The plant PDR family of ABC transporters

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Abstract The plant pleiotropic drug resistance (PDR) family of ATP-binding cassette (ABC) transporters has been implicated in the transport of antifungal agents. In this paper, we provide an analysis of the entire family of PDR genes present in the Arabidopsis thaliana (L.) Heynh. genome. This analysis both resolves discrepancies in published inventories of plant ABC proteins and provides an expression analysis of all the annotated Arabidopsis PDR genes. The results indicate that the Arabidopsis genome contains 15 genes encoding PDR proteins and that these genes show a spectrum of specific expression patterns, both at the organ level and in response to various hormonal, environmental and chemical factors. These data provide a scaffold for the future molecular genetic analysis of this important family of ABC transporters. In addition, we demonstrate the usefulness of such data by using them to identify an Arabidopsis PDR protein that may play a role in the extrusion of the antifungal diterpene sclareol. Electronic Supplementary Material is available if you access this article at http://dx.doi.org/10.1007/s00425-002-0889-z. On that page (frame on the left side), a link takes you directly to the supplementary material.

Keywords ABC transporter · Arabidopsis · Oryza · Pleiotropic drug resistance · Sclareol · Spirodela

Abbreviations ABA: abscisic acid · ABC: ATP-binding cassette · CHX: cycloheximide · 2,4-D: 2,4-dichlorophenoxyacetic acid · EST: expressed sequence tag · MATDB: MIPS Arabidopsis thaliana database · MDR: multidrug resistance · PDR: pleiotropic drug resistance · TMS: transmembrane-spanning

Introduction

The completion of the Arabidopsis genome sequencing project represents a milestone in plant biology (Arabidopsis Genome Initiative 2000) but brings with it the challenge of accurately identifying genes and characterising their function. This challenge is particularly great for those proteins encoded by relatively large gene families, since classical approaches of reverse genetics to identify mutant phenotype may be confounded by the potential for functional redundancy (Bouche and Bouchez 2001). These problems are compounded in gene families encoding relatively large proteins in which precise annotation of the genome sequence is required to ascertain and identify functional genes and the structure of the genomic loci encoding these proteins. The ABC superfamily of transporters represents a significant challenge in this area.

ATP-binding cassette (ABC) transporters have been implicated in the active movement of a wide variety of substrates across cellular membranes (Higgins 1992). The proteins are characterised by the possession of one or two cytosolically orientated nucleotide-binding folds (NBFs) or ATP-binding cassettes (ABCs) linked to multiple (usually six) hydrophobic transmembrane-spanning (TMS) domains. The ABC domains are highly conserved and contain an ATP-binding site consisting of a Walker A box and a Walker B box separated by approximately 120 amino acids (Walker et al. 1982) and, between the two boxes, a consensus sequence specific for ABC transporters known as the ABC signature (Bairoch 1992). In eukaryotes these molecular characters are arranged in a modular fashion within the ABC transporter...
protein. Although a number of ABC transporters consist only of a single TMS–ABC or ABC–TMS module, larger proteins exist which contain repeats of these modules, i.e. (TMS–ABC)_2 and (ABC–TMS)_2. These proteins have been designated ‘full-size’ ABC transporters, whereas those containing single modules have been defined as ‘half-size’ (Higgins 1992). The family of full-length ABC transporters can be subdivided into four major subfamilies – MDR (multidrug resistance; Gottesman and Pastan 1993), MRP (MDR-associated protein; Borst et al. 1999; Rea 1999), ABCA (Broccardo et al. 1999) and PDR (pleiotropic drug resistance). The PDR family is characterised by a configuration in which the ABC module is nearer the N-terminal end of the protein than the TMS domain (ABC–TMS). This is the reverse form of the configuration observed in MDR, ABCA and MRP proteins in which the TMS module is towards the N terminus of the protein (TMS–ABC).

The PDR family of transporters is distinguished not only by the specific modular configuration of its members, but also by their association with the transport of antifungal agents. This has been most fully characterised for the PDR genes in yeast (Decottignies and Goffeau 1997) and *Candida albicans* (Del Sorbo et al. 2000). Thus, in yeast the PDR transporters have been shown to export xenobiotics (Kolaczkowski et al. 1996) and expression of the PDR genes is associated with antifungal drug resistance (Kolaczkowski et al. 1998; Bauer et al. 1999; Wolger et al. 2001). In plant pathogenic fungi, members of this transporter group play a role in resistance to antifungals (Nakaune et al. 1998; Schoonbeek et al. 2001; Vermeulen et al. 2001) or have been shown to be necessary for pathogenicity (Urban et al. 1999).

The first plant PDR ABC transporter to be identified was SpTUR2 from the water plant *Spirodela polyrrhiza* (Smart and Fleming 1996). The expression of the gene at the transcript level was subject to a complex hormonal and environmental regulation. In particular, although transcripts were present at low levels in control plants, addition of the hormone abscisic acid (ABA) led to the rapid and high accumulation of *SpTUR2* mRNA. In addition to ABA, a number of factors associated with plant stress led to induction of *SpTUR2* gene expression (such as high salt and cold), leading to the proposal that SpTUR2 might play a role in a general plant response to conditions inhibiting plant growth. Data have accumulated indicating that plant PDR proteins may also play a role in the excretion of antifungal agents. Thus, recent work on an SpTUR2-related PDR protein from *Nicotiana plumbaginifolia* (NpABC1) showed that induction of the gene in cell cultures by the antifungal diterpenes sclareol and sclareolide was correlated with a decreased accumulation of a synthetic sclareolide analogue, suggesting that the protein might function to transport this substrate (Jasinski et al. 2001). Moreover, the expression of the SpTUR2 transporter in *Arabidopsis* leads to the acquisition of resistance to sclareol (van den Brule et al. 2002). Taken together, these data suggest that proteins encoded by PDR genes in plants may play a key role in plant interactions with fungi.

As a result of the sequencing of the *Arabidopsis* genome, a number of PDR genes have been identified. However, there is some discrepancy in the annotation of the AtPDR gene family between the published reports, including differences in the expected number of functional AtPDR genes (Davies and Coleman 2000; Sánchez-Fernández et al. 2001; Martinioa et al. 2002). In addition, expression data on these gene members has been limited to those available from expressed sequence tag (EST) data, which provide only a rough estimate of the conditions under which the relevant genes are expressed. Such expression data are especially important for the analysis of the AtPDR gene family. Firstly, due to the size of the gene family there is a high potential for functional redundancy that complicates phenotypic analysis using reverse genetics (Bouché and Bouchez 2001). Expression data can provide an important clue as to when and where any phenotype for a given gene mutant can be expected. Secondly, a number of ABC transporters have been shown to show elevation of transcript level in response to chemicals that are themselves substrates for the transporter (Hirata et al. 1994; Miyahara et al. 1996; Del Sorbo et al. 1997; Piper et al. 1998). Transcript profiles can, thus, also provide a clue as to the biochemical function of a transporter.

Inventories of the entire *A. thaliana* ABC protein superfamily (Davies and Coleman 2000; Sánchez-Fernández et al. 2001) and of the full-size *Arabidopsis* transporters (Theodoulou 2000; Martinioa et al. 2002) have been compiled recently. In this paper, we provide an inventory of all the plant PDR proteins so far characterised and a detailed analysis of the annotated PDR genes in *Arabidopsis*. This analysis resolves the discrepancies in the previously published reports on this gene family in *Arabidopsis* (Sánchez-Fernández et al. 2001; Martinioa et al. 2002). Secondly, we provide an analysis of the expression pattern of all the annotated PDR genes in *Arabidopsis* at the organ level and in response to various environmental, hormonal and chemical factors. These data help delimit the potential function of the individual PDR genes and provide the basis for a future molecular genetic analysis to characterise the function of this important family of transporters in plants. Finally, using data from *Nicotiana plumbaginifolia* and *Spirodela polyrrhiza* identifying PDR proteins involved in transport of the antifungal agent sclareol, we identify an *Arabidopsis* PDR transporter that might perform a similar function.

**Materials and methods**

**Plant growth conditions**

*Arabidopsis thaliana* (L.) Heynh. plants (Col-2 seeds obtained from the Nottingham *Arabidopsis* Stock Centre, http://nasc.nott.ac.uk) were either grown in soil under long-day conditions of 16 h light at 21 °C and 8 h darkness at 17 °C or aseptically in liquid culture on