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Intra- and extra-cellular excretion of carboxylates

Stefan Meyer\textsuperscript{1,8}, Alexis DeAngeli\textsuperscript{1}, Alisdair R. Fernie\textsuperscript{2} and Enrico Martinoia\textsuperscript{1}

\textsuperscript{1}Institute of Plant Biology, Zollikerstrasse 107, University of Zurich, CH-8008, Zurich, Switzerland

\textsuperscript{2}Max-Planck-Institut of Molecular Plant Physiology, Am Mühlenberg 1, 14476 Potsdam-Golm, Germany

\textsuperscript{8}Corresponding author: Meyer, S. (stmeyer@botinst.uzh.ch).

Abstract

Carboxylates such as malate and citrate are widely acknowledged to play a central role in plant metabolism. They are involved both in the production of energy and its storage as well as contributing to the cellular osmolyte pool and participating in regulating cellular pH. Recent research has demonstrated the immense functional importance of carboxylate excretion into the soil, apoplast and vacuole particular with respect to the regulation of stomatal and root function.

CARBOXYLATES-GENERAL CONSIDERATIONS
Plants produce a multitude of mono-, di- and tri-carboxylates. Most of them, such as citrate, malate or fumarate are implicated within several metabolic processes, whilst others such as oxalate represent end-products of metabolic pathways. This article will focus on the role of the excretion of carboxylates outside the cell into either the soil or the apoplast as well as their intracellular partitioning into the vacuole. For details readers interested in carboxylate metabolism should refer to several reviews published during the last years [1, 2, 3, 4, 5]. Notwithstanding their independent biosynthesis citrate, malate, fumarate and oxalate exhibit similar functions when excreted from the cytosol to the soil, apoplast or vacuole. This is due to the commonality of their carboxylic groups - which are negatively charged at neutral pH although to a lesser extent at acidic pH. It thus follows that their function may alter with respect to the pH of the solution into which they are excreted. That said, despite the fact that these compounds are mainly exported from the cytosol in the charged form their excretion is often accompanied by the release of protons- a process which leads to acidification of the soil, apoplast and vacuole, respectively.

PHYSIOLOGICAL FUNCTIONS OF CARBOXYLATES

Root exudation

Root exudates constitute a diversity of compounds derived from both primary and secondary metabolism including carbohydrates, carboxylates, phenolics and terpenoids [6, 7, 8, 9, 10, 11]. During the last years they have attracted considerable interest given their impact on a multitude of processes including communication between organisms, providing C-sources for microbial-soil communities and roles in plant nutrition and protection [12, 13, 14]. Citrate and malate are the major
carboxylates implicated in these processes, however some plants have been characterised to excrete large quantities of oxalate [15, 16].

About 80% of land plants harbour mycorrhizal associations [17]. In such symbioses the plant receives mineral nutrients, in particular Pi and N from the fungus, in exchange for the provision of photosynthate which is essential for the strictly biotrophic fungus. Plants which do not form mycorrhiza have had to develop alternative strategies to survive on phosphate deprived soils. Australian soils are particularly poor in phosphate but members of one of the largest plant families present on this continent, Proteaceae, very rarely form mycorrhizal associations [14, 18]. These plants developed a special root structure, proteoid or cluster roots, which are characterised by a short, very densely packed lateral root structure [13, 14]. On maturity, cluster roots excrete large amounts of citrate, which serves as an anion exchanger and catalyses the release of rock-bound phosphate. The cluster root structure not only increases the root surface area for adsorption, but also facilitates the production of very high local concentrations of carboxylates thus facilitating efficient extraction of phosphate from the soil. Other plants from diverse families which do not form mycorrhiza such as Fabaceae, Betulaceae, Casuarinaceae produce cluster roots in order to improve their phosphate status. Given its ease of cultivation the model plant most commonly used to study the function of cluster roots is white lupin. Interestingly, root tips of this species mainly excrete malate, while mature cluster roots, which are responsible for the burst of carboxylate release, mainly excrete citrate. Langlade et al (2002) observed a strong correlation between the activity of the ATP-dependent citrate lyase (ACL) and malate excretion indicating that this enzyme is responsible for the switch between malate and citrate excretion [19]. Shane et al. (2003) investigated whether the root or shoot phosphate content is
responsible for cluster root formation and citrate excretion using foliar Pi application and a split-root system [20]. They concluded that phosphate status of the shoot is more important than that of the roots. Carboxylate excretion is often, but not always, accompanied by soil acidification through plasma membrane localized proton pumps. It was recently observed that acidification and excretion follow a diurnal rhythm starting a few hours into the light phase [21]. Since excretion of citrate requires a large provision of C, it is likely that sugars must first accumulate in the root tissue prior to carboxylate production and release. Intriguingly, the expression pattern of transporters responsible for the uptake of NO$_3^-$, NH$_4^+$ and SO$_4^{2-}$ also follow similar diurnal cycles [22]. Carboxylate excretion is, however, not restricted to cluster roots but is rather a common strategy used by plants which do not form mycorrhiza to improve their phosphate status, although in terms of levels of local concentrations cluster roots outstrip other root types by far [14].

Excretion of carboxylates, mainly citrate, is additionally beneficial for iron nutrition and plants producing cluster roots also induce the production of this root type under low iron availability. However, the relationship between Fe$^{3+}$/Fe$^{2+}$, soil composition and soil pH is extremely complex - interested readers are referred to several excellent recent reviews for details [23, 24, 25]. Although aluminum is extremely toxic and a prominent element of the Earth's crust it generally does not pose a problem for plants since at neutral pH on alkaline soils, the solubility of aluminum is extremely low. However, about one third of arable soils are acidic and in these regions aluminum is present partially as Al$^{3+}$, which inhibits root growth and development, creating serious toxicity symptoms and yield losses [26]. One way plants deal with aluminum toxicity is via the excretion of carboxylates which form complexes with aluminum and thus reduce the free Al$^{3+}$ concentration [26]. Due to their lower pK
citrate and oxalate are more effective in complexing heavy metals, however, most plants mainly excrete citrate and malate, via independent processes, in response to Al\(^{3+}\) [27]. That said some plants, such as poplar predominantly excrete oxalate instead of malate when exposed to aluminum or other heavy metals [28], and a recent study comparing different rice cultivars for lead tolerance revealed that this trait correlated with the amount of oxalate excreted [29]. The response of roots to Al\(^{3+}\) is very rapid and considerable amounts of malate are excreted during this process. This observation suggests that the malate and citrate pools of the vacuole can be readily mobilized when the plant is exposed to aluminum, in order to release sufficient amounts of carboxylates to complex Al\(^{3+}\) ions.

Malate and citrate are excellent C-sources for a large number of microorganisms. Bacteroids living in symbiosis with plants receive their energy supply through the peribacteroidal membrane largely in the form of malate. Similarly their symbiotic counterparts, free living nitrogen fixing bacteria, preferential use malate and citrate as C-sources. However, other pathogens depend on citrate within the soil as reported for *Pectobacterium atrosepticum* which requires citrate uptake for full bacterial virulence. Accordingly, the absence of a functional citrate transporter in *Pectobacterium atrosepticum* renders potato relatively tolerant to inoculation with this pathogen [30]. In a complementary study Rudrappa et al. (2008) made the highly interesting observation that inoculation of Arabidopsis leaves with the foliar pathogen *Pseudomonas syringae* (Pst DC 3000) induced malate excretion in roots [31]. This study revealed that malate induces the chemotactic motility of the beneficial *Bacillus subtilis* strain FB17 which moves to the roots of leaf-infected plants and protects the root from infection by *Pseudomonas syringae*. The authors further demonstrated that infection of Arabidopsis leaves upregulated AtALMT1, a
plasma membrane located malate channel. During the last few years the interaction between plant roots and microbes has attracted much attention, since it has been recognized in white lupin that this interaction plays an important role for plant nutrition [32]. Carboxylate excretion and acidification at the root surface may thus have a strong general impact on microbial communities [33, 34].

**Carboxylates in the apoplast**

Transport of iron, zinc and other heavy metals from the root to the shoot occurs mainly in the form of complexes rather than in their free form. Nicotianamine complexes several heavy metals, such as iron or zinc [35, 36, 37]. In addition to nicotianamine, citrate has been found to complex heavy metals and to be required for their long term transport in plants [38, 39, 40]. Reduced citrate contents in the xylem, as observed in knock-out mutants of the MATE transporter FRD3 results in the accumulation of iron in the root and leaves and in the constitutive expression of genes required for iron uptake [39]. Despite the fact that the frd3 knock-out mutants contain excess amounts of iron, they are chlorotic, suggesting that the intra-plant distribution of iron has been disrupted. Indeed, detailed analysis revealed that iron accumulates to large amount in the vascular cylinder in the roots of the frd3 mutants, that iron contents in the xylem were reduced and that in leaves iron was most probably precipitated in the cell wall and could thus not be delivered into the cells. These observations strongly suggest that reasonable amounts of citrate are required in the apoplast and xylem to sustain normal rates of root-to-shoot iron delivery.

In the leaf apoplast malate can be found at concentrations of 1-2 mM, whereas racing CO₂ levels result in increased apoplastic malate concentrations [41]. It has
been proposed that apoplastic malate released in response to elevated [CO$_2$] at least partially mediates closure of stomata under these conditions by shifting the current-voltage curve of anion channels towards resting membrane potentials, and thereby increasing the probability of opening of the anion channels [42, 43, 44]. Opening of anion channels results in the release of anions from guard cells and consequently in stomatal closure. In favour of this hypothesis it has recently been shown, that in the absence of the malate import protein AtABCB14 stomata opening is retarded and that malate-induced stomata closure is more efficient [45]. In addition biochemical analyses of plants deficient in the expression of fumarase, which likely display an increased apoplastic concentration of malate which would be anticipated to inhibit the anion channels, exhibited defective stomatal opening [46].

Internal excretion – vacuolar storage

In mature plant cells, the central vacuole occupies between 80 and 90% of the cell volume. Vacuoles contain a large number of inorganic ions, soluble carbohydrates, organic acids, amino acids, secondary compounds and modified xenobiotics [47]. In most plants the predominant carboxylate is malate, but citrate and in some plants such as *Arabidopsis thaliana* fumarate may also accumulate to high levels [48]. Carboxylates stored within the vacuole play an important role in osmoregulation and as counter-ions for vacuolar potassium, sodium and magnesium. This role is most pronounced in guard cells, where malate is the predominant anion in most plants during stomata opening and closure [49, 50]. Plants exhibiting C4 or CAM photosynthesis temporarily store malate as a carbon source, since this metabolic pathway can release CO$_2$ to be fixed during the C3 photosynthetic step. C3 plants also utilize vacuolar malate and citrate to support respiration at night. Surprisingly, plants impaired in vacuolar malate transport did not display an obvious phenotype.
However, a more detailed analysis uncovered that the respiratory coefficient was substantially altered from 1.16 to 1.26, indicating that when malate cannot be efficiently transported into the vacuole it enters the respiratory chain [52]. As in roots, carboxylates excreted into the vacuole also play an important role in complexing essential and toxic heavy metals stored in this compartment. Complexes of carboxylates with heavy metals such as zinc, iron or copper allow the plant to store considerable amounts of essential heavy metals which might be toxic if present at high concentration in the cytosol [53]. However, the exact nature of the signal transmitted from the cytosol to the vacuole in order to release micronutrients required by the plant remains unknown. One possibility is that alteration of the vacuolar pH would stimulate the dissociation of the heavy metal-carboxylate complex. Alternatively free carboxylates could first be released into the cytosol and the consequent shift in equilibrium between complexed and free micronutrients could be anticipated to facilitate the availability of the metal ions. It should, however, be noted that the release of micronutrients seems unlikely to be highly specific since the nramp3 and nramp4 mutants which are responsible for the release of iron are also permeable to the highly toxic cadmium [54].

A special case is oxalate which always forms Ca-oxalate crystals in vacuoles, albeit of different shapes and Ca$^{2+}$-oxalate ratios [55]. Such crystals allow the plant to store large amounts of Ca$^{2+}$ within the vacuole and at the same time to maintain low levels of free Ca$^{2+}$, in order to limit the concentration gradient between the vacuole and the cytosol. To facilitate crystal formation, transport of Ca$^{2+}$ and oxalate must be strictly coordinated, a question which still awaits elucidation. The exact source of oxalate is still not well established - in leaves oxalate may derive from photorespiration [56] but
ascorbate is also a potential substrate to fuel its production [57]. The latter pathway is probably the major pathway in C4 plants and in roots [55].

TRANSPORTERS INVOLVED IN CARBOXYLATE EXCRETION

So far three classes of exporters for malate and citrate have been identified. Malate excretion at the plasma membrane occurs by ALMT (aluminum metal tolerance) channels. Citrate excretion is catalized by members of the MATE (multidrug and toxins efflux) transporters. Two types of transporters/channels are implicated in the internal excretion of malate into the vacuole, the tDT transporter and ALMT channels.

ALMTs

As mentioned above most plants excrete carboxylates into the surrounding soil in response to Al$^{3+}$ to form non-toxic complexes. TaALMT1 was the first malate channel identified in wheat [58]. It is constitutively expressed in root apices but further activated by apoplastic aluminum thus conferring tolerance to wheat cultivars. Expression of TaALMT1 in heterologous systems such as Xenopus oocytes, rice (Oryza sativa), tobacco (Nicotiana tabacum) and barley (Hordeum vulgare) mediated an Al$^{3+}$-activated efflux of malate and increased Al$^{3+}$ resistance [58, 59]. TaALMT1 encodes a hydrophobic protein containing six to seven transmembrane spanning domains [60]. It was suggested that both the amino and carboxyl termini are located on the extracellular side of the plasma membrane. Homologous proteins have been identified in many other plant species (AtALMT1, [61]; BnALMT1/BnALMT2, [62]; ZmALMT1, [63]; ScALMT1, [64]) and several studies demonstrated that these proteins perform similar functions as TaALMT1. However, functional analysis of
ZmALMT1 in Xenopus oocytes interestingly suggests that this transporter is probably involved in general anion mineral nutrition and homeostasis processes (i.e. Cl-) rather than mediating the specific release of carboxylates in response to Al$^{3+}$ [63]. In Arabidopsis the AtALMT protein family consists of 14 members which can be subdivided into three clades. AtALMT1 belongs to the same clade as TaALMT1 (44% similarity) and also localizes to the plasma membrane of the root where it mediates an Al$^{3+}$-activated malate extrusion into the rhizosphere [61]. Al$^{3+}$ is not merely required to activate AtALMT1 but also to induce expression of this protein, a mechanism also described for BnALMT1 and 2 [62]. By contrast AtALMT9 a member of the second clade is targeted to the tonoplast and highly expressed in the mesophyll tissue of leaves [65]. Deletion mutants of AtALMT9 exhibited a reduced current density in vacuoles isolated from mesophyll protoplasts whereas tobacco cells overexpressing AtALMT9 exhibited strongly enhanced malate currents. The high expression of AtALMT9 in the mesophyll tissue indicates a function in malate homeostasis rather than in Al$^{3+}$ tolerance although that said Al$^{3+}$ activation could be demonstrated in Xenopus oocytes. Beside AtALMT9 other members of clade II also localize to the tonoplast (S. Meyer, unpublished), it thus seems reasonable to speculate that the intracellular localization of the ALMT proteins is largely uniform within a clade and depends on slight differences in sequence and topology between members of the different clades. Interestingly other members of the AtALMT protein family may well fulfill further as yet unknown functions since data from microarray experiments indicate the accumulation of their mRNA levels in many other plant tissues (http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi). One such example is the guard cell (AtALMT6, At2g17470; AtALMT12, At4g17970; AtALMT5, At1g68600), wherein malate largely functions as a charge balancing anion for potassium [66]. In general, anion channels function as central regulators of stomata movements since
opening and closure is driven by uptake or release of osmotically active anions and organic metabolites [67]. In respect to malate, anion currents have been reported for the stomatal plasma membrane and the vacuolar membrane but despite the important role of malate in guard cell regulation little is known about molecular identity of malate transport systems in guard cells and particularly no malate channels have been identified at the genetic level. It is, therefore, conceivable that members of the AtALMT family could be involved in the regulation of stomatal movement either as vacuolar or as plasma membrane localized anion channels.

**MATE transporters**

Multidrug And Toxic Compound Extrusion (MATE) proteins were first described in bacteria as transporters which can export several antimicrobial agents and therefore confer multidrug resistance [68]. They have subsequently been found in all eukaryotes. While in bacteria export of drugs by MATE transporters is coupled either to the antiport of Na\(^+\) or H\(^+\), in eukaryotes only H\(^+\) coupled antiport activity has been described so far. As in bacteria, MATE transporters in animals are also part of the detoxification pathway. In bacteria 9 to 13 genes coding for MATE transporters are present on the genome, whereas mammal genomes contain only a few for example there are two in humans and mouse and three in dogs [69]. In plants MATE transporters, as ABC transporters are overrepresented. This is probably due to the large number of diverse secondary compounds which can be regarded as toxic compounds produced by the plant cell itself and which must be detoxified, either by extrusion into the apoplast or the vacuole. *Arabidopsis thaliana* contains 56 MATE transporters. Members of this family have been demonstrated to be localized to the plasma membrane, the vacuolar membrane and the chloroplast envelope. They are involved in functions as diverse as detoxification and lateral root formation, salicylic-
dependent signaling and transport of secondary metabolites such as catechin

glucosides, anthocyanins and nicotine [70, 71, 72]. In Arabidopsis two MATE
transporters excrete citrate out of the cytoplasm. AtMATE/AtDTX42 (At1g51340), as
its closest sorghum homolog - SbMATE, confers aluminum tolerance by excretion of
citrate into the soil in response to the exposure to Al$^{3+}$ [73, 74, 27]. By contrast, its
closest intraspecific homolog FRD3 is implicated in the excretion of citrate in the
xylem, and is required for long distance iron transport and uptake in the leaf cells [75,
76, 77]. The function of most plant MATE proteins has yet to be established,
however, it is somewhat surprising that a primary metabolite such as citrate is
transported by this class of proteins.

The tonoplast Dicarboxylate Transporter (tDT)

The vacuolar malate transporter (Arabidopsis thaliana tonoplast Dicarboxylate
Transporter, AttDT) has been identified by investigating the plant homolog of the
human sodium-dependent dicarboxylate transporter HsNaDC-1 [51]. This human
transporter is a member of the solute carrier family 13A2 (SLC13A2) which
comprises sodium coupled sulfate and carboxylate transporters [78]. SLC13A2 in
turn belongs to the large transporter family of the solute carrier family 13. In humans
SLC13A2 transporters are localized in the plasma membrane of many different
organs. The carboxylate transporters of this subfamily can be subdivided into three
clades, NaDC1-3 which differ in their sensitivity to pH and the affinity to the substrate,
but are all electrogenic since the transport of one, generally divalent anion is coupled
to that of three Na$^+$ [78]. Arabidopsis contains only one homologue of the NaDC
family which exhibits 65.6 similarity and 38.4% identity with HsDC1, while two
members of this family have been annotated in rice and Brassica oleracea
(Aramemnon; http://aramemnon.botanik.uni-koeln.de/index.html). By contrast to the
animal NaDcs, AttDT is localized to the vacuolar membrane and its transport activity is not sodium dependent. Accumulation of malate is energized by the electrochemical gradient and in the case of vacuolar malate transport it is believed that the driving force for vacuolar accumulation is the difference in the electrical membrane potential between the cytosol and the vacuole. Despite the fact, that biochemical analysis showed competitive inhibition of malate by citrate and *vice versa*, deletion mutants of Arabidopsis for AttDT were not impaired in citrate transport [79]. Shimada et al. (2006) identified a homologue of AttDT, CsCit1, in citrus juice sac cells [80]. The highest expression of CsCit1 coincided with the decrease in vacuolar citrate contents and increase in pH. By heterologous expression in yeast the authors provided convincing results that implicated CsCIT1 in the $\text{H}^+$-dependent efflux of citrate from the vacuole. This result is in line with some physiological observations that AttDT can act also as a malate exporter [52]. Therefore it will be interesting to elucidate how AttDT is regulated in order to act at the same time as importer as well as an exporter.

### Conclusions and Outlook

The central importance of carboxylates in plant metabolism and physiological processes have long been recognized. However, the post-genome era has led to rapid advances with a number of transporters being identified which mediate the excretion of carboxylates to the soil, apoplast and vacuole. The facile acquisition of knock-out mutants and transgenics in Arabidopsis has accelerated the realisation of the precise function and importance of several of these transport proteins with studies on aluminium tolerance, iron and phosphate nutrition being of particular note. Despite these crucial advances a number of vital open questions remain. These
include (i) what is the nature of vacuolar citrate import? (ii) what are the mechanisms of oxalate transport across the plasma and tonoplast membrane? (iii) how are the transporter activities physiologically regulated? (iv) what is the cue that initiates root excudation and (v) which channels are responsible for increases observed in apoplastic malate. Once the entire complement of carboxylate transporters have been identified the grand challenge remains to understand how they are regulated in concert and how they are hierarchically controlled in order to accommodate the myriad of responses which plants, as sessile organisms, require to adjust to environmental circumstance.

Acknowledgements

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References


9 Weisskopf, L. et al. (2006) Isoflavonoid exudation from white lupin roots is influenced by phosphate supply, root type and cluster-root stage. *New Phytol.* 171, 657-668


29 Yang, Y.Y. et al. (2000) Identification of rice varieties with high tolerance or sensitivity to lead and characterization of the mechanism of tolerance. *Plant Physiol.* 124, 1019-1026


41 Hedrich, R. et al. (1994) Malate-sensitive anion channels enable guard cells to sense changes in the ambient CO₂ concentration. *Plant J.* 6, 741-748


54 Lanquar, V. *et al.* (2005) Mobilization of vacuolar iron by AtNRAMP3 and AtNRAMP4 is essential for seed germination on low iron. *EMBO J.* 24, 4041-4051


61 Hoekenga, O.A. et al. (2006) AtALMT1, which encodes a malate transporter, is identified as one of several genes critical for aluminum tolerance in Arabidopsis. *Proc. Natl. Acad. Sci. U. S. A.* 103, 9738-9743


65 Kovermann, P. et al. (2007) The Arabidopsis vacuolar malate channel is a member of the ALMT family. *Plant J.* 52, 1169-1180


**FIGURE LEGENDS**

**Figure 1.** Tissue-specific and intracellular localization of the different plant carboxylate transport systems. (a) AtALMT9 and AttDT are localized to the tonoplast of the leaf mesophyll tissue mediating the flux of malate from the cytosol into the vacuole [51, 65]. AttTD has been identified as a malate uptake transporter, however physiological experiments strongly suggest that it can also catalyze the efflux of malate from the vacuole to the cytoplasm [52]. In citrus fruits an AttDT homolog was shown to be responsible for efflux of citrate from the vacuole to the cytosol [80]. (b) Different transport systems are suggested to be involved in the efflux and influx of malate during stomatal opening and closure, respectively. Whereas ABCB14 has been functionally described as a plasma membrane targeted malate importer [45] the presence of several ALMT proteins in guard cells is reported only at the level of the presence of mRNA ([http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi](http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi)) and the putative role of these proteins in stomatal movement remains to be elucidated. On one hand ALMT-type
channels could mediate the malate flux into the vacuole during stomatal opening but it can not be excluded that ALMTs could also function in the efflux of malate into the cytosol during guard cell closure. Efflux of malate through the plasma membrane could also be mediated by ALMT proteins or alternatively by the SLAC1 protein which is likely to represent at least part of the S-type channel, however the substrate specificity for this channel has still to be established [43, 44]. We also propose that AttDT could act as an importer during stomatal opening and as an exporter during guard cell closure since published data suggest a similar function in mesophyll tissue [52, 80]. However, nothing is known about regulatory mechanisms allowing this transporter to facilitate import and export. (c) Iron is mobilized by excretion of citrate into the apoplastic space of the vasculature tissue catalyzed by the FRD3 transport protein and subsequently the iron-citrate complex is translocated via the xylem into the shoot tissue [77]. (d) In acidic soil conditions the abundance of the phytotoxic Al$^{3+}$ increases and becomes a major factor limiting plant growth. Diverse carboxylates are excreted from root tissues into the rizosphere to bind with the Al$^{3+}$ cations and form non-toxic complexes which ameliorate aluminum toxicity. The most prominent carboxylates are malate and citrate which are released by the well described ALMT1 channel and MATE protein, respectively [58, 61, 73, 74]. (V=Vacuole; E=Endodermis; P=Pericycle; VP=Vascular Parenchyma; Xy=Xylem)

**Figure 2.** Putative topology and currents of two selected ALMT channels. (a) Topology was deduced by comparison of the Arabidopsis ALMTs in the Aramemnon database (http://aramemnon.botanik.uni-koeln.de/) and the topology presented by Motoda et al. (2007) [60]. We have inverted the topology proposed by Motoda et al. (2007), since a recent report reveals activation of TaALMT1 via phosphorylation of serine residue 384 (red dot; [62]). Since no apoplastic protein
kinases has been identified to date, we propose that the N- and C-terminus of the protein is likely located in the cytosol. (b - c) Representative currents of TaALMT1 ((b), taken from [81]) and AtALMT9 ((c); taken from [65]), exemplarily shown for plasma membrane (TaALMT1) and vacuolar membrane (AtALMT9) located ALMTs. While TaALMT1 mediates instantaneously activated currents, vacuolar malate currents of AtALMT9 exhibit an instantaneous and a time-dependent component.

**Figure 3.** MATE transporters are involved in excretion of citrate into the soil and xylem.

(a) Topology was deduced by comparison of the Arabidopsis MATEs in the Aramemnon database (http://aramemnon.botanik.uni-koeln.de/). A long cytosolic loop is present between the second and the third α-helix only in MATE proteins reported to transport citrate (FRD3 and AtMATE). This loop has also been found in citrate transporting MATE proteins of other plant species [27]. (b) HvAACT1, a MATE transporter of barley excreting citrate into the soil is predominantly localized in epidermal root cells (taken from [73]; Scale bar: 100 µm; Mu = cultivar Murasakimochi). (c) The Arabidopsis citrate transporter FRD3 is localized in the pericycle and responsible to keep iron soluble in the apoplast. (d) The frd3 mutant plants exhibit a pale phenotype. (e - f) Absence of FRD3 leads to the precipitation of iron in cell walls (e) while in the presence of FRD3 iron can be translocated to the shoot (f) (blue coloration corresponds to iron content; taken from [76] and [77]).

**Figure 4.** Topology and amino acids possibly determining the sodium-dependency of DTC transporters. Hydrophobicity plot analysis (http://www.expasy.ch/cgi-bin/protscale.pl) predicts that DTC transporters are formed of 12 transmembrane domains with the N- and C-terminus facing the cytosol. Differently from the animal
DC transporters, the plant AttDT has been shown to transport dicarboxylates in a Na\(^+\) independent manner [51]. In the inset a region of the DTC proteins involved in Na\(^+\) binding is highlighted (numbering of amino acids corresponds to AttDT). The T509 of the human hNaDC1 as well as the S512 of the RbNaDC1 or rabbit has been shown to be involved in Na\(^+\) binding [82]. Interestingly, in an equivalent position the plant DTC transporters contain an alanine as opposed to the polar residue of their animal counterparts. It could be possible that this polymorphism is involved in determining the dependency on Na\(^+\).
Figure 1

(a) 

(b) open closed

(c) 

(d) 

Legend:

- Fe
- malate
- citrate
- FRD3
- ABCB14
- S-type (SLAC1)
- AttDT
- ALMT9
- ALMT1
- ALMTx
- MATE