RESEARCH HIGHLIGHT

Alluminating structure key to stress tolerance

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Plant tolerance to aluminium is encoded by root-localized aluminium-activated malate transporter 1 (ALMT1). In a recent study published in *Cell Research*, Wang et al. resolved the structure of ALMT1, which provides intriguing insights into its function and opens new research opportunities.

In acidic soils (pH < 5), aluminium trivalent cations (Al^{3+}) are mobilized into the soil solution and can cause cell and tissue damage.[1] To thrive in acidic soils, plants must protect themselves against Al^{3+} through detoxification or compartmentalization. A key aluminium tolerance trait is Al^{3+} exclusion from roots via exudation of carboxylates, which immobilizes Al^{3+} preventing its cellular entry or interaction with cell walls.[1] The first protein identified encoding this trait was wheat aluminium-activated malate transporter 1 (ALMT1),[2] which is localized to the growing root apex. Despite considerable genetic and physiological insights already being revealed for ALMT1 in wheat and other plants,[1,2] key details of its function at the molecular level have remained unresolved.

Recently, a study published in *Cell Research* determined structures for *Arabidopsis thaliana* ALMT1 using cryo-electron microscopy (cryo-EM),[3] shedding new light on the mechanism of Al^{3+}-activated malate transport. ALMT1 has a short amphipathic N-terminal α-helix, six transmembrane α-helices (TM1–TM6) creating the transmembrane domain (TMD), and six cytoplasmic α-helices comprising a large C-terminal domain (CTD) (Fig. 1a). The functional anion channel is a homo-dimer with a single ion conduction pore assembled by 12 transmembrane α-helices from each TMD, with an internal pseudo-two-fold symmetry of TM1–TM3 and TM4–TM6 repeats in each chain (Fig. 1b).

Multiple advances are provided by Wang et al.[3] of which we highlight four: (1) ALMT1 malate channel function requires dimerization. Multimeric ALMT have been predicted,[4] including when expression of a truncated rice ALMT simultaneously with the full-length protein inhibited currents.[5] The malate-conducting pore of ALMT1 is formed at the interface between specific residues of the transmembrane α-helices from the individual subunits. Association between transmembrane α-helices from different subunits enabling channel functionality is not uncommon. However, it is unlike the structure of anion channels known currently in plants, such as the trimeric Slow Anion Channel Associated 1 (SLAC1) where each subunit allows the conduction of ions.[6] (2) Malate is coordinated in the pore by pairs of arginine residues. Previously, the positively charged amino acid residues arginine (R) and Lysine (K) were predicted to line the ALMT pore.[5] Here, interactions in the pore between the R165 pair were corroborated as essential,[5] while the R80 pair affected current magnitude but may not be essential for function (Fig. 1). K76 and R125 facing the cytosol were predicted to assist in recruiting malate but are yet to be tested in ALMT1. (3) Al^{3+} binding activates ALMT1 via structural re-arrangement of an extracellular gate. Al^{3+} was found to associate with residues on the extracellular side of the protein on the loop adjoining TM1–TM2 (D49) and the N-terminus of TM6 (E156, D160). Their association with Al^{3+} induces conformational changes (outward movements) of the TM1–TM2 and TM5–TM6 loops that carry I53 and F51, or F153 residues, respectively, opening the extracellular gate for malate conduction (Fig. 1c). Previously, residues near N- and C-termini were found to be important in the Al^{3+} response[1]; Wang et al.'s structural data[3] suggest that their impacts are indirect. (4) ALMT1 topology. The topology of ALMT1 has been long debated, with several alternative models presented.[7] While correct predictions of the six transmembrane α-helices, a large CTD and both the N- and C-termini facing the cytosol have been made,[5] the orientation of α-helices and how they fold were unclear. Figure 1a shows the previously undetermined interaction between the TMDs, and that the N-terminal amphipathic α-helix is positioned alongside the membrane cytosolic surface.

Now that the *Arabidopsis* ALMT1 structure is revealed, it is possible to examine whether the key residues identified for malate and Al^{3+} binding, and activation are universal to other ALMT1. As wheat ALMT1 shares 63% similarity with *Arabidopsis* ALMT1, it is almost certain that they will share a similar mechanism. Beyond these insights the new ALMT1 structure has broader significance than revealing the mechanism of Al^{3+} activation, as it provides a blueprint by which the structure-function relationship for other ALMT proteins can be explored.

Since the first member of this family was identified,[2] many more ALMT proteins have been discovered.[7] For instance, the model plant *Arabidopsis* has fourteen ALMT proteins.[7] ALMTs share high sequence similarity, and thus are likely to form similar homo-dimeric structures,[3] but this needs to be confirmed, as does whether ALMT can form hetero-dimers. Another question that can be examined in fine detail is the role of the identified, or additional, coordinating residues in ALMT transport selectivity,[3,5] i.e., for other anions transported by ALMTs such as Cl^-, NO_3^-[7], other carboxylates[5] or non-charged solutes such as GABA.[8]

ALMTs are now known to have wide-ranging physiological roles. Intriguingly, only ALMT1 is activated by Al^{3+}; other ALMTs have known or proposed roles in nutrition, pollen tube growth, stomatal aperture control, pathogen defence and GABA signaling,[9] amongst others. Most ALMTs are activated by the presence of external anions, and are further regulated by pH, phosphorylation, ATP, voltage and GABA.[7] In fact, both wheat and *Arabidopsis* ALMT1 are also activated by external anions at alkaline pH.[10] Examining

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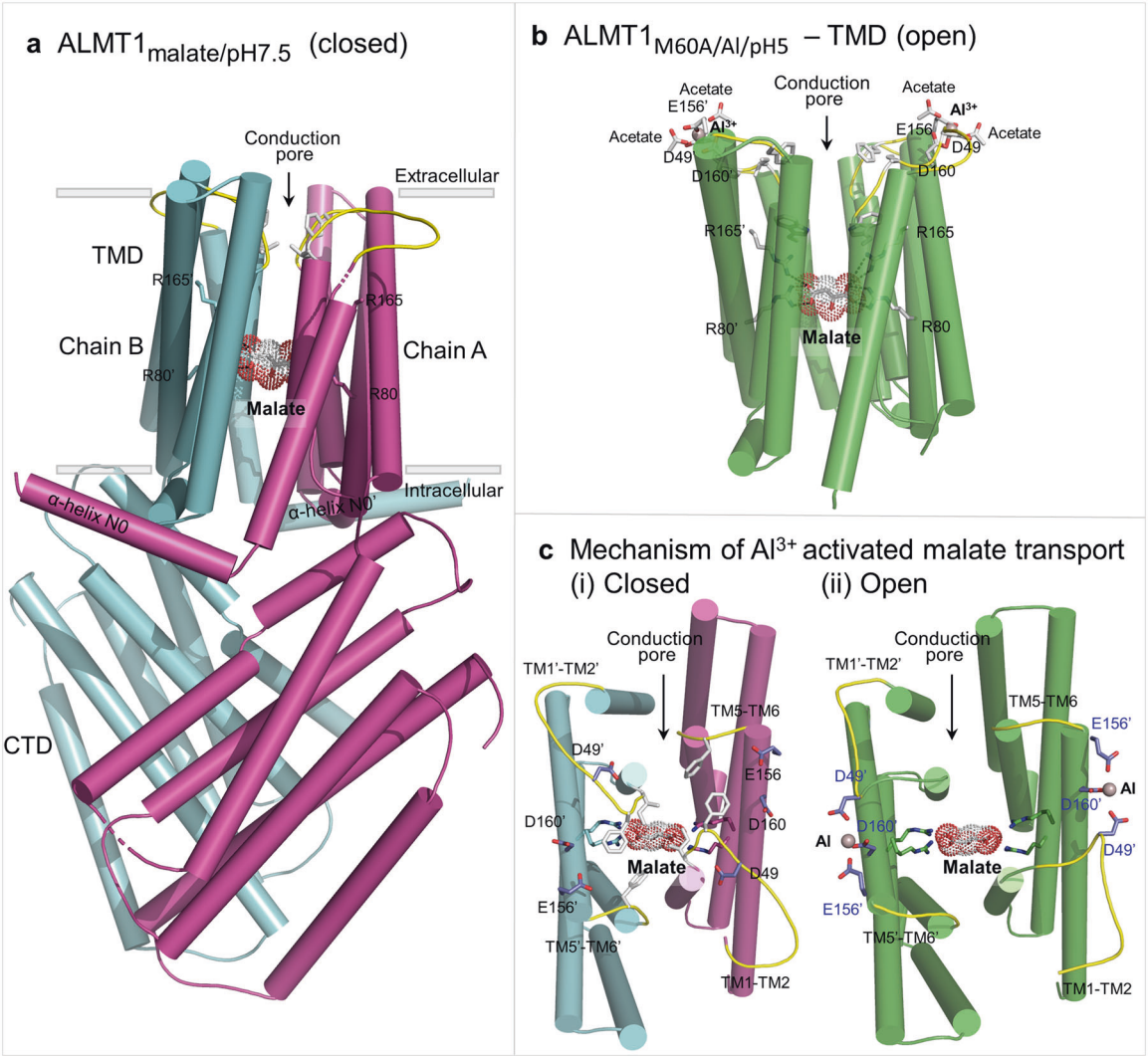

Fig. 1 Cryo-EM structures of the *Arabidopsis thaliana* ALMT1. a Representation of $ALMT1_{malate/pH7.5}$ (PDB: 7VQ5) in closed conformation with cylindrical α-helices illustrating dispositions of A (magenta) and B (cyan) chains, folding into TMD and CTD, respectively, where TMDs from each chain form a conduction pore (arrow) containing the malate molecule (cpk sticks with dots). TM1–TM2 and TM5–TM6 loops (yellow) that play roles in Al^{3+}-activated malate transport are positioned near the extracellular side of the structure. N-terminal α-helices N0 in both chains are labeled. **b** Representation of TMD of $ALMT1_{M60A/Al/pH5}$ (green) in open conformation illustrating dispositions of two Al^{3+} ions (brown spheres) at the extracellular side of TMD that are coordinated each by D49, E156, and D160, and two acetate molecules (cpk sticks). **c** Conformational re-arrangements of ALMT1 underlie the mechanism of Al^{3+}-activated malate transport. Left panel shows $ALMT1_{malate/pH7.5}$ structure (A and B chains in magenta and cyan, respectively). Under acidic conditions Al^{3+} binds at the extracellular gate of each chain. Right panel ($ALMT1_{M60A/Al/pH5}$; A and B chains in green) shows structural re-arrangement due to Al^{3+} binding.

whether anion activation occurs through a similar modification of the extracellular gate for both Al^{3+}- and non-Al^{3+}-activated ALMT requires further experimentation. It is likely that the large C-terminus plays a regulatory role and functions in dimerization; this can now be explored in a targeted way. Furthermore, the ALMT1 structure will provide insights beyond plants. ALMTs are not specific to vascular plants (with related proteins in bacteria, fungi, algae and non-vascular plants), but are absent in animals; therefore the ALMT1 structure provides a new tool by which the significance of ALMTs in other organisms can be studied.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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