDKILL VHEGTVKEEG 636-820
humrp1 HVGKHIFENV IGPKGMLKNK TRILVTHSMS YLPQVDVIIV MSGGKISEMG 666-850
hucftr LTEKEIFESC VC..KLMANK TRILVTSKME HLKKADKILI LNEGSSYFYG 446-628
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B

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atpgp1  AGKTLALVGP SGCGKSSVIS LIQRFYEPSS GRVMIDGKDI RKYNLKAIRK
humdr1  KGQTLALVGS SGCGKSTVVQ LLERFYDPLA GKVLLDGKEI KRLNVQWLRA
atmrp1  PMDKVGIVGR TGAGKSSLLN ALFRIVELEK GRILIDECDI GRFGLMDLRK
humrp1  GGEKVGIVGR TGAGKSSLTL GLFRINESAE GEIIIDGINI AKIGLHDLRF
hucftr  PGQRVGLLGR TGSGKSTLLS AFLRLLN.TE GEIQIDGVSW DSITLQQWRK
                Walker A

atpgp1  HIAIVPQEPC LFGTTIYENI AYGHEC..AT EAEIIQAATL ASAHKFISAL
humdr1  HLGIVSQEPI LFDCSIAENI AYGDNSRVVS QEEIVRAAKE ANIHAFIESL
atmrp1  VVGIIPQAPV LFSGTVRFNL ...DPFSEHN DADLWESLER AHLKDTIRRN
humrp1  KITIIPQDPV LFSGSLRMNL ...DPFSQYS DEEVWTSLEL AHLKDFVSAL
hucftr  AFGVIPQKVF IFSGTFRKNL ...DPYEQWS DQEIWKVADE VGLRSVIEQF


atpgp1  PEGYKTYVGE RGVQLSGGQK QRIAIARALV RKAEIMLLDE ATSALDAESE
humdr1  PNKYSTKVGD KGTQLSGGQK QRIAIARALV RQPHILLLDE ATSALDTESE
atmrp1  PLGLDAEVTE AGENFSVGQR QLLSLARALL RRSKILVLDE ATAAVDVRTD
humrp1  PDKLDHECAE GGENLSVGQR QLVCLARALL RKTKILVLDE ATAAVDLETD
hucftr  PGKLDFVLVD GGCVLSHGHK QLMCLARSVL SKAKILLLDE PSAHLDPVTY
                                        Walker B

atpgp1  RSVQEALDQA CSGRTSIVVA HRLSTIRNAH VIAVIDDGKV AEQGSHSHLL 1051-1248
humdr1  KVVQEALDKA REGRTCIVIA HRLSTIQNAD LIVVFQNGRV KEHGTHQQLL 1062-1252
atmrp1  VLIQKTIREE FKSCTMLIIA HRLNTIIDCD KVLVLDSGKV QEFSSPENLL 1263-1459
humrp1  DLIQSTIRTQ FEDCTVLTIA HRNLTIMDYT RVIVLDKGEI QEYGAPSDLL 1319-1515
hucftr  QIIRRTLKQA FADCTVILCE HRIEAMLECQ QFLVIEENKV RQYDSIQKLL 1236-1431
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Fig. 1. Sequence alignment of selected ATP binding domains. (A) N-Terminal nucleotide binding domain. (B) C-Terminal nucleotide binding domain. Five ATP binding domains have been aligned to illustrate conserved motifs common to ABC transporters. Numbers at the bottom of the figure indicate the amino acid residues used in the alignment. Walker A and B motifs (common to all ATP binding proteins) are underlined. The ABC signature motifs are marked by double underlining. The accession numbers of sequences used to construct this figure are: human CFTR M28668 [7], human MRP L05628 [10], human P-gp1 M14758 [11], *Arabidopsis* MRP1 AF008124 [12], *Arabidopsis* P-gp1 E1339433 [13].

unique nature. Many ABC transporters are relatively specific, but others are able to handle several chemically dissimilar compounds, and for this reason are of considerable academic and practical interest [1,5].

The first ABC transporters to be extensively characterised were the so-called prokaryotic periplasmic permeases, involved in nutrient uptake by bacteria [6], but recently, much attention has focussed on members of the superfamily with clinical significance, including the cystic fibrosis transmembrane conductance regulator (CFTR [7]), sulphonylurea receptor (SUR [8]) and transporters from humans, parasites, yeast and bacteria which mediate multiple drug resistance (MDR [9]). In the past decade, ABC transporters have emerged as an important and fascinating group of proteins in plants. Before it is possible to review the plant literature, it is necessary to introduce some background information on the ABC superfamily which has been obtained from mammalian and microbial systems.

2. Structure of ABC proteins

2.1. Domain organisation

Typically, an ABC transporter contains two copies each of two structural units: a highly hydrophobic transmembrane domain (TMD), and a peripherally located ATP binding domain or nucleotide binding fold (NBF), which together are often necessary and sufficient to mediate transport. The TMD domains form the pathway via which the substrate crosses the membrane, and in some cases, have been shown to contribute to the substrate specificity. The NBFs are oriented towards the cytoplasmic side of the membrane and couple ATP hydrolysis to transport. Within the NBF is a conserved region of approx. 200 amino acids, consisting of the Walker A and B boxes separated by the ABC signature motif (Fig. 1). It is this signature motif which distinguishes ABC transporters from other NTP binding proteins, such as kinases, which also contain the Walker sequences [14,15]. Sequence homology over the whole gene can be negligible between different ABC transporters, but in the conserved areas of the NBF it is typically 30–40% between family members, and this has proved useful in the isolation of ABC genes by approaches such as PCR and hybridisation with degenerate nucleotides [16].

The organisation of ABC genes is almost as varied as their function [4,17]; representative examples of eukaryotic ABC genes are presented in Fig. 2. In prokaryotes, the different domains are commonly encoded as separate subunits, with the component genes of the ABC transporter arranged in a single operon [1,4]. However, there are many prokaryotic ABC genes in which two or more domains are fused to form a single polypeptide. ABC transporters of eukaryotic organelles are also expressed as separate subunits, which may reflect the endosymbiotic origin of plastids and mitochondria, but fusion of domains is more common in nuclear-encoded eukaryotic ABC genes. The most frequent arrangement is four domains fused in a single polypeptide, although the sequential order of domains may vary, for example: transmembrane domains precede the nucleotide

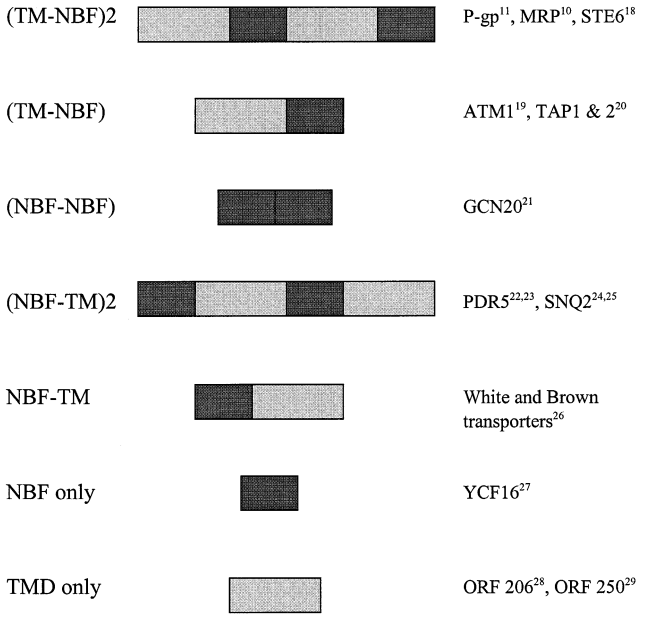

Fig. 2. Domain organisation of eukaryotic ATP binding cassette genes. The diagram shows the arrangement of domains in the RNA transcript. The transmembrane domains are represented as lightly shaded boxes, and the nucleotide binding domains are represented as dark shaded boxes. Examples for each arrangement are given. Note that *ycf16* and *ORF206, 250* are components of putative organellar ABC transporters, and require other subunits to form a functional transport protein. References [10,11,18–29] are given as superscripts above the gene names.

binding domains in MRP, STE 6 and P-glycoprotein (TMD-NBF-TMD-NBF), but PDR5 and SNQ2 exhibit the 'mirror' topology (NBF-TMD-NBF-TMD) (Fig. 2). Some eukaryotic ABC proteins, such as the HLA Class I antigen transporter (TAP) and the *Drosophila* white and brown gene products each consist of an ATP binding domain fused to a hydrophobic domain; these 'half-size' transporters are believed to function as dimers [20,26]. This flexibility of organisation is such that functional proteins can be obtained both by experimental separation and by fusion of domains [1], but whether four domains, either expressed as a single polypeptide or bound together to form an oligomer, represent the minimal structural unit for all ABC transporters is not yet confirmed (for a discussion, see [1]). Some members of the ABC superfamily which do not have transport functions, appear to have a different organisation e.g. OAB, which forms part of the ribonuclease L complex [30] and GCN20 which regulates protein kinase activity [21]. Additional domains occur in several ABC transporters; these may serve a regulatory function, for example, the CFTR R-domain [31,32]. In the case of the bacterial periplasmic permeases, an extra subunit serves to bind substrate and deliver it to the transporter [6]: these periplasmic binding proteins also have the ability to interact with membrane bound receptors as part of a signalling cascade [3,33].

2.2. Secondary structure

Given their varied domain organisation, it is difficult to make general statements about the secondary structure of ABC transporters [1]. As in the case of other membrane proteins, hydropathy analysis has indicated the likely disposition of transmembrane helices and hydrophilic loops, and biochemical studies to test theoretical models have been conducted for some proteins such as human MDR P-gp and MRP. P-gp is predicted to contain a tandem repeat of six transmembrane helices, each set followed by an ATP binding domain – the 'two-times-six paradigm'. Whilst this model has strong experimental support [34–36], the topology of MRP has been found to be significantly different [37]. Members of the MRP subfamily have a large, hydrophobic N-terminal extension, in addition to the core structure of two membrane associated and two ATP binding domains possessed by most ABC transporters. Secondary structure predictions combined with biochemical analyses suggest that MRP has 11 transmembrane spans in the N-terminal half of the protein and four or six in the C-terminal part [38,39]. Recent epitope insertion studies favour the latter model and indicate that the N-terminus is extracellular [40,41]. No hard evidence exists concerning the secondary structure of plant ABC transporters, but, given strikingly similar hydropathy profiles, parallels have been drawn from studies of homologues in other organisms (e.g. [5]).

2.3. Tertiary structure

In comparison to the abundance of biochemical and genetic data, little is known about the three-dimensional structure of ABC transporters. In a recent study, electron microscopy and image analysis of both reconstituted and detergent-solubilised human P-glycoprotein gave the first experimental insight into the 3D architecture of an ABC transporter [42]. The authors were able to propose a structural model which was in agreement with available biochemical and genetic data: the shape and size of the protein were consistent with the proposal that P-gp functions as a monomer containing two transmembrane domains, each consisting of six α-helices. Two 3 nm lobes exposed at the cytosolic face of the membrane were thought to correspond to the nucleotide binding domains. Three-dimensional reconstructions suggest that P-gp forms a large aqueous chamber within the membrane, open to the extracellular medium, but closed at the cytoplasmic face of the membrane, with an opening to the lipid phase [42]. The data were obtained in the absence of ATP; further information awaits the acquisition of data in the presence of ligands.

Crystallisation of membrane proteins is notoriously difficult, but attempts to crystallise ABC transporters or their separate domains are underway. In the absence of crystal data, an alternative approach has been to model ABC proteins on related, known structures, for example: folding patterns for the cytoplasmic NBDs of CFTR based on the mitochondrial F_1-ATPase [43,44] and aspartate aminotransferase [45], have been proposed, and are consistent with biochemical data. However, Hung et al. were re-

cently able to exploit the fact that ABC transporter domains are encoded as separate proteins in the histidine permease of *Salmonella typhimurium*, and determined the crystal structure for the ATP binding subunit, HisP [46]. Results from the more challenging task of crystallising an ABC transporter transmembrane domain are eagerly awaited; such information would greatly increase our understanding of the molecular mechanisms of transport.

3. ABC transporters and multidrug resistance: mechanistic implications

Cancer cell lines selected for resistance to specific cytotoxic drugs such as colchicine or doxorubicin often simultaneously acquire resistance to a number of structurally and functionally unrelated compounds – this phenomenon is known as multidrug resistance (MDR) and is of considerable clinical importance, since it renders tumours refractory to a number of chemotherapeutic agents [1]. A major advance in understanding MDR was the discovery that the human P-glycoprotein gene, *MDR1*, is overexpressed in a number of drug resistant cell lines. P-gp is thought to mediate ATP-dependent efflux of anticancer drugs such as *Vinca* alkaloids, anthracyclines, actinomycin D and taxol, thereby reducing their cytoplasmic accumulation. Since these compounds have little in common except for amphipathicity, a novel mechanism has been proposed to account from this unusual specificity – the 'flippase' model [47]. It has been proposed that P-gp binds an amphipathic molecule in the cytoplasmic leaflet of the plasma membrane and flips its polar group across the plasma membrane to deliver the molecule to the exocytoplasmic leaflet. The amphipathic molecule can now diffuse into the extracellular medium, effecting net removal from the cytoplasm. ATP is required to overcome the concentration gradient, which in this model is the differential substrate concentration between the two leaflets of the lipid bilayer. Not all members of the P-gp subfamily mediate drug transport, and perhaps ironically, support for the flippase model was obtained when the function of one such member was discovered. Mouse mdr2, although highly homologous to human MDR1 P-gp, does not pump drugs, but functions as a phosphatidylcholine translocase [48,49]. The equivalent protein in humans, MDR3 P-gp, also specifically translocates phosphatidylcholine, but MDR1 P-gp is a lipid translocase of broad specificity, which explains why it is able to handle multiple lipophilic drugs [50]. Structural data have offered further insight into the molecular basis of the flippase mechanism: three-dimensional reconstructions of electron microscopic data suggest that the aqueous chamber formed by P-gp has an opening to the lipid phase, allowing substrate access from the membrane to the pore translocation pathway (see above and [42]).

Other ABC transporters with low homology to P-gp are responsible for certain drug resistance phenotypes: for example, *MRP* was identified as being overexpressed in a multidrug resistant small cell lung cancer cell line, in which P-gp levels were normal [10]. Evidence from transfection experiments and studies of cell lines overexpressing *MRP* demonstrated that it is a glutathione conjugate transporter [51,52]. In addition to negatively charged glutathione conjugates, MRP also pumps lipophilic, neutral or mildly cationic cytotoxic drugs, in an unmodified form, with a substrate specificity which is overlapping, but distinct from that of P-gp [53,54]. The hydrophobic N-terminal extension of MRP has been postulated to play a special role in the interaction with anionic substrates [37], and to what extent structural and mechanistic models for P-gp can be applied to MRP in the light of this is yet to be determined.

ABC genes responsible for resistance phenotypes in the yeasts *Saccharomyces cerevisiae* and *Candida albicans* have also been well characterised in genetic and biochemical studies [2,55]. In *S. cerevisiae*, two linked networks of genes are responsible for the multidrug resistant phenotype: the PDR (pleiotropic drug resistance) and the YAP1 networks [56,57]. YAP1 is a leucine zipper transcription factor which mediates drug resistance through increased transcription of major facilitator superfamily (MFS) transporters, and also plays a role in resistance to oxidative stress. One of its targets is the vacuolar MRP homologue, YCF1 [58], which mediates resistance to Cd^{2+} [59] and acts as a transporter of glutathione conjugates and complexes [60,61]. The PDR network consists of two zinc finger transcriptional regulators Pdr1p and Pdr3p, which together activate the expres-

sion of the ABC transporters: PDR5, SNQ2 and YOR1. Whilst YOR1 is similar in organisation to human MRP [62], PDR5 and SNQ2 form a new class of ABC transporters, exhibiting a mirror topology with respect to MDR and MRP ([22–24,63–65]; Fig. 2). Despite a low level of sequence homology, and different organisation, they perform analogous roles to mammalian MDR P-gp, which has interesting structure/function implications. The precise specificities of Yor1p, Pdr5p, and Snq2p have been established in careful and elegant studies by Goffeau and co-workers, using a series of mutants in which combinations of the five PDR network genes are deleted [57]. The results of screening 359 toxins revealed that these three ABC transporters confer resistance to an extremely broad spectrum of compounds with distinct, but overlapping specificities. Not only antifungals, but also plant defence secondary metabolites were identified in the catalogue of transporter substrates. The existence of additional targets for the PDR1 and PDR3 transcriptional regulators was also evident from this work – possible candidates include several as yet uncharacterised ABC transporters identified in the yeast genome project [2,66]. Whilst comparisons of unicellular and multicellular organisms are not always appropriate [67], the amenability of yeast to genetic studies has revealed a complex and flexible defence system which may provide useful models for studies of detoxification in higher organisms.

4. The emergence of plant ABC transporters

In a 1992 review, only one plant ABC transporter was identified [1]. Currently, there are published reports of over 20 ABC genes from plants (Table 1), several others are represented in the EST databases, and various transport processes which may represent the activity of ABC transporters have been characterised. The role of the ABC superfamily in multidrug resistance phenomena has been an important factor in the development of plant transport research, since analogous roles for plant ABC genes in cross-resistance to herbicides have been postulated [13,89]. However, the diversity of roles in other taxa suggests that ABC transporters will certainly perform many other functions in plants. This potential, coupled with the possibility of employing highly conserved sequences from the NBF in the identification and isolation of genes, has led several groups to search for members of this gene family in plants. Two pioneering reports mark the start of plant ABC research in earnest: the isolation of an *MDR* homologue from *Arabidopsis thaliana* [13], and a biochemical study describing transport of glutathione conjugates into barley vacuoles, which could be ascribed to the activity of an MRP-like transporter [90]. Since then, reports of ABC transporter genes and activities have followed rapidly; these are discussed in detail below, and the reader is also referred to two excellent recent reviews on vacuolar conjugate transport [5,91].

5. MDR family

5.1. Molecular cloning of plant P-gp homologues

The first plant ABC transporter to be cloned was *AtPGP1*, an *MDR P-gp* homologue from *Arabidopsis* [13]. Degenerate oligonucleotide probes based on conserved regions of ABC transporters were used to screen an *Arabidopsis* genomic library, resulting in the isolation of a full-length genomic clone. The corresponding cDNA was subsequently isolated by PCR, permitting comparison of both coding sequence and gene structure with ABC transporter genes from other organisms. The *AtPGP1* clone exhibited similar intron structure to mammalian MDR genes, and encoded a protein with similar organisation of structural domains, suggesting that the P-gp subfamily evolved prior to the divergence of plants and animals [13]. Southern blot analysis of *AtPGP1* revealed the existence of a second, diverged member of the gene family; subsequently, a second homologue, *AtPGP2*, was cloned [13,16]. AtPGP1 and 2 share 44% amino acid identity, and are each 42% identical to human MDR1 [16]. Further similar, but non-identical *MDR*-like *Arabidopsis* genes have also been reported, though their sequences are not yet publicly available ([5]; Rea et al., unpublished results).

P-gp homologues have been identified in other plant species: screening of a potato stolon cDNA expression library with ^{35}S-labelled calmodulin re-

Table 1

a. Plant ABC proteins

Gene	Species	Functional information	Ref.
AtPGP1	*Arabidopsis*	role in light-dependent hypocotyl cell elongation	[13,68]
AtPGP2	*Arabidopsis*	?	[16]
PMDR1	potato	CaM binding site	[69]
HvMDR2	barley	?	[70]
AtMRP1	*Arabidopsis*	GS-X transport (DNP-GS, MOC-GS, C-3-G-GS)	[12]
AtMRP2	*Arabidopsis*	GS-X and chlorophyll catabolite transport (DNP-GS, *Bn*-NCC1), xenobiotic inducible	[71,72]
AtMRP3	*Arabidopsis*	GS-X and chlorophyll catabolite transport (DNP-GS, *Bn*-NCC1) confers Cd^{2+} resistance to *ycf1* mutant, xenobiotic inducible	[73,74]
AtMRP4	*Arabidopsis*	xenobiotic inducible	[75]
AtMRP5	*Arabidopsis*	GS-X transport	[76]
TaMRP1	wheat	xenobiotic inducible	[77]
TUR2	*Spirodela*	*PDR5* homologue, transcriptional regulation by hormones and stress	[78]
AtTUR2	*Arabidopsis*	*PDR5* homologue	[78]
SNQ2 homologue	alfalfa	induced in somatic embryogenesis	[79]
GCN20 homologues	rice, *Arabidopsis*	regulation of kinase activity?	[21]

b. Organellar ABC proteins

Gene/ORF	Species	Function	Mit/c.plast?	Ref.
orf277	*Marchantia*	haem transport, *helB* homologue	M	[80]
orf206	*Oenothera*, *Arabidopsis*, carrot, tomato	haem transport, *helB* homologue	M	[28,81]
?	*Marchantia*	haem transport, *helC* homologue	M	[80]
orf240	wheat, rice	haem transport, *helC* homologue	M	[29,82]
orf250	*Oenothera*, *Arabidopsis*, carrot	haem transport, *helC* homologue	M	[83]
ycf16	*Odontella*	located in stroma	C	[27]
Aorf2	*Antithamnion* sp.	?	C	[84]
orf257	*Galdiera sulphuraria*	?	C	[85]
mbpX	*Marchantia*	?	C	[86,87]
mbpY	*Marchantia*	forms complex with *mbpX*?	C	[88]

sulted in the isolation of *PMDR1*, which is 86% identical to *AtPGP1* and 41% identical to human *MDR1* P-gp [69]. The cloning method employed suggests an intriguing link to calcium-dependent signalling pathways, but the function of *PMDR1* has not yet been reported; the authors tentatively propose a role in tuberisation, based on high levels of expression in stolon tips [69]. A P-gp homologue has also been cloned from barley, using a semi-nested degenerate PCR approach [70]. Two PCR products with homology to ABC transporters (*HvMDR1* and *2*) were originally identified, and a full-length cDNA isolated for *HvMDR2*. Examination of the nucleotide sequence indicates that *HvMDR1* represents a separate putative ABC transporter, and is not a fragment of *HvMDR2*. The predicted amino acid sequence of *HvMDR2* is 43% identical to both *AtPGP1* and *2*, and 38% identical to human *MDR1*, suggesting that it is a novel P-gp homologue from plants. *HvMDR2* is expressed at very low levels in roots and shoots, and Southern analysis of different barley cultivars suggests that there is a small family of related genes in this species, with some polymorphism between cultivars. The function of *HvMDR2* has not yet been determined.

Comparison of these plant *P-gp* homologues reveals many similarities to other *MDR* genes: in primary sequence, predicted topology and domain organisation. However, the proteins encoded by the plant genes are significantly smaller than their mam-

malian counterparts: between 134 and 144 kDa, compared with 170–180 kDa for human P-gp [1], and whilst all plant *P-gp* homologues isolated to date have potential sites for N-linked glycosylation, none of these correspond to the glycosylation site of human P-gp in the first predicted extracellular loop [13,69,70]. Whether the plant transporters are glycosylated remains to be determined experimentally.

5.2. Functions of MDR homologues

The first and, to date, only, functional characterisation of a plant P-gp was achieved with transgenic plants. In this study, Dudler and coworkers manipulated the expression of *AtPGP1* in transgenic *Arabidopsis* plants using sense and antisense constructs, and their results demonstrated the involvement of AtPGP1 in hypocotyl cell elongation in the light [68]. The authors propose that AtPGP1 is involved in the export of a signal compound, possibly a peptide hormone, from the shoot apical region. In agreement with this hypothesis, expression of *AtPGP1* was analysed using reporter gene constructs and in situ hybridisation, and found to be located in apical regions of shoots and roots. Overexpression of *AtPGP1* in transgenic plants facilitated its subcellular localisation: c-myc tagged protein was visualised in the plasma membrane using immunofluorescence confocal microscopy, and results were confirmed by Western blotting of membrane fractions. A plasma membrane location for AtPGP1 is also consistent with a signalling/export role.

Since it is clear that plants have at least a small gene family of MDR-like transporters, the question of whether they perform functions analogous to those of homologues in other organisms arises. Determining the function of cloned ABC transporters is problematic: where available, full-length cDNAs can be expressed in heterologous systems, such as the yeast *S. cerevisiae*, but without additional information to guide experiments, systematically testing the list of possible substrates is prohibitive. Examination of the ABC transporter literature suggests a range of putative functions: for example, yeast STE 6 transports peptides [18], mammalian MDR2 functions as a phospholipid translocator [48,49], and P-gp/MDR1 exports cytotoxic drugs from the cells and acts as a channel regulator [92] – all these are plausible functions for P-gp homologues in plants. Moreover, the fact that plant secondary products such as vincristine and taxol are often substrates for, or inhibitors of, MDR proteins suggests a role of plant P-gp in synthesis and compartmentation of these compounds. It is not possible to predict substrate based on primary structure/sequence homology: the products of even closely related genes can have markedly different functions [1] and site-directed mutagenesis of many types of transport protein has shown that single amino acid changes can radically alter substrate specificity. Therefore, for transporters isolated without reference to their function, other approaches are needed: for example, a transgenic strategy first successfully identified the function of murine MDR2. Here, plant scientists have the advantage that generation of transgenic plants is relatively straightforward in many species, and the opportunity for generating tagged mutants exists for model plants such as *Arabidopsis*, petunia and maize [93]. However, phenotypes of antisense plants may not be immediately obvious, and may only be evident under specific conditions, such as stress, or at specific developmental stages.

6. MRP subfamily

Whilst the first higher plant ABC transporter to be cloned was a member of the MDR subfamily, investigations of the role of vacuolar transport in xenobiotic detoxification, together with important insights from ABC transporters implicated in heavy metal transport and drug resistance have led to the identification and characterisation of plant members of the MRP subclass [5]. Progress in characterising the roles of MRP in plants has been rapid, since the isolation of *MRP* genes has explained and extended results from detailed biochemical studies.

6.1. Detoxification: biochemical studies

The detoxification of lipophilic xenobiotics such as herbicides is a multistage process, commonly comprising activation (phase I), conjugation (phase II) and compartmentalisation (phase III) of a toxic compound [94,95]. Phases I and II are relatively well characterised in plants: activation may involve hy-

drolysis by esterases or amidases, but more commonly is an oxidation reaction catalysed by a cytochrome *P*-450 [95]. Often these reactions result in products of increased toxicity, but they serve to generate functional groups for the protective conjugation reactions of phase II. In phase II, the xenobiotic or its activated metabolite is covalently linked by a transferase enzyme to an endogenous, hydrophilic substance: glucose, glutathione or malonate in plants, and glutathione, glucuronate or sulphate in animals. These inactive, water-soluble conjugates are then transported from the cytoplasm in phase III. In animals, conjugates are excreted from the cell across the PM by a specific ATPase [96–98], but in plants, which have no excretion system, they are thought to be deposited in the vacuole, and may be further metabolised, eventually appearing as 'bound residues' in the extracellular matrix [99].

In 1993, Martinoia and coworkers demonstrated that intact vacuoles isolated from barley mesophyll mediated MgATP-dependent accumulation of glutathione conjugates [90]. Both the model substrate, *N*-ethylmaleimide-GS (NEM-GS) and a glutathione conjugate of the herbicide, metolachlor (metolachlor-GS) were investigated. Uptake was driven by MgATP, but not by non-hydrolysable ATP analogues, or by PPi. Transport of NEM-GS and metolachlor-GS was sensitive to vanadate, but unaffected by inhibitors of the vacuolar H^+-ATPase, and chemicals which collapse the tonoplast proton gradient, indicating that uptake of glutathione conjugates into the vacuole is mediated by a specific ATPase, and not by a secondary active process. Oxidised glutathione (GSSG) was also shown to be a substrate for this transporter, whereas reduced glutathione (GSH) was not [100]. The characteristics of this activity were strikingly similar to the ATPase in the canalicular membrane of liver, which exports glutathione conjugates and GSSH into the extracellular medium [101], and this important finding provided the first experimental evidence for the identity and location of the phase III transport step in plants.

Subsequently, confirmation of this phenomenon was obtained for other systems: Rea and co-workers demonstrated MgATP-energised transport of the model conjugate dinitrophenol-GS (DNP-GS) by vacuolar membrane vesicles from *Arabidopsis*, beet, maize and mung bean [102]. Interestingly, they also reported the partial sensitivity of DNP-GS transport activity to P-glycoprotein inhibitors, vinblastine and verapamil [102], in contradiction to Blake-Kalff and Coleman, who did not detect any notable effect of these compounds on NEM-GS uptake into barley vacuoles [103].

Thus, a substantial body of evidence suggested the participation of a glutathione conjugate (GS-X) transporter in the detoxification of xenobiotics. Evidence for vacuolar accumulation and further processing of herbicide-GS conjugates was obtained by Wolf et al., who demonstrated rapid accumulation of alachlor-GS in barley vacuoles, and characterised a carboxypeptidase which catalyses the first step in conjugate degradation [104]. Operation of the detoxification pathway in vivo, from conjugation to vacuolar sequestration, was elegantly confirmed by Coleman et al. using a monochlorobimane-based fluorescent assay in whole maize and carrot cells [105]. Further evidence that GS-X transport is part of an integrated detoxification pathway came from studies with herbicide safeners and xenobiotics. Safeners are a group of chemically diverse compounds which increase the tolerance of monocot crops to specific herbicides [106]. Gaillard et al. found that the cereal safener, cloquintocet mexyl, doubled the vacuolar transport activity for both glutathione and glucoside conjugates of herbicides in barley [107]; glutathione *S*-transferase (GST) activity was also increased by the treatment. Similarly, Li et al. found that pretreatment of mung bean seedlings with model GST substrate, 1-chloro-2,4-dinitrobenzene, increased the activity of DNP-GS transport in tonoplast vesicles [108]. In both studies, application of the exogenous compound increased the V_{max}, but did not affect the K_m, suggesting that the higher activity was due to increased expression of the transporter and not altered affinity for substrate [107,108].

6.2. Molecular cloning of GS-X transporters

The seminal paper of Martinoia and the reports that followed from the groups of Rea and Coleman provided strong evidence for an *MRP* homologue as the candidate gene encoding the plant vacuolar glutathione conjugate transporter [90,102,103,108]. *MRP* was originally isolated from a drug resistant small cell lung cancer line [10], and a related gene,

the canalicular multispecific organic anion transporter, *cMOAT*, has since been identified [109]. Biochemical studies with membrane vesicles suggest that an export ATPase for glutathione conjugates resides at the plasma membrane of several mammalian cell types [101], and functional studies demonstrate that *MRP1* [51,52] and *cMOAT* [97,109] are responsible for these activities. Moreover, the yeast cadmium factor, *YCF1*, an *MRP* homologue isolated by its ability to mediate cadmium resistance [59], was shown to be a vacuolar glutathione *S*-conjugate pump [60]. Functional complementation of a *ycf1* mutant with human *MRP1* lent further support to the importance of this ABC subclass in GS-X transport [110].

Thus began the search for *MRP* homologues in plants. Several *Arabidopsis* expressed sequence tags (ESTs) were identified as putative *MRP* homologues [73,95], and Tommasini et al. demonstrated that transcripts corresponding to certain *MRP*-like ESTs were up-regulated in response to xenobiotic treatment, in agreement with a probable role in detoxification [73]. The genes corresponding to these ESTs have now been cloned: *AtMRP1* and *2* as both full-length genomic and cDNA clones [12,71,72], and *AtMRP3* and *4* as cDNA and genomic clones respectively [73–75]. Identification of the yeast MRP homologue, YCF1, as a GS-X pump, and the availability of a *ycf1* deletion mutant DTY167, provided the intellectual basis and genetic background for the heterologous expression of plant MRP homologues, and hence *AtMRP 1–3* were characterised in yeast. All three genes encode glutathione conjugate transporters, with similar characteristics to GS-X uptake activities previously studied in tonoplast vesicles and isolated vacuoles [12,71,74]. There are significant differences between the isoforms, for example: the overall GS-X transport capacity of AtMRP2 greatly exceeds that of AtMRP1 [71], and AtMRP3, but not AtMRP1 or AtMRP2, was able to alleviate the Cd^{2+} sensitivity of ycf1 [71,74]. YCF1 has been shown to mediate Cd^{2+} resistance by vacuolar sequestration of $(Cd.GS)_2$ [61], but human *MRP1*, which also rescues the *ycf1* mutant, does not [110]. It is not currently clear whether AtMRP3 is competent in $(Cd.GS)_2$ transport [5,74].

Recently, a further *Arabidopsis* MRP homologue (*AtMRP5*) was cloned, and also shown to encode a glutathione conjugate transporter ([76]; N. Gaedeke, B. Müller-Röber, pers. comm.). A partial *MRP* homologue (*TaMRP1*) has also been isolated from wheat, in a screen for herbicide safener-induced genes [77]. The wheat gene bears closest sequence similarity to *AtMRP4*, which is consistent with the finding that *AtMRP4* also exhibits a transcriptional response to herbicide safeners [75]. *AtMRP4* and *TaMRP1* have yet to be characterised functionally.

6.3. Other substrates for MRP?

The ability of MRP to transport glutathione conjugates and the inducibility of MRP isoforms by herbicide safeners argues strongly for a role in herbicide metabolism. However, the application of herbicides is a recent event in evolutionary history [111], and therefore postdates the emergence of MRP, which appears to have evolved prior to the divergence of plants and animals. Since *Arabidopsis* has a small family of MRP genes encoding transporters with different kinetic properties, this raises the question of alternative/ancestral functions for MRP in plants. All plant MRPs characterised to date mediate GS-X transport, but few natural glutathionylated compounds have been demonstrated convincingly in plants. This may reflect low steady-state levels, due to further processing of glutathione conjugates once they have been sequestered in the vacuole: a vacuolar carboxypeptidase which cleaves the glycine residue from metolachlor-GS has been characterised [104], and further steps leading to the deposition of bound residues can be postulated [95,99]. However, an alternative explanation is that non-glutathionylated compounds also serve as MRP substrates. This is indeed the case for human MRP isoforms: substrates for cMOAT include bile acids [112], glucuronides [113] and unconjugated organic acids [114], and MRP1 transports glucuronate and sulphate conjugates in addition to glutathione conjugates [113, 115]. Similarly, a number of vacuolar transport activities which may be attributable to MRP have recently been characterised, and are summarised below.

6.3.1. Chlorophyll catabolites

During leaf senescence, the porphyrin moiety of chlorophyll is cleaved into linear tetrapyrroles, which

are eventually deposited in the vacuoles of mesophyll cells [116]. ATP-dependent uptake of these chlorophyll breakdown products into isolated barley vacuoles has been demonstrated by Hinder et al., and showed striking similarities with GS-X transport [117]. Following cDNA cloning of *Arabidopsis* MRP genes, yeast expression studies showed that AtMRP2 and 3 transport not only GS-X, but also chlorophyll catabolites such as *Bn*-NCC-1 [71,74]. Whilst *Bn*-NCC-1 is not glutathionylated, it is conjugated to malonate, and it is possible that malonylation may also serve to 'tag' compounds for sequestration in the vacuole, the action of a malonyltransferase playing an analogous role to that of glutathione *S*-transferases [118]. Competition experiments employing isolated vacuoles suggested that GS-X and *Bn*-NCC1 are sequestered by distinct transporters, but the heterologous expression experiments provide unequivocal proof that a single transporter is competent in the transport of both substrates. However, *AtMRP2* is not necessarily an orthologue of the barley transporter, since their K_m values for *Bn*-NCC1 differ by 10-fold [71]. Interestingly, DNP-GS and *Bn*-NCC1 did not compete for uptake when supplied simultaneously to tonoplast vesicles from yeast expressing *AtMRP2*, but were accumulated to levels comparable to those observed when only a single substrate was supplied. This unusual kinetic observation has important mechanistic implications, suggesting the presence of two distinct functional domains which operate independently in AtMRP2. A model accounting for all the kinetic data is developed in [71], and discussed further in [5]. It will be informative to determine whether dual transport can be reconciled with the flippase model proposed for MDR-type transporters.

6.3.2. Glucosides and glucuronides

Studies with isolated barley vacuoles indicate that different energisation mechanisms drive the uptake of flavonoid glucosides and herbicide glucosides [119]. The endogenous flavonoid glucoside, isovitexin, was taken up via a ΔpH, V-ATPase dependent mechanism, whereas hydroxyprimisulphuron-glucoside was transported by a vanadate-sensitive pump [119]. Induction of hydroxyprimisulphuron-glucoside transport activity by herbicide safeners [107], and induction of *AtMRP3* by primisulphuron [73] lend further support to the hypothesis that an MRP could be implicated in xenobiotic-glucoside transport, although inducing compounds do not necessarily serve as substrates. Similarly, glucuronides, which are generally rare in plants, but are abundant endogenous conjugates in rye, were shown to be transported by an MRP-like pump in isolated rye vacuoles [120].

6.3.3. Taurocholate

At the time of the discovery of the glutathione conjugate ATPase, a second vacuolar ATP-dependent anion transporter, which functioned as a taurocholate pump was identified in liver [121–123]. Perhaps surprisingly, Hörtensteiner et al. were able to demonstrate a similar activity in barley vacuoles [124]. Since bile acids, such as taurocholate, do not occur naturally in plants, the plant taurocholate transport activity must represent the operation of a transporter with an unknown, probably bulky, anionic substrate. As for the transport of glutathione conjugates, uptake of cholate conjugates, taurocholate and glycocholate, was ATP-dependent, inhibited by vanadate, but not by bafilomycin and could not be supported by non-hydrolysable ATP analogues, suggesting a primary active process. NEM-GS and taurocholate transport could be distinguished by their substrate specificity, response to alternative nucleotides and sensitivity to different inhibitors [103,124], and glutathione conjugates stimulated, rather than inhibited taurocholate transport [124]. It was therefore proposed that taurocholate and GS-X transport activities represented distinct transporters [124]. However, Blake-Kalff and Coleman found that taurocholate inhibited the uptake of NEM-SG into barley vacuoles [103]. The ability of vacuoles to maintain a proton gradient in the presence of taurocholate was verified by quinacrine fluorescence quenching, and taurocholate was supplied at a concentration below its critical micelle concentration, thereby eliminating inhibition due to detergent effects. In accordance with these observations, neither AtMRP1 nor AtMRP2 transported taurocholate when expressed in yeast, but taurocholate selectively inhibited DNP-GS uptake by AtMRP2 with an IC_{50} well below its CMC [71]. An important corollary of this finding, and of the facility of AtMPR2 for simultaneous, parallel transport (see above) is

that competition experiments must be interpreted with care where MRP homologues are concerned: competition does not imply that the competing compound is a bona fide substrate and conversely, absence of competition does not imply that a compound is not transported. This makes it difficult to assign significance to potentially interesting results of competition studies.

Probenecid is an anion transport inhibitor which competitively inhibits taurocholate transport in isolated vacuoles [103], and has also been reported to block vacuolar transport of anionic fluorescent dyes such as fura 2, Quin-2 and Lucifer yellow [125–128]. Lucifer yellow has been employed as a model substrate to study uptake of sulphated and sulphonated compounds into isolated rye by Klein et al. who demonstrated that transport was mediated by a MOAT-like ATPase, and was inhibited by probenecid, sulphated compounds and glucuronates, but not by glutathione conjugates [129]. Whether or not fluorescent dyes represent exogenous substrates for the taurocholate transporter, or for another vacuolar transport system, will only be confirmed by the isolation and functional expression of the relevant gene or the isolation of transport mutants. Fluorescent dyes are commonly used for probe cellular parameters such as intracellular free Ca^{2+} (fura 2, Quin-2) and pH (BCECF), and their eventual vacuolar sequestration is a significant hindrance to their use in plant cells [125,126,130]. Identification of the appropriate transporter gene(s) may lead to the construction of transgenic plants with reduced vacuolar accumulation of fluorescent probes.

Whilst the precise molecular nature of the plant taurocholate transporter remains unknown, other systems provide clues to its possible identity. Taurocholate transport has been demonstrated in secretory vesicles and a vacuole-enriched fraction of *S. cerevisiae*; the same populations of vesicles were also competent in the transport of DNP-GS, but the two substrates did not compete [131,132]. Since, as in plants, the characteristics of taurocholate transport suggested that it might be encoded by an *MRP* homologue, and yeast has several such genes, Ortiz et al. attempted to identify a taurocholate transporter genetically, by creating yeast deletion mutants lacking ABC transporters. Three candidate genes were identified by PCR, and ATP-dependent bile acid transport was abolished when one of the genes, *BAT1*, was deleted from the genome, and restored upon reintroduction of the gene [132]. BAT1 exhibited homology to *S. cerevisiae* putative ABC proteins of unknown function, to rat cMOAT, and human MRP1. Strict *BAT1* homologues have not yet been identified in plants, but the finding that rat cMOAT, which shares only 32% amino acid identity with BAT1, is able to transport bile acids [112], suggests that there may not necessarily be a great deal of sequence homology between bile acid transporters. In agreement with this suggestion, the human gene responsible for taurocholate transport, *BSEP* (bile salt export pump), has been identified by its role in disease and found to be identical to a *P-gp* homologue, *sister of P-glycoprotein* [133]. Thus, it appears that ABC transporters belonging to two different subfamilies are competent in bile acid transport.

6.3.4. Secondary products

The *Bronze-2* (*bz2*) mutation is the last genetically defined step in anthocyanin pigmentation in maize, resulting in accumulation of anthocyanins in the cytoplasm, where they are oxidised and cross-linked to form brown products. *Bz2* encodes a GST, which has been postulated to tag cyanidin-3-glucoside with glutathione, prior to sequestration by the GS-X pump [134]. In agreement with this hypothesis, the *bz2* phenotype can be mimicked by application of vanadate to wild-type protoplasts [134], and AtMRP1 and 2 have been proposed to transport a synthetic glutathionylated derivative of the anthocyanin cyanidin-3-glucoside [12,71]. A divergent, but functionally equivalent GST, *An9*, has been isolated from *Petunia*, suggesting widespread involvement of GST in anthocyanin pigmentation [135]. However, the precise role of *Bz2* is unclear, since glutathione conjugates of anthocyanins have not yet been detected in vivo, and conjugation of glutathione to cyanidin-3-glucoside by *Bz 2* has not been demonstrated. Alternative pathways for the vacuolar sequestration of anthocyanins have also been proposed: Hopp and Seitz demonstrated uptake of acylated anthocyanins into isolated carrot vacuoles [136] and a vesicular transport system for anthocyanins has been described by Grotewold et al. ([137], and refs. therein).

Other possible endogenous MRP substrates are allelochemicals such as phenolics and phytoalexins

[5,138]. The legume phytoalexin, medicarpin, when glutathionylated, has been shown to be transported into tonoplast vesicles with high efficiency [138]. Li et al. have therefore proposed a role for MRP in storage of antimicrobial compounds in healthy plant tissue [138]. Interestingly, an ABC transporter from pathogenic strains of rice blast fungus is postulated to efflux phytoalexins; however, the gene (*ABC1*) is more closely related to the yeast ABC transporter *PDR5*, than *MRP* [139].

6.4. Antiquity of GSH-mediated detoxification

The evidence for secondary metabolites as MRP substrates should not detract from a likely primary role for MRP in detoxification: whilst plants have been systematically exposed to man-made chemicals such as herbicides for the last 100 years only [111], the need to protect against cytotoxic electrophiles is ancient [95]. The occurrence of glutathione in all eukaryotes and its restriction in prokaryotes to cyanobacteria and purple bacteria suggest that it arose at the same time as oxygenic photosynthesis, as part of a mechanism to protect cells from damage by active oxygen species. Transport of oxidised glutathione (GSSG – a special form of glutathione conjugate) by GS-X pumps may thus function in defence against oxidative stress.

Many biotic and abiotic compounds are susceptible to conjugation to glutathione, since the chemical determinants of GST substrates are common and widely distributed (for discussions, see [95,140,141]), and it seems that the subsequent evolution of gene products involved in detoxification such as GSTs and GS-X pumps to perform additional functions is a consequence of the versatility of glutathione chemistry.

6.5. Tissue specificity and subcellular location

Northern analysis reveals that *AtMRP1*, *2* and *3* do not appear to have restricted distributions, being constitutively expressed in several tissues [12,71, 72,74]; expression does, however, increase in response to xenobiotic treatments [73,75,107]. Since plant MRPs accept multiple substrates, it will be informative to investigate whether a particular isoform performs different functions in different tissues or whether roles are also delineated by cell specific expression. It is already evident that certain functions of MRP are restricted to specific cell types, for example: in leaves, transport of chlorophyll catabolites is specific to the mesophyll, since the epidermis does not contain chloroplasts. However, the MRP responsible for chlorophyll catabolite transport may also be present in epidermis where it performs a different function, such as glutathione conjugate transport. Evidence for this is limited, as biochemical studies have concentrated on mesophyll vacuoles and tonoplast vesicles from whole organs; however, using leaf protoplasts, Coleman and coworkers observed that, whilst glutathione conjugates of monochlorobimane were formed in the cytosol of barley epidermal cells, they did not accumulate in the vacuole [95,142]. This may also reflect different substrate specificities of epidermal and mesophyll transporters, or may indicate that an appropriate isoform of MRP is absent from the tonoplast in epidermal cells. Experimental evidence to date strongly suggests the tonoplast as the prime location for MRP, but this does not rule out the existence of MRP isoforms in other subcellular locations.

Studies of plasma membrane GS-X transport (e.g. using inside-out plasma membrane vesicles) have not yet been reported, but recent immunological evidence points to a plasma membrane location for certain isoforms of MRP. An expression library was screened with polyclonal antibodies raised against total proteins from *Arabidopsis* plasma membrane and tonoplast, and the positive clones were used to construct two cDNA libraries enriched in genes encoding plasma membrane and tonoplast proteins respectively [143]. Sequence analysis of the plasma membrane library identified a clone 100% homologous to *AtMRP1*. Whilst this result does not necessarily indicate that AtMRP1 itself resides in the plasma membrane, it suggests that *Arabidopsis* does possess plasma membrane proteins which are antigenically related to AtMRP1. Testing of this hypothesis awaits the availability of isoform-specific antibodies, or plants transformed with epitope tagged constructs, as has been achieved for *AtPGP1* [68].

6.6. Special features of MRP

To date, cloning of plant representatives of one

ABC subclass – MRP – has accounted for several vacuolar transport activities: MRP isoforms have been shown to transport chlorophyll catabolites, and glutathione conjugates of xenobiotics, anthocyanins and phytoalexins. For other substrates, such as glucosides, involvement of an MRP is suspected, but definitive proof will require molecular cloning of the corresponding genes or analysis of mutants. The multispecific nature of MRP, and its unique ability to transport dissimilar substrates simultaneously have been significant discoveries: interestingly, unlike MDR, which can transport many substrates, but at relatively low affinity, K_m values for putative MRP substrates are all in the micromolar range [5]. Studies of how these properties are related to the specific structural features of MRP will form the basis for future investigations.

7. The search for channel regulators

The majority of ABC proteins characterised to date function as ATP-dependent pumps. However, the finding that the product of the cystic fibrosis gene is not only a chloride channel, but also a regulator of several other ion channels, opened up a new field of ABC transporter research, and challenged conventional views on the distinction between channels and pumps [92]. It was subsequently demonstrated that several other ABC proteins such as P-glycoprotein and the sulphonylurea receptor (SUR) regulate heterologous channels in addition to possessing their own intrinsic transporter activities [92], and evidence for similar phenomena has recently been sought in plants.

7.1. SUR and CFTR

The cystic fibrosis gene of animals encodes an ABC transporter with homology to MRP, but which functions as an outwardly rectifying Cl^- channel – the cystic fibrosis transmembrane conductance regulator (CFTR [144]). CFTR is regulated by cAMP-dependent phosphorylation and by ATP [144,145] and is inhibited by diphenylamine-2-carboxylic acid (DPC) [146]. CFTR itself regulates other channels in cystic fibrosis cells, including a second, distinct, outwardly rectifying Cl^- channel, [147], a Ca^{2+}-activated chloride channel [148], a sodium channel [149] and inwardly rectifying K^+ channels [150–152].

Sulphonylureas are drugs used to treat non-insulin-dependent diabetes: sulphonylurea blockade of an ATP-sensitive K^+ channel (K-ATP) in pancreatic β cells modulates insulin release. The sulphonylurea receptor (*SUR*) has been cloned and found to be an ABC transporter [8], which associates with an inwardly rectifying K^+ channel to render it sensitive to sulphonylureas and to form an ATP-sensitive K^+ channel [153]. SUR is also a receptor for K^+ channel openers, e.g. chromakalim, which can reverse the inhibitory effect of sulphonylureas [154]. Interestingly, CFTR is also blocked by the sulphonylurea glibenclamide [155,156].

Attempts have been made to clone *CFTR* and *SUR* homologues from *Arabidopsis*; however, the genes cloned by screening libraries with appropriate ESTs exhibited greater overall identity to *MRP* than *CFTR* or *SUR* and have been shown to be glutathione conjugate transporters (see above; [76]; C. Forestier, pers. comm.). These transporters may nevertheless have a second function, as MRP has been shown to alter Cl^- and K^+ currents in some mammalian cell types [157], and a recently isolated ABC channel protein, the epithelial basolateral cAMP-regulated Cl^- channel conductance regulator (EBCR), exhibits greater homology to MRP isoforms than to CFTR [158].

Electrophysiological approaches to study plant ABC transporter/channel relations have proved more informative: guard cells possess a number of well-characterised channel activities implicated in stomatal movements [159], some of which may represent ABC transporters: for example, anion channels have been proposed to be *CFTR* homologues [160,161]. Thus they comprise an excellent model system to study ABC transporter/channel interactions in plants [162].

Forestier and coworkers have exploited the specific pharmacological profile of K-ATP and CFTR to probe possible analogous systems in stomatal guard cells. In one study, the effect of sulphonylureas and K^+ channel openers on guard cell K^+ channels was investigated [162]. The sulphonylureas, tolbutamide and glibenclamide induced stomatal opening in epidermal strip bioassays, and in whole cell patch clamp experiments, glibenclamide was found to inhibit the

outward K^+ current, but did not affect the inward K^+ current. Conversely, K^+ channel openers such as chromakalim triggered stomatal closure under light and prevented stomatal opening, suggesting that K^+ permeability through the outward K^+ channel could be increased. Sulphonylureas and K^+ channel openers competed in the epidermal strip bioassays, and taken together, the results suggest the presence of a SUR-like protein in guard cell plasma membrane, which is involved in the regulation of the outward K^+ channel during stomatal movements. The experimental conditions employed did not permit unequivocal identification of an ATP-dependent K^+ channel, since ATP was present in pipette solutions, and in a previous study, the guard cell outward K^+ channel was not found to be ATP dependent [163]. Further investigation of the presence of K-ATP in guard cells awaits the molecular characterisation of the K^+ outward rectifier from guard cells. A potassium outward rectifier (*KCO1*) has been cloned from *Arabidopsis*, where it is expressed in leaves [164]. This gene belongs not to the ABC superfamily, but to a new group of 'two-pore' K^+ channels [164] – it would be interesting to test whether KCO1 interacts with ABC transporters to yield an ATP-dependent K^+ channel. A Shaker-type outward rectifying K^+ channel gene, *SKOR*, has also been isolated from plants, but its expression is specific to the root stele [165]. When making comparisons between ABC/K^+ channel interactions in plant and animal systems, it should be noted that the K^+ channel induced by SUR in mammalian cells is an *inward* rectifier [153] and belongs to a different gene family to *KCO1* [164]. A potassium inward rectifier has been shown to play a critical role in stomatal opening [166], and Shaker-type *Arabidopsis* and potato genes encoding K^+ inward rectifiers are indeed expressed in guard cells [167,168]. Although the K^+ inward rectifier was not affected by sulphonylureas in the experiments of Leonhardt et al. [162], the question of whether this channel is modulated by ABC transporters remains open.

In guard cells, slow anion channels are activated by Ca^{2+}, and are thought to mediate the prolonged anion efflux necessary for stomatal closure [169,170]. Again, using whole-cell patch clamp experiments and epidermal strip bioassays, Leonhardt et al. studied the effect of ABC/channel modulators on the slow anion channel [171]. CFTR blockers glibenclamide and DPC were found to inhibit the slow anion current, and triggered stomatal opening in darkness. The potassium channel opener, chromakalim, suppressed glibenclamide-induced stomatal opening, and recovered the glibenclamide-inhibited slow anion current. Glibenclamide prevented stomatal closure triggered by Ca^{2+} and ABA, and, accordingly, ABA partially relieved glibenclamide inhibition of the slow anion current, suggesting interactions with Ca^{2+} and ABA signalling cascades. Overall, the results of the two guard cell studies confirm that the guard cell slow anion channel is similar to CFTR, and suggest that it mediates sulphonylurea block of the outward K^+ channel. This interaction may allow coordinated efflux of K^+ and anions during stomatal closure, and is analogous to the interaction between mammalian CFTR and Na^+ channels [92].

7.2. P-gp channel activity

In addition to its role as an ATP-dependent drug efflux pump, mammalian P-glycoprotein has been shown to be associated with a volume-activated chloride channel [172,173]. P-gp alters the sensitivity of channel activation to osmotic gradients and can impose protein kinase C dependency on channel activation. Three lines of evidence confirm that channel regulator and drug transport functions are distinct activities: requirements for ATP hydrolysis (drug transport requires ATP hydrolysis, whilst channel function can be supported by non-hydrolysable analogues [173]), pharmacology [174] and differential regulation by phosphorylation: protein kinase C-mediated phosphorylation regulates channel activity, but not drug transport [175,176]. No channel regulating activity has yet been ascribed to P-gp plant homologues.

7.3. Prospects for plants

Given the presence of P-gp and MRP transporters in plants, it is possible that some of these ABC proteins interact with ion channels, as has been demonstrated in mammalian systems. Despite a growing body of pharmacological evidence for ABC/channel interactions in guard cells [162,171], no *cloned* plant ABC transporter has yet been shown to exhibit chan-

nel or channel regulator activity. Several groups are currently investigating this possibility by expressing ABC transporters in heterologous expression systems such as *Xenopus* oocytes. However, since effects of ABC transporters on channel activity can be quite subtle, e.g. increased sensitivity to ATP, as opposed to activation/deactivation, channel-transporter interactions may prove difficult to detect in heterologous systems, without clues from other experiments to the identity of the channel activity in question. Also, not all ABC proteins are expected to have dual functions: in view of the size and diversity of the superfamily, this would clearly be deleterious for the cell [1]. Thus, the extent of this phenomenon remains to be determined, both in mammalian and plant systems, and an unequivocal estimation will be hard to achieve. Application of techniques such as two hybrid systems, 'green Westerns', and coexpression of libraries with cloned genes in heterologous systems has already helped to identify proteins which interact with various membrane transporters – development and use of these techniques to study ABC transporters may lead to progress in the field of channel regulation in the future.

8. PDR5 subfamily

Whilst several plant ABC transporters have been cloned by homology to genes from yeast and humans, or following their identification in genome sequencing projects, differential screening studies of two developmental processes have led to the identification of a new class of plant ABC transporter homologues. In the aquatic plant, *Spirodela polyrrhiza*, formation of dormant buds, termed turions, is induced by ABA treatment and low temperature, and the effect of ABA is antagonised by cytokinins [177,178]. In a screen for transcripts induced by ABA, Smart and Fleming isolated a homologue of the yeast drug resistance gene: *PDR5* [78]. PDR5 is a 'mirror image' four-domain ABC protein, which mediates plasma membrane efflux of numerous unrelated toxins in yeast ([23]; see above). The plant homologue, *TUR2*, was expressed at very low levels in untreated tissue, but expression appeared to be correlated to factors involved in the control of turion formation, since transcripts were induced by ABA and repressed by kinetin. However, in situ analysis revealed that *TUR2* mRNA accumulated in all parts of the plant, not just in those involved in turion formation, and could also be induced by stress treatments such as low temperature and high salt. The authors concluded that the accumulation of *TUR2* transcripts was associated with the decrease in growth which accompanies turion formation, rather than a causal event.

Similarly, a study of changes in gene expression during the onset of somatic embryogenesis led to the identification a *SNQ2* homologue in alfalfa [79]. SNQ2 is a plasma membrane-bound ABC transporter, which is closely related to PDR5, and also mediates drug resistance in yeast [24,25]. The alfalfa *SNQ2* homologue was expressed 10 days after induction of embryogenesis by 2,4-D and wounding; it would therefore be interesting to investigate further the response of this gene to stress and hormones, in the light of the expression patterns of *TUR2* [78].

At present, the role of the PDR5 subfamily in plants is not known. The regulation of *TUR2* and alfalfa *SNQ2* by plant hormones is an interesting feature, since yeast SNQ2 has been shown to transport steroid hormones in addition to cytotoxins [179]. However, it is premature to suggest similar roles for the plant transporters. Homologues of *TUR2* have been identified in the *Arabidopsis* and rice EST databases, indicating their widespread presence in higher plants, and permitting detailed molecular analysis, which may help elucidate their precise roles in physiology ([78]; C. Smart, pers. comm.).

9. Organelle ABC transporters

9.1. Chloroplast

Genome sequencing projects have led to the discovery of several putative organellar ABC transporters; indeed, the first plant ABC gene to be identified was a 1.1 kb reading frame, *mbpX*, in the *Marchantia* chloroplast genome [86,87]. *MbpX* encodes a bacterial permease-like membrane-spanning subunit, and a candidate for another component of a putative complex containing the *mbpX* product is encoded in a second, linked reading frame, *mbpY* [88]. Reading frames encoding ATP binding subunits have also

been identified in the plastid genomes of red algae [84,85] and diatoms [27]. Not all organellar genes are transcribed, but immunoprecipitation studies indicated that the diatom protein, YCF16, is synthesised in plastids and located in the stroma [27]. Analysis of sequenced chloroplast genome of higher plants has not revealed the presence of *mbpX* or *ycf16* homologues, suggesting either that the proteins encoded by these genes are not required in higher plants, or that they have been transferred to the nuclear genome during the course of evolution [27]. Such 'migration' of sequences from organellar genomes to the nucleus is well documented [180], and thus the latter explanation is plausible.

9.2. Mitochondria

ABC transporter subunit genes implicated in the biogenesis of cytochrome *c* have recently been identified in higher plant mitochondrial genomes. Cytochromes *c* are located outside the cytoplasmic membrane of bacteria, in the intermembrane space of mitochondria, and in the lumen in chloroplasts and their assembly therefore requires transmembrane transport of haem [181]. The photosynthetic bacterium *Rhodobacter capsulatus* has been employed as a model organism to study cytochrome *c* biosynthesis, and *helABC* genes encoding components of an ABC transporter necessary for haem export and ligation have been characterised in detail [182]. As *Rhodobacter* is phylogenetically related to endosymbiont ancestors, it is perhaps not surprising that orthologues of the two transmembrane subunits encoded by *helB* and *C* have been identified in the mitochondrial genome of *Marchantia* [80]. Orthologues are also present in several higher plant species (see Table 1; [28,29,81–83,183]). However, the gene encoding the ATP binding subunit, *helA*, has not been identified in any of the mitochondrial genomes sequenced to date, and it has been proposed that this and other genes involved in cytochrome biogenesis have been transferred to the nucleus [181].

9.3. Nuclear-encoded ABC transporters

Despite the paucity of organellar ABC transporter genes, the presence of and requirement for further ABC proteins in organelles can be postulated. Firstly, preliminary experimental evidence suggests that additional ABC transporters exist, for example, antibodies raised to conserved regions of P-glycoprotein react positively in immunoblots of chloroplast outer envelope membranes [184]. Furthermore, chloroplast MRP homologues may be implicated in chlorophyll degradation. As discussed above, it has been shown that AtMRP2 and 3 are capable of mediating transport of the chlorophyll metabolite *Bn*-NCC-1 into the vacuole when expressed in transgenic yeast [71,74] and biochemical studies have identified an MRP-like *Bn*-NCC-1 vacuolar transport activity in barley [117]. Since chlorophyll breakdown products must somehow exit the plastid before this can take place, a second ABC transporter may also exist in the chloroplast envelope. In agreement with this, chlorophyll catabolites have been shown to be released from intact barley gerontoplasts into the medium in an ATP-dependent fashion [185].

10. ABC transporters in plant-microbe interactions

In addition to endogenous transporters, a number of ABC proteins are relevant to plant biology by virtue of their role in symbiotic and pathogenic relationships. *Rhizobium* and *Agrobacterium* are closely related bacteria whose associations with higher plants have become important paradigms for studying plant-microbe interactions: agrobacteria are tumour-inducing pathogens of dicotyledonous plants, and rhizobia are involved in symbiotic nitrogen fixation in legume root nodules. Such intimate associations require the exchange of signals and nutrients between plant and microorganism, and by definition must involve numerous transport processes. Biochemical and genetic studies have demonstrated a number of ABC transporters in the Rhizobiaceae family which operate at different stages of the association.

10.1. Agrobacterium

During *Agrobacterium*-mediated gall formation, a piece of bacterial DNA – the T-DNA – is transferred to the plant cell, where it becomes stably integrated into the host genome. Components encoded by the *vir* regulon are responsible for this process. *Vir* genes

are induced in response to chemical signals of the plant wound site, for example, monosaccharides are bound by a periplasmic binding protein, ChvE, which then interacts with the membrane-spanning VirA molecule of the VirA-VirG sensor-regulator pair to activate transcription of the *vir* regulon. As part of this process, ChvE also mediates chemotaxis towards sugars, through interaction with an unidentified receptor. Additionally, analysis of the region downstream from the *ChvE* gene indicates that it is also part of an ABC sugar transport operon, thus one periplasmic binding protein serves three related functions in establishment of the association: signalling, chemotaxis and sugar uptake [33].

Overlap between nutrition and signalling also occurs later in tumour development: the integrated T-DNA directs the synthesis of opines – amino acid and sugar derivatives which are produced by the plant and taken up and catabolised by the infecting bacteria. ABC genes encoding periplasmic transport systems for octopine and nopaline have been cloned, and found to be homologous to the histidine permease of *S. typhimurium* [186,187]. In addition to acting as nutritional sources, a subclass of opines function as signal molecules, and have a specific ABC uptake system [188].

Interestingly, the induction of tumours by *Agrobacterium tumefaciens* and the production of nitrogen-fixing nodules by *Rhizobium meliloti* require a related set of genes. Several linked chromosomal genes (*chvA*, *chvB*, *exoC*), are required for attachment of *A. tumefaciens* to plant cells and have homologous, functionally interchangeable counterparts (*ndvA*, *ndvB*, *exoC*) in *R. meliloti* [189]. Mutagenic analysis has implicated *chvB*/*ndvB* in the synthesis of a low molecular weight, cyclic β-1,2-glucan required for attachment, but mutants of *chvA* and *ndvA* also fail to produce extracellular polysaccharide. The *chvA*/*ndvA* loci have been sequenced and found to encode a polypeptide homologous to the haemolysin export protein of *E. coli*, suggesting a role in polysaccharide export, and this has been confirmed experimentally [190,191].

10.2. Rhizobium

Further transport parallels between tumour and nodule formation also exist: the capacity to utilise specific carbon sources and the ability to respond to signals are also mediated by ABC transporters in rhizobia. One of the first steps in plant-*Rhizobium* signal exchange is the induction of plasmid-borne *nod* genes by plant-exuded flavonoids or betaines – a phenomenon which requires as yet unidentified transporters in both plant and bacterium. In light of the ability of flavonoids to act as substrates for mammalian and yeast ABC transporters, it is tempting to speculate that an ABC transporter might be responsible for export of flavonoids from the root.

Nod genes are involved in the synthesis of nod factors, which trigger the nodulation process. Most nod factors are lipochitooligosaccharides, but nodO, which is specific to *R. leguminosarum* bv. *viciae*, is a protein. Downie and coworkers have demonstrated that nodO is transported from the cell by an ABC system: nodO could be exported by a strain of *E. coli* carrying the *nodO* gene, the outer membrane protein gene, *tolc*, plus either the haemolysin transporter genes *hlyBD*, or the protease secretion genes, *prtDEF* from *Erwinia chrysanthami* [192]. Although *nodO* homologues are not found in other rhizobia, several strains were found to have the ability to export nodO, which is consistent with the finding that the protein transporter genes are unlinked to the *nod* genes, and may represent a general protein transport apparatus [192]. Interestingly, three genes encoding a ABC transporter with homology to *prtDE* were recently isolated from the genome of *R. leguminosarum* [193].

Other *Rhizobium* loci related to nodulation but not involved in nod factor production have been described; some of these act to ensure the survival of the bacteria in the rhizosphere, such as the *R. meliloti* locus *ndvF* which encodes an ABC phosphate uptake system, and appears to play a purely nutritional role in nodulation [194]. Others may be involved in signalling processes between bacteria and plants to establish colonisation of the root, for example: in a search for *Rhizobium tropici* genes inducible by host exudates, Rosenblueth et al. isolated an ABC transport complex with homology to ribose transport proteins, thought to mediate host-specific exudate uptake [195].

10.3. Fungal pathogens

Whilst the role of fungal ABC transporters in drug resistance phenomena is well established [55], their involvement in pathogenic interactions with plants is only beginning to emerge. An ABC transporter (*ABC1*) was recently identified in an insertional mutagenesis screen for pathogenicity mutants of the rice blast fungus, *Magnaporthe grisea* [138]. ABC1 is 47% identical to the well-characterised yeast ABC transporter, PDR5, which is known to operate as a drug efflux pump [55]. Both *ABC1* and *PDR5* transcript levels are dramatically elevated in response to metabolic poisons, and up-regulation of ABC1 was required for pathogenicity. However, unlike *PDR5*, deletions in *ABC1* did not result in sensitivity to metabolic poisons and antifungals, implying a specialised pathogenic function, rather than a general role in drug resistance. Homology to PDR5 suggests that ABC1 may act to export a fungal toxin during pathogenesis, but this seems unlikely since there is no evidence for toxin production in rice blast disease, and growth of abc1 mutants was arrested following penetration of the plant, a phenomenon which is not observed in Tox minus strains of other fungal pathogens. The authors therefore proposed that ABC1 acts as an efflux pump to remove antimicrobial compounds, such as phytoalexins present in rice, and may therefore represent a common phenomenon in plant pathogenesis.

10.4. General relevance

Given the examples described above, it seems axiomatic that the transport complement of a microorganism can contribute to its symbiotic and saprophytic competence. These observations can be extended to other systems, for example an ABC transporter has been shown to play a defensive role in protecting lactobacilli from toxins produced by hops [196] and an ABC transporter has been implicated in the *Pseudomonas*-oilseed rape mutualism [197].

11. Conclusions and future perspectives

In the last decade, the existence of several classes of plant ABC transporters has been established. Whilst much has been learned already, many questions remain: What are the prospects for the identification, cloning and characterisation of further transporters? What are the roles of those transporters already isolated? How do the properties of these proteins reflect their structure? How are the ABC transporters regulated? To what extent is the complement of ABC transporters specific to plants? These questions and others will help form the background of plant ABC transporter research in the future.

11.1. Cloning and characterisation of plant ABC transporters

Plant ABC transporters have been identified and cloned by a variety of means: many have been identified as a result of genome sequencing and EST projects, a number have been isolated by PCR; other, novel clones have emerged in differential screens. Many plant ABC transporters will continue to be identified as plant genome sequencing projects approach completion. Characterisation of these transporters, for which only sequence information is available, presents a challenge for biologists. The increasing availability of tagged mutants is a tool with which to address this problem, but identifying or isolating clones with reference to their function circumvents problems with characterisation. Subtractive cloning has the advantage that it may afford clues to the identity of the transporter, over and above its homology to other proteins. Also, yeast complementation has been a popular and successful method for cloning many plant transporter cDNAs in recent years [198], and the superb variety of available yeast ABC transporter mutants (e.g. [57]) makes functional cloning appear appealing for plant members of this superfamily. However, the large size (120–170 kDa; 3–6 kb) of four-domain ABC transporters such as MDR and MRP may prove prohibitive in functional cloning experiments. In an attempt to clone an *Arabidopsis* STE 6 homologue by complementation, Covic and Lew isolated a serine/threonine protein kinase [199]. While this result is interesting and has yielded a novel non-transport gene, it highlights the difficulty of obtaining transporter clones. The use of size-selected libraries may facilitate functional cloning, but isolating full-length

transporters from a directional library may prove problematic due to leaky, toxic expression when the library is propagated in *E. coli*. Whilst yeast may not be the first choice for cloning, it nevertheless remains a useful host for heterologous expression of cloned ABC transporter genes, as has been the case for MRP homologues. For other transporter subclasses, judicious use of transcription factor mutants to remove several endogenous ABC transporters will facilitate analysis. Also, yeast ABC transporter genes are also a potential source of useful promoters for heterologous expression: the PDR5 promoter having proved particularly strong [65].

11.2. Extent of the ABC superfamily in plants; lessons from other organisms

Given the size of ABC superfamilies in microbes and humans, and the range of suspected roles for ABC transporters, the identification of further plant ABC genes is highly likely. However, it is risky to base speculations concerning the size of a gene family on comparisons with other organisms: within bacteria alone, the size of the ABC family varies 5-fold [2]. It is also difficult to speculate accurately on the possible roles of the ABC transporters. Nevertheless, comparative genomics can be extremely instructive when interpreted with caution, as has been shown for comparisons of the yeast and *Caenorhabditis* genomes [67]: it is expected that the ABC transporter complement of an organism will include 'housekeeping' proteins necessary for cellular maintenance – these will have orthologues throughout eukaryotes, but it will also include proteins which have specialised roles in the host organism. This is the case for other gene families, for example: the complete *Saccharomyces* genome sequence reveals only three genes encoding cytochromes *P*-450, whereas 275 have been identified to date in *Arabidopsis* [200,201]; such discrepancies reflect the different metabolic activities of the two groups of organisms, in this case, the extensive ability of plants to synthesise secondary metabolites. The ABC transporter complement of plants may similarly play a special role in secondary metabolism.

In other cases, housekeeping functions common to eukaryotes may be achieved by different means in different organisms, depending on their lifestyles: ABC transporters which mediate detoxification are a good example of this. Since yeast is unicellular, it deals with toxic insults largely by exclusion, effluxing compounds into the extracellular medium, and therefore has a complex network of inducible plasma membrane ABC transporters. Multicellular animals can also effectively exclude toxins from their cells, since these can be excreted from the body. However, toxins are metabolised prior to transport and are effluxed by rather different ABC proteins. Plants, in contrast, have evolved to accommodate their sessile habit: they contain a similar complement of detoxification enzymes to that found in mammalian liver [94], but rather than being excreted, toxins are metabolised and sequestered intracellularly. Such differences cannot be deduced from sequence databases alone.

11.3. A final word

To a large extent, research on plant ABC transporters has been directed by analogy to mammalian and microbial systems. Whilst much has been learned from comparative studies, there are aspects of biology which are specific to plants, and cannot be addressed by comparison with other organisms. It may be that some of the most interesting ABC transporters are those which are specific to plants. It may also be the case that novel information relevant to all organisms emerges from plant ABC transport research.

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