FEBS Letters 581 (2007) 2255–2262

Minireview

The roles of organic anion permeases in aluminium resistance and mineral nutrition

Emmanuel Delhaize*, Benjamin D. Gruber, Peter R. Ryan

CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia

Received 13 February 2007; revised 14 March 2007; accepted 16 March 2007

Available online 30 March 2007

Edited by Julian Schroeder and Ulf-Ingo Flügge

Abstract **Soluble aluminium (Al^{3+}) is the major constraint to plant growth on acid soils. Plants have evolved mechanisms to tolerate Al^{3+} and one type of mechanism relies on the efflux of organic anions that protect roots by chelating the Al^{3+}. Al^{3+} resistance genes of several species have now been isolated and found to encode membrane proteins that facilitate organic anion efflux from roots. These proteins belong to the Al^{3+}-activated malate transporter (ALMT) and multi-drug and toxin extrusion (MATE) families. We review the roles of these proteins in Al^{3+} resistance as well as their roles in other aspects of mineral nutrition.**

Keywords: Aluminium tolerance; ALMT; MATE; Malate; Citrate; Transport

1. Introduction

Acid soils are a world-wide problem for agriculture with the main constraint to plant growth being the aluminium (Al) that is solubilised by the acidity into the toxic cation Al^{3+}. The problems of acid soils and the physiology of the mechanisms that allow plants to grow on these soils have been covered in detailed reviews [1–4]. We give a brief overview of the earlier literature described in these reviews and focus on the recent identification and characterisation of organic anion permeases; their genes and the roles they play in conferring Al^{3+} resistance to plants. Although the terms ''resistant'' and ''tolerant'' are often used interchangeably in the literature when referring to mechanisms in abiotic stresses, here we use ''resistance'' when referring to a mechanism that excludes Al^{3+} from entry into the plant whereas ''tolerance'' refers to a mechanism that detoxifies or sequesters Al^{3+} internally. A detailed discussion of Al^{3+} resistance or tolerance mechanisms that do not rely on the efflux of organic anions is beyond the scope of this review, however we do include a discussion of the emerging roles of transport proteins involved in mechanisms of Al^{3+} tolerance. Al^{3+} inhibits root growth by disrupting the root apex, the part of the plant most susceptible to Al^{3+} toxicity and this can occur within minutes of exposure to toxic concentrations. Genotypes of many plants vary in their ability to withstand Al^{3+} toxicity and one mechanism that increases the resistance of some species involves the efflux of one or more organic anions from the root apex. These organic anions are thought to chelate Al^{3+} either in the immediate vicinity or even within the apoplasm of the root apex [5]. Many plants increase the efflux of organic anions from roots when exposed to Al^{3+} and the most convincing evidence that these organic anions confer resistance comes from genotypes within a species that show contrasting levels of resistance. Detailed studies of the processes involved in organic anion efflux have uncovered a central role for transport proteins. The field has progressed more recently from an understanding of the mechanisms involved in Al^{3+} resistance to the cloning of the genes responsible. Here we describe Al^{3+} resistance genes from plants that encode proteins belonging to the ALMT and MATE families of membrane proteins. Although members of these protein families differ from one another in sequence and structure they confer Al^{3+} resistance in a similar fashion: by facilitating organic anion efflux from roots. In addition, we review recent evidence that indicates a role for these proteins in other aspects of mineral nutrition.

2. The ALMT organic anion permeases

2.1. The Al^{3+} resistance gene of wheat *TaALMT1*

The *TaALMT1* (for Al^{3+}-activated malate transporter) gene of wheat (*Triticum aestivum*) was the first Al^{3+} resistance gene to be cloned from any plant species [6]. Evidence that *TaALMT1* is the major gene for Al^{3+} resistance in wheat includes the findings that it co-segregates with resistance, that its expression in several heterologous systems confers an Al^{3+}-activated efflux of malate and when expressed in tobacco (*Nicotiana tabacum*) suspension cells and intact barley (*Hordeum vulgare*) plants, increases their Al^{3+} resistance [6,7]. Furthermore, *TaALMT1* is located within a single major quantitative trait locus (QTL) for Al^{3+} resistance identified in several different doubled-haploid populations of wheat [8,9]. These observations coupled with previous findings that Al^{3+}-activated efflux of malate is a general mechanism controlling Al^{3+} resistance in wheat [10] suggests that *TaALMT1* is a major and widespread gene for Al^{3+} resistance in this species. The *TaALMT1* gene encodes a hydrophobic protein with five to seven predicted membrane spanning domains consistent with its proposed role as a transport protein that facilitates malate efflux.

Transgenic barley plants expressing *TaALMT1* display many of the phenotypes typical of Al^{3+}-resistant wheat. Al^{3+}

*Corresponding author. Fax: +61 2 6246 5270.
E-mail addresses: Manny.Delhaize@csiro.au (E. Delhaize), Ben.Gruber@csiro.au (B.D. Gruber), Peter.Ryan@csiro.au (P.R. Ryan).

0014-5793/$32.00
doi:10.1016/j.febslet.2007.03.057

activates malate efflux from the roots of both which confers comparable levels of resistance [7,11]. Apparently the presence of a transport mechanism in the plasma membrane is the only factor that limits malate efflux from barley with the existing biosynthetic machinery being sufficient to replenish malate pools within the apical root cells. The kinetic properties of malate efflux in Al^{3+}-resistant wheat and transgenic barley are comparable and both show a remarkably similar activation by erbium, a rare-earth element [7,10,12]. Direct evidence that TaALMT1 is localised to the plasma membrane was obtained by transiently expressing TaALMT1 fused to green fluorescent protein in tobacco and onion (*Allium cepa*) and by immunodetection of TaALMT1 in membrane fractions derived from wheat and stably transformed tobacco cells [13].

The two alleles of the *TaALMT1* coding region that have been identified differ by six nucleotides with only two of these differences encoding for different amino acids in the predicted proteins [6]. The alleles occur in both Al^{3+}-resistant and Al^{3+}-sensitive wheat genotypes indicating that the different amino acids do not confer differences in resistance. Instead, the level of expression of either allele appears to be the major determinant of Al^{3+} resistance in wheat [8]. *TaALMT1* is expressed constitutively in root apices and the level of expression in different genotypes correlates positively with Al^{3+} resistance [14]. Interestingly, this correlation is much weaker among some Japanese genotypes examined which are more sensitive to Al^{3+} than expected from their levels of *TaALMT1* expression. Nevertheless the strong relationship between Al^{3+} resistance and malate efflux is maintained in these Japanese genotypes indicating that malate efflux is still the major mechanism of resistance in wheat and that additional factors are required for post-transcriptional regulation of TaALMT1 function.

To investigate how *TaALMT1* expression is regulated in wheat, sequences upstream and downstream of the *TaALMT1* coding region, as well as the introns, were compared in Al^{3+}-resistant and sensitive genotypes [14]. Polymorphisms in the introns and downstream sequences do not correlate with Al^{3+} resistance. By contrast, the promoter region upstream of *TaALMT1* is highly polymorphic between genotypes. One class of polymorphism consists of duplicated or triplicated blocks of sequence 97, 108 or 172 bp long (Fig. 1). A general relationship exists between the number of repeats and the level of *TaALMT1* expression and Al^{3+} resistance. Two different types of duplications and triplications exemplified by the type V and type VI sequences are related in that they both perfectly repeat a B block but differ in that they each repeat an additional, but different block [14] (Fig. 1). It is not known how these repeated sequences are derived but the tandem repeats occurring in a mutant form of the *Mlo* locus of barley are thought to result from the chance replication of genomic DNA by "rolling circle" machinery used by some viruses and transposons [15]. Whatever the mechanism, the relationship between the type V and VI sequences can be simply explained if the tandem repeats are generated from the progenitor type I promoter from opposite directions along the genome with an overlapping sequence that in this case defines the B block (Fig. 1). This model assumes that the two promoter types arose independently whereas it is conceivable that promoter types that differ only in the number of tandem repeats are derived from one another.

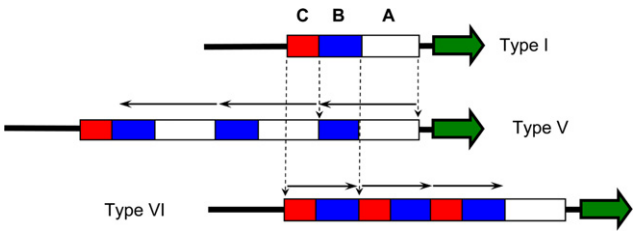

Fig. 1. The relationships and derivations of *TaALMT1* promoter variants. The type I promoter, primarily associated with Al-sensitive wheat genotypes, is the putative progenitor of the type V and VI promoters whereby different tandem repeats within the type I promoter gave rise to the Al^{3+}-resistant variants. The A, B and C blocks of sequence can result if the tandem repeats are generated in opposite directions as shown by the solid black arrows. The vertical dashed arrows show the different tandem repeats in relation to the three promoter types. A consequence of this mechanism is that the B block is perfectly repeated in the promoter types V and VI. The green arrow denotes the start of the first exon.

Other polymorphisms upstream of *TaALMT1* include a number of single nucleotide polymorphisms, a small repeated region of 31 bp and a relatively large repeated region of 803 bp in the genotype Chinese Spring. The type V sequence can function as a promoter [14] but, to date, evidence is lacking to show that the polymorphisms in the promoter of *TaALMT1* are responsible for the different levels of expression.

2.2. *TaALMT1-like genes of other species*

TaALMT1-like proteins are also implicated in the Al^{3+} resistance mechanisms of other plant species. Differences in Al^{3+} resistance between the two *Arabidopsis thaliana* accessions Columbia (Al^{3+}-resistant) and Landsberg (Al^{3+}-sensitive) can be attributed to differences in Al^{3+}-activated malate efflux and several QTLs contribute to the phenotype [16]. A gene (*AtALMT1*; At1g08430) encoding a TaALMT1-like protein is located within an Al^{3+} resistance QTL located on chromosome 1 [17]. Although AtALMT1 is only 44% identical to TaALMT1 it appears to function in a similar way. Al^{3+} not only activates AtALMT1 to trigger malate efflux but is also required to induce *AtALMT1* expression ([17,18]; Fig. 2). A mutant with a disrupted *AtALMT1* gene in the Columbia ecotype has lost the capacity for Al^{3+}-activated malate efflux and is Al^{3+}-sensitive. Despite the direct link between AtALMT1 function and Al^{3+} resistance established by mutant analysis, fine-scale mapping within the QTL on chromosome 1 showed that *AtALMT1* is not the gene responsible for the differences in Al^{3+} resistance between the two *Arabidopsis* accessions [17]. The authors of this study concluded that although AtALMT1 is an essential component of the Al^{3+} resistance mechanism, another factor that interacts with AtALMT1 is required for its activation. The gene encoding this factor would define the Al^{3+} resistance QTL but has not yet been identified and might encode a protein involved in transducing a signal to AtALMT1.

Two cDNAs (*BnALMT1* and *BnALMT2*) encoding proteins with 80% amino acid sequence identity to AtALMT1 were recently cloned from *Brassica napus* [19]. Similar to *AtALMT1*, Al^{3+} is required to both induce expression of the gene and to activate malate efflux (Fig. 2). When expressed in tobacco suspension cells both *BnALMT*s confer an Al^{3+}-activated efflux of malate and increase Al^{3+} resistance. Although these observations suggest a role for these genes in Al^{3+} resistance, direct links between *BnALMT* expression, Al^{3+}-activated malate efflux and Al^{3+} resistance remain to be established in *B. napus* plants.

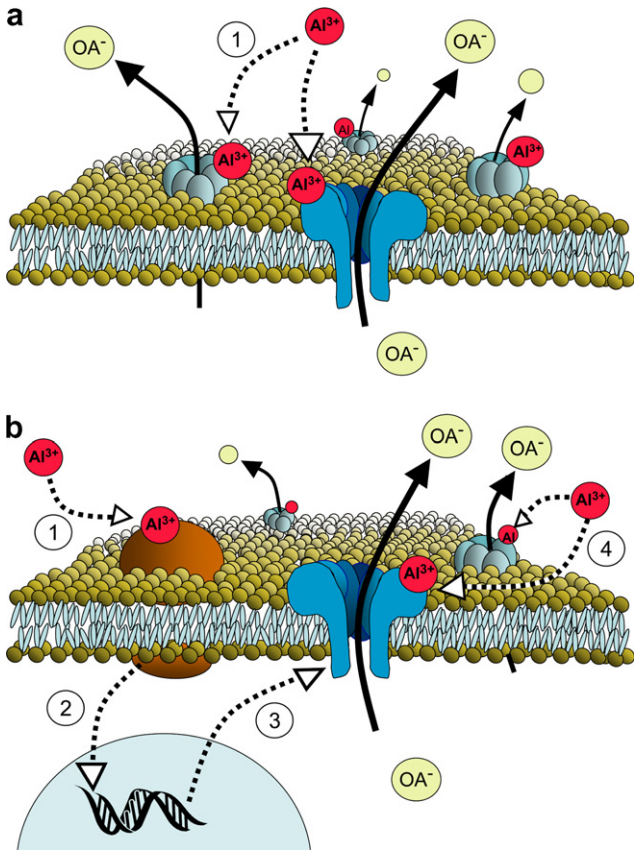

Fig. 2. Hypothetical models for the Al^{3+}-activated efflux of organic anions by members of the ALMT and MATE families of proteins. (a) Pattern I: The protein is expressed constitutively in root apices with Al^{3+}-resistant genotypes showing greater expression than Al^{3+}-sensitive genotypes. Al^{3+} (red circles) activates organic anion (OA^-) efflux by interacting directly with the pre-existing proteins in the plasma membrane (blue proteins; arrow 1). This model explains the Al^{3+}-activated malate efflux via TaALMT1 in wheat. (b) Pattern II: Al^{3+} first induces the expression of the proteins through a signal transduction pathway possibly involving a specific receptor (arrows 1, 2 and 3) or non-specific stress responses. Al^{3+} then activates organic anion efflux by interacting with the newly synthesised proteins in the plasma membrane (arrow 4). This model explains the Al^{3+}-activated malate efflux via AtALMT1 in *Arabidopsis* and via BnALMT1 in *B. napus* as well as Al^{3+}-activated citrate efflux via Alt_{SB} in sorghum.

Recently Fontecha et al. [20] isolated alleles of a gene (*ScALMT1*) from Al^{3+}-resistant and sensitive rye (*Secale cereale*) cultivars that encode proteins with 86% identity to TaALMT1. The Al^{3+}-resistant allele cosegregates with the *Alt4* Al^{3+} resistance locus in three different F_2 populations. Al^{3+} induces the expression of *ScALMT1* and expression is induced to a greater level in root apices of an Al^{3+}-resistant cultivar compared to a sensitive cultivar. These observations provide circumstantial evidence that ScALMT1 plays a role in the Al^{3+} resistance mechanism encoded by *Alt4*. However, direct evidence to show that ScALMT1 transports malate is lacking as is evidence that the gene is able to confer Al^{3+} resistance by genetic transformation of a sensitive species.

2.3. ALMT function

Detailed biochemical data has only been collected from four members of the ALMT family: TaALMT1 of wheat, AtALMT1 of *Arabidopsis* and the two BnALMT proteins of *B. napus*. We can now compare the properties of these proteins because all four have been expressed in *Xenopus laevis* oocytes and three of the four have been expressed in tobacco suspension cells [6,17,19]. Oocytes expressing each of these genes show an Al^{3+}-dependent increase in inward current. The currents generated by TaALMT1 and BnALMT proteins occur when oocytes are pre-injected with several millimolar of malate but are absent when the oocytes are pre-injected with either water or citrate. This demonstrates some specificity of the wheat and *B. napus* proteins for malate over citrate and indicates that in the absence of supplementation the endogenous malate concentration in oocytes is too low to generate large currents. Smaller currents were also detected in oocytes expressing AtALMT1 in the absence of Al^{3+} [17] which contrasts with the strict requirement for Al^{3+} to activate malate efflux in wild type plants. Oocytes expressing the *BnALMT* genes generate larger currents than oocytes expressing either *TaALMT1* or *AtALMT1* but this may reflect experimental differences (e.g. amount of cRNA injected) rather than protein function because when expressed in tobacco suspension cells, the wheat and *B. napus* genes generate similar phenotypes [6,19]. For example, Al^{3+}-activated malate efflux in tobacco cells for both genes is about 0.5 μmole/g fresh weight/hour.

Several questions concerning the function of these ALMT proteins remain unanswered: are they permeases that facilitate malate transport across membranes or do they encode receptors that control this process? If they are permeases, what type do they encode, and how are they activated by Al^{3+}? None of these questions can be resolved with certainty until the proteins are examined in an artificial lipid bilayer system. Nevertheless we can start to make some educated guesses. For instance, if *TaALMT1* encodes a receptor for Al^{3+}, and not a permease, it follows that all species that show enhanced Al^{3+}-activated malate efflux when transformed with *TaALMT1* including barley roots, tobacco suspension-cells, rice roots, and *Xenopus* oocytes must also possess endogenous membrane proteins that facilitate malate efflux. In addition, these endogenous proteins need to be able to respond to a signal triggered by the interaction between Al^{3+} and the TaALMT1 receptor protein. Therefore, it is more likely that *TaALMT1* encodes a malate permease, and most likely a ligand-gated ion channel. Ion channels facilitate the transfer of ions across membranes down their electrochemical gradient. They are usually comprised of multiple protein subunits arranged to form a selective pore which can exist in open or closed states. Ligand-gated channels are ion channels that are opened or activated (gated) after binding to a specific signalling molecule or ligand such as an inorganic ion (in this case Al^{3+}) or organic molecules. In either case, the ligand is not the substrate of the channel.

The hypothesis that TaALMT is a ligand-gated anion channel in wheat is supported by physiological studies that compare malate efflux from wheat roots with the Al^{3+}-activated anion currents (ALAAC) measured in root protoplasts [21]. These Al^{3+}-activated inward currents behave similarly to the malate efflux measured from intact roots as well as excised root apices [11,22]. Both are activated by Al^{3+} but not La^{3+}, both occur in cells from the root apex but not from mature root cells and both are sensitive to the same range of channel antagonists. Furthermore, when Cl^- is the main permeable anion in the pipette solution, single-channels events have been detected in intact wheat protoplasts and outside-out patches excised from protoplasts pre-exposed to Al^{3+} [21]. The conductance

of these channels is 66 pS at −50 mV (~100 mM $[Cl^-]_{pipette}$, ~20 mM $[Cl^-]_{external}$). Subsequent whole-cell studies described large inward currents that are carried by malate [22]. Assuming the whole-cell currents are mediated by channels then the permeability for malate relative to Cl^- (P_{mal}/P_{Cl-}) is 7.8 in wheat protoplasts (corrected from Zhang et al. [22]). Measurments of tobacco-suspension cells expressing *TaALMT* indicates that P_{mal}/P_{Cl-} is 38 (W.-H. Zhang, personal communication) which make this the most malate-permeable plant channel described to date. Surprisingly, single-channel events have not been detected when malate is the major permeable anion present in wheat protoplasts [22] or in tobacco-suspension cells expressing TaALMT1 (W.-H. Zhang, personal communication).

Exactly how Al^{3+} activates this protein remains unknown. Models for ligand-gated channels often propose that the proteins change their conformation when bound by the ligand which increases their probability of existing in the open state thus facilitating ion movement. The four ALMT proteins examined in detail are hydrophilic over their *C*-terminal half (Fig. 3) and it is tempting to speculate that Al^{3+} interacts with amino acid residues in that region. Indeed many residues are conserved in the *C*-terminal half of the TaALMT1, AtALMT1 and *B. napus* proteins and these provide a starting point for structure–function analyses using site-directed mutagenesis (Fig. 3). However, it is also uncertain whether Al^{3+} interacts directly with these ALMT proteins or whether intermediates are involved. ALAACs have not been observed in excised patches of wheat apical root membrane [21,22] which could indicate that an intermediate is necessary or that some post-translational protein modification is required [23]. Alternatively it is possible that the density of the channel protein is low or that the function of the channel is physically restricted in the excised patches.

Al^{3+}-activated release of organic anions from plant roots has also been reported in a range of other plant species [1]. The proteins controlling these responses have not yet been identified and so it is unknown whether they are encoded by *TaALMT1*-like genes. However, two groups have described ALAACs in root cells of maize (*Zea mays*) [24,25]. The currents described in these studies have a 7-fold disparity in their single-channel conductances (~100 mM $[Cl^-]_{pipette}$, ~10 mM $[Cl^-]_{external}$) highlighting the possibility that a range of different Al^{3+}-activated channels occur in maize. Al^{3+} was able to trigger single-channel currents in excised membrane patches [25] indicating that a direct interaction between Al^{3+} and the protein is sufficient to activate the channels. Another study showed that one ALAAC found to be restricted to cells from a specific region of the root apex is permeable to citrate and malate, albeit with an approximately 5-fold lower permeability than Cl^- [24]. Pellet et al. [26] first proposed that the Al^{3+}-activated efflux of citrate is the Al^{3+} resistance mechanism in maize. However, it is now clear that the level of citrate efflux is poorly correlated with the level of Al^{3+} resistance among a wide range of cultivars [27] which indicates that citrate efflux is not the main Al^{3+} resistance mechanism operating in maize.

3. The ALMT family

The ALMT proteins implicated in mechanisms of Al^{3+} resistance belong to a larger group of related proteins that share an uncharacterised protein family five domain (UPF0005; pfam01027; http://www.sanger.ac.uk/Software/Pfam/). The 467 proteins identified by Pfam with the UPF0005 domain are found in the archaea, viruses, prokaryotes and eukaryotes. Of these proteins, 64 are found in plants and most of these are between 140 and 670 residues long and contain four to seven transmembrane regions although one subgroup consists of plant proteins that are 89–309 residues long and are predicted to be soluble or to posses only one or two transmembrane domains (SOSUI; http://bp.nuap.nagoya-u.ac.jp/sosui/). Functions and names assigned to various members of the UPF0005 group of proteins include ALMT, Bax-inhibitor1, putative glutamate/aspartate receptor peptide, putative receptor-associated protein, and putative *z*-protein.

Here we define ALMT proteins as those that contain the UPF0005 domain, possess five to seven transmembrane

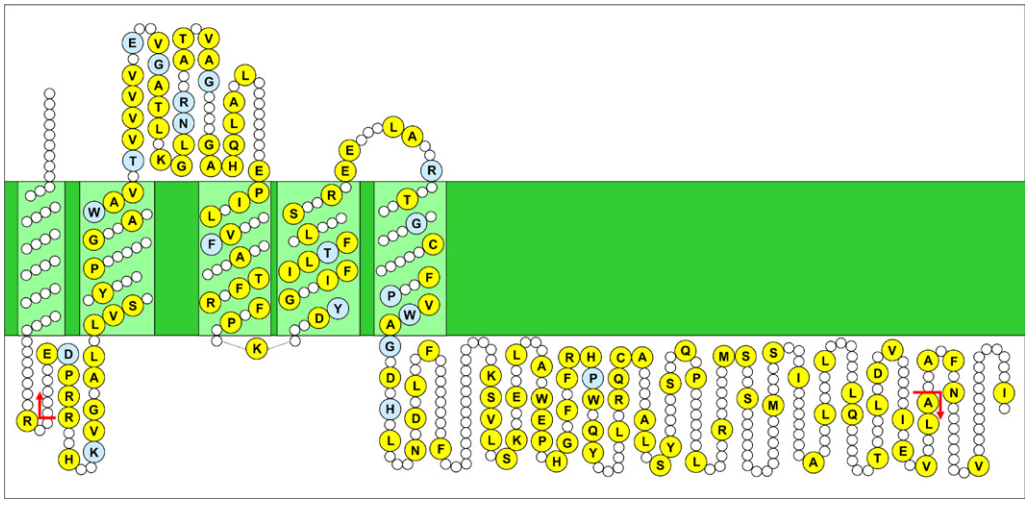

Fig. 3. Secondary structure of ALMT proteins. Most ALMT proteins are predicted to have 5–7 membrane spanning regions in the amino terminal half of the protein. The membrane topology of TaALMT1 (above) is predicted to contain five membrane spanning regions (SOSUI: http://bp.nuap.nagoya-u.ac.jp/sosui/). Yellow circles denote the residues conserved between the four proteins (TaALMT1, AtALMT1, BnALMT1 and BnALMT2) shown to confer Al^{3+}-activated malate efflux in heterologous systems. Blue circles denote residues conserved in all members of the ALMT family (Fig. 4). Red arrows indicate the start and end of the UPF0005 domain.

domains and do not belong to groups that contain Bax-inhibitor 1, putative glutamate/aspartate or receptor-associated proteins. According to this definition the ALMT family consists entirely of plant proteins. The protein encoded by *AtALMT11* (*At4g17585*) of *Arabidopsis* was originally included as a member of the ALMT family [17] but is excluded here because it is predicted to be soluble. A phylogenetic tree of all known full length ALMT members is shown in Fig. 4 and all of these proteins share 19 fully conserved amino acids (Fig. 3). There are also numerous expressed sequence tags (ESTs) and partial cDNAs derived from many plant species that are predicted to encode proteins of the ALMT family. The transmembrane domains of ALMT proteins are usually located in the *N*-terminal half of the protein with a hydrophilic *C*-terminal region forming a "tail", although this can vary according to the prediction program used. The conserved amino acids are mostly located in the *N*-terminal region within or near the membrane spanning regions with relatively few conserved amino acid located in the hydrophilic *C*-terminal region (Fig. 3).

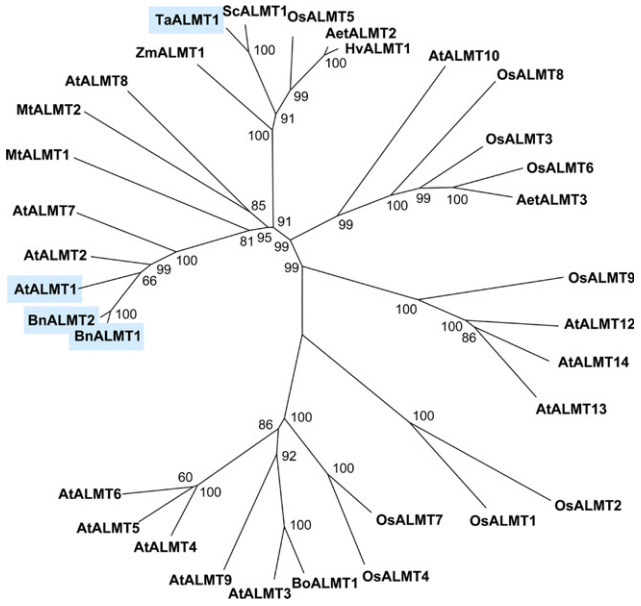

Fig. 4. Unrooted phylogenetic tree of the ALMT family. The highlighted proteins are those that have been functionally characterised as Al^{3+}-activated malate transporters. An alignment of known full-length proteins was performed using ClustalW as part of the MEGA3.1 software package [45]. The phylogenetic tree was constructed using the neighbour-joining method, and the bootstrap test of phylogeny performed using 10,000 replicates. ALMT proteins of rice and *Medicago truncatula* are assigned ascending numbers according to the relative positions of the corresponding genes starting from the proximal end of chromosome 1 through to the distal end of chromosome 12 for rice and chromosome 8 for *M. truncatula*. The *Arabidopsis* proteins are named according to Hoekenga et al. [17]. Proteins from other species are named according to their Genbank accessions except for an ALMT protein from *B. oleracea* which is named BoALMT1. Species prefixes and Genbank accession numbers: Aet, *Aegilops taushii* (AetALMT2: EF424085, AetALMT3: EF424086); At, *A. thaliana* (AtALMT1: AAF22890, AtALMT2: NP_172320, AtALMT3: AAF25997, AtALMT4: NP_173919, AtALMT5: NP_564935, AtALMT6: NP_179338, AtALMT7: NP_180292, AtALMT8: NP_187774, AtALMT9: NP_188473, AtALMT10: CAB80900, AtALMT12: NP_193531, AtALMT13: AAX55201, AtALMT14: NP_199473); Bn, *B. napus* (BnALMT1: BAE97280, BnALMT2: BAE97281); Bo, *B. oleracea* (BoALMT1: AAW81734); Hv, *H. vulgare* (HvALMT1: EF424084); Mt, *M. truncatula* (MtALMT1: ABD32183, MtALMT2: ABD32184); Os, *O. sativa* (OsALMT1: NP_001042433, OsALMT2: BAD87020, OsALMT3: BAD29455, OsALMT4: BAD16367, OsALMT5: CAE01530, OsALMT6: CAE02072, OsALMT7: BAD54395, OsALMT8: BAD32958, OsALMT9: AAL86482); Sc, *S. cereale* (ScALMT1: ABA62397); Ta, *T. aestivum* (TaALMT1: BAD10882); Zm, *Z. mays* (ZmALMT1: ABC86748). For ScALMT1 the ScALMT1-1 allele is shown (differs from the ScALMT1-2 allele at 6 amino acids) and for TaALMT1 the TaALMT1-1 allele is shown (differs from the TaALMT1-2 allele at 2 amino acids). AetALMT1 (AAZ22853) is not shown as it is identical to TaALMT1-1.

Within the ALMT family there are a number of sub-families. For example, TaALMT1 and other monocot members separate from other groups in the phylogenetic tree such as those containing the AtALMTs from *Arabidopsis* (Fig. 4). The large diversity within the ALMT family suggests functional roles that extend beyond Al^{3+} resistance. For example, the majority of the *ALMT* genes of *Arabidopsis* have not been associated with Al^{3+} resistance QTLs suggesting they have other roles. However, only one population has been investigated to date and it is possible that a wider examination of ecotypes will identify further *ALMT* genes that are involved in Al^{3+} resistance. In addition, the observation that many of the ESTs encoding ALMT-like proteins submitted to the databases are derived from tissues other than roots also suggests that the roles of the ALMT family are not restricted to Al^{3+} resistance. Since the ALMT proteins are predicted to be membrane-bound we speculate that some of them will function as transport proteins in processes other than Al^{3+} resistance, possibly using organic anions as substrates.

4. *MATE* genes encoding organic anion transport proteins

The MATE proteins comprise a large family widespread across all domains of life with 58 members encoded by *Arabidopsis* genes alone [28]. The proteins were first characterised for their capacity to confer drug resistance to microbes by an efflux mechanism. On the basis of this function the proteins were named the multi-drug and toxin extrusion (or efflux) family and given the acronym MATE. While the vast majority of MATEs remain uncharacterised some members of the family were shown to function as drug/cation antiporters that remove toxic compounds and secondary metabolites from the cytosol by exporting them out of the cell or sequestering them to the vacuole. The recent characterisation of two additional *MATE* genes, *Frd3* and Alt_{SB}, has established that citrate is also a substrate of some members of the family as discussed below.

The *frd3* mutant (originally named *man1*) of *Arabidopsis* was first identified by its metal accumulating phenotype and constitutively high Fe-reductase activity in roots [29]. Subsequently Rogers and Guerinot [30] showed that *frd3* is perturbed in Fe homeostasis because it expresses Fe-starvation genes constitutively and, under some conditions, also accumulates excess Fe in shoots. The constitutive expression of *IRT1*, a transporter of Fe and other metals whose expression is normally down regulated with adequate Fe nutrition, provides an explanation for the metal-accumulator phenotype of the *frd3* mutant. The *FRD3* gene isolated by map-based cloning encodes a MATE [30]. A role for the FRD3 protein in the long distance transport of Fe in the xylem was inferred from elegant experiments involving reciprocal grafts between roots and shoots of wild type and mutant plants [31]. It became apparent that instead of transporting Fe directly, the function of FRD3

is to load citrate into the xylem so that the Fe:citrate complex can move up the xylem and become available to shoot cells [32]. Indeed, the expression of FRD3 in the root pericycle and vascular tissue is consistent with this proposed role [31] as is the ability of FRD3 to transport citrate when expressed in *Xenopus* oocytes [32]. Furthermore, ectopic expression of *FRD3* confers constitutive citrate efflux from *Arabidopsis* roots which is associated with enhanced Al^{3+} resistance.

A role for MATE proteins in Al^{3+} resistance mechanisms involving organic anion efflux has recently come to light from studies on sorghum (*Sorghum bicolor*; Leon Kochian personal communication). High resolution mapping in sorghum identified a *MATE* gene (Alt_{SB}) as the most likely candidate encoding an Al^{3+} resistance gene. Subsequently Alt_{SB} was confirmed to be the Al^{3+} resistance gene because when expressed in *Arabidopsis* it confers both Al^{3+} resistance and an Al^{3+}-activated efflux of citrate. These findings are consistent with the physiology of Al^{3+} resistance in sorghum which relies on Al^{3+}-activated efflux of citrate. Alt_{SB} is expressed primarily in root apices, its expression is induced by Al^{3+} and it is more highly expressed in resistant genotypes than in sensitive genotypes. The different levels of Alt_{SB} expression are associated with a region in the Alt_{SB} promoter that contains a miniature inverted-repeat transposable element (MITE). This region is highly structured and repeated, with the number of repeats correlated with Alt_{SB} expression. Hence this region may act as an enhancer of gene expression.

A third study provides preliminary evidence that citrate efflux from the roots of Al^{3+}-resistant barley plants is also controlled by a *MATE* gene (H. Raman, personal communication). Although barley is relatively sensitive to Al^{3+} toxicity some variation in resistance is present among genotypes. Like sorghum, Al^{3+} resistance in barley is correlated with citrate efflux, a phenotype controlled by the *Alp* locus on chromosome 4H [33]. Recent work has exploited the synteny between the rice and barley genomes to identify a gene encoding a MATE within the *Alp* locus (H. Raman, personal communication). Expression of this *MATE* gene is greater in the root apices of an Al^{3+}-resistant genotype than a sensitive genotype and is correlated with Al^{3+}-activated citrate efflux and the resistance phenotype in a set of selected lines derived from a cross between these parental genotypes.

5. The *ALS* genes encoding ABC transporters

The ABC transporters constitute a large family with over 130 genes encoding these proteins in the *Arabidopsis* genome [34]. Of the plant ABC transporters that have been functionally characterised some detoxify organic and inorganic substances by sequestering these substances into vacuoles [34]. Two Al^{3+}-sensitive mutants of *Arabidopsis* (*als1* and *als3*) are mutated in genes that encode proteins of the ABC transporter family [35,36]. The functions and substrates of ALS1 and ALS3 are not known but from the mutant phenotypes, Larsen et al. [35,36] inferred that the proteins act to mobilise and sequester Al^{3+} within the plant to confer tolerance. ALS1 is located at the tonoplast and the gene is expressed primarily in root apices and the vascular system. By contrast, expression of *ALS3* is induced by Al^{3+} and the protein is primarily located at the plasma membrane of leaf hydathode cells, the phloem and the root cortex. In some plant species Al^{3+} can be either mobilised or stored when complexed by organic anions (see [3]) but to date the transport proteins that mediate these processes are unknown. An intriguing possibility is that ALS1 and ALS3 are involved in these processes and capable of transporting Al^{3+} chelated to small peptides [35] or organic anions such as oxalate and citrate.

6. Organic anion efflux during phosphorus (P) deficiency

Some species release organic anions from their roots in response to P deficiency [2] allowing the plants to exploit poorly-soluble forms of P. Citrate, in particular, increases the availability of P in the soil by replacing inorganic P from insoluble complexes or by displacing it from ligands on the surfaces of soil minerals [2]. Electrophysiological studies on white lupin (*Lupinus albus*) and *Arabidopsis* show that citrate efflux is likely to be mediated by anion channels (see [37]). Zhang et al. [38] described two citrate conductances capable of facilitating citrate efflux from the roots of white lupin. The first, an inwardly-rectifying anion conductance that is activated by membrane hyperpolarisation, is 26-fold more selective for citrate over Cl^-. The second outwardly-rectifying conductance is activated by membrane depolarisation and more permeable to Cl^- than to citrate ($P_{Cl^-}/P_{citrate} = 3.7$). These currents were detected in both P-starved and P-replete white lupin but their frequency of detection and current magnitude tended to be greater under P-deficiency. By contrast, a citrate-permeable channel identified in *Arabidopsis* roots was only observed in P-starved plants [39]. This channel is significantly more permeable to citrate than Cl^- ($P_{citrate}/P_{Cl^-} = 26$) and shows a distinctive voltage-dependence that is similar to the R-type anion channel from guard cells [37,40]. It is unknown whether members of the MATE or ALMT families are involved in the organic anion efflux from P-deficient plants.

7. Evolutionary relationships

The ability of specific *ALMT* and *MATE* genes to confer Al^{3+} resistance to various plant species represents a clear case of convergent evolution. That is, different genes co-opted to yield a similar phenotype of organic anion efflux to protect plants from Al^{3+} toxicity. This is not unexpected as it is likely that numerous solutions to the problem of Al^{3+} toxicity have evolved and organic anions with the capacity to bind Al^{3+} represent one ''obvious'' way of detoxifying this toxin. Malate and citrate are common carbon currencies in the biochemistry of living organisms and in many cases it appears that it is not their biosynthesis that is rate limiting for efflux but their transport across the plasma membrane [2]. Less clear is the relationship between the *ALMT1* genes of *Arabidopsis* and wheat which to date represent the only two *ALMT* genes with established roles in Al^{3+} resistance. Magalhaes [41] interpreted this similarity to represent conservation of gene function across a wide range of plant species with the implication that the *ALMT1* genes are derived from a common ancestor with a similar function in Al^{3+} resistance. Support for this idea comes from the observations that the chromosomal positions of Al^{3+} resistance genes appear to occur on similar regions in wheat, barley, rye and rice. For instance, in the case of rye the *Alt4* locus co-segregates with *ScALMT1* on a chromosomal region

related to chromosome 4D of wheat where *TaALMT1* is located [20]. An alternative interpretation for more divergent species is that Al^{3+} resistance based on *ALMT* or other genes arose independently in which case the location of resistance loci at related chromosomal regions in some species would be co-incidental. For example, rice does not possess an *ALMT* gene on chromosome 3 even though a QTL exists for Al^{3+} resistance in a region of this chromosome thought to be synteneous to chromosome 4 of wheat [42]. Furthermore, no QTLs for Al^{3+}resistance have been identified on chromosome 4 of rice which is the region where the closest homolog to *TaALMT1* is located suggesting that *ALMT1*-like genes are not responsible for the Al^{3+} resistance in this species. Additional work is required to establish whether *ALMT* genes are responsible for the Al^{3+} resistance at loci thought to be orthologous in other species and the example of barley might help clarify the situation. It is notable that the four ALMT proteins characterised to date specifically transport malate whereas the MATE proteins described here from sorghum and *Arabidopsis* transport citrate. An Al^{3+} resistance gene in barley maps to chromosome 4HL at an apparently orthologous locus to that of *TaALMT1* on chromosome 4DL in wheat [33,43]. The recent report of an *ALMT* sequence located on barley chromosome 4H appears consistent with conserved gene function between wheat and barley [20]. However, the mechanism encoded at 4HL in barley involves the Al^{3+}-activated efflux of citrate and not malate [33]. This observation implies that either an *ALMT* gene encodes the Al^{3+}-activated citrate transporter or that the chromosomal location is co-incidental such that the Al^{3+} resistance gene encodes a different protein, for example a MATE, which preferentially transports citrate. The mapping of a gene encoding a MATE to the *Alp* locus in barley (see above) is consistent with the second hypothesis. The *ALMT* sequence located to chromosome 4H in barley relied on PCR amplification of genomic DNA and remains to be verified by other techniques such as Southern blots.

8. Applications

The availability of genes encoding organic anion transport proteins provides opportunities for improving crop yields on acid soils. The potential of the *TaALMT1* gene to confer Al^{3+} resistance to plants has been demonstrated in short-term experiments [7]. The future challenges are to demonstrate yield benefits in greenhouse and ultimately field trials in a range of acid soils. The recent cloning of the sorghum Alt_{SB} gene provides another related strategy to improve Al^{3+} resistance by genetic modification. Since citrate forms a stronger complex with Al^{3+} than malate, expression of Alt_{SB} in transgenic plants might confer a higher level of Al^{3+} resistance than can be achieved by *TaALMT1*. In addition to their value as tools for the genetic modification of plants, the Al^{3+} resistance genes can be used as markers in breeding plants better suited to acid soils. For instance, regions of the coding, intron and promoter of *TaALMT1* have been used as markers for Al^{3+} resistance in wheat [6,14,44]. To date all of the organic anion transport proteins involved in Al^{3+} resistance require Al^{3+} for activation which limits their application for other purposes such as improving the P nutrition of plants. Since organic anions can improve the availability of P in the soil (see above) increasing the efflux of organic anions from roots by genetic modification could be a strategy for improving the P efficiency of plants. It would be desirable to control efflux of organic anions from roots under conditions where Al^{3+} is not present and the FRD3 protein provides a unique opportunity in this regard because it does not require Al^{3+} for its activation.

9. Concluding remarks

The last five years has seen rapid progress in our understanding of the molecular basis for Al^{3+} resistance mechanisms in plants that rely on the efflux of organic anions. The ALMT and Alt_{SB} proteins that belong to entirely different families are implicated in these resistance mechanisms. A feature that is common to the ALMT and MATE proteins involved in resistance is that Al^{3+} is required for their activation. Evidence suggests that TaALMT1 functions as a ligand-gated anion channel but the modes of transport for the other proteins are not known and need to be investigated. Other topics for future research include a study of protein structure and function to determine which residues define substrate specificity and to identify those critical for Al^{3+} activation. Further study of the gene sequences will provide clues as to the relationships between different species, how the genes have evolved and in defining the molecular bases for the differences between genotypes in gene expression and gene induction. The ALMT family of proteins, like the MATE family, are likely to have functions that extend beyond Al^{3+} resistance mechanisms and there is a need to define these functions. The roles of ABC transporters in tolerance mechanisms need to be explored further with a focus on determining the substrates for these proteins and establishing whether the genes are the underlying basis for natural variation in Al^{3+} tolerance. Finally, the availability of cloned Al^{3+} resistance and tolerance genes provides researchers with the tools to begin to deliver on the promises of developing plant-based solutions for acid soils through genetic engineering and the implementation of molecular markers to facilitate breeding.

Acknowledgements: We acknowledge the Grains Research and Development Corporation for funding (B.D.G) and thank colleagues for generously providing personal communications of their recent unpublished research.

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