RESEARCH HIGHLIGHT

Sensing the toxic aluminum cations in acidic soils

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Plant cells are regularly challenged by harmful pathogens and toxins in the environment, and they must detect these stressors to induce resistance and avoidance responses. A recent study in *Cell Research* identifies a leucine-rich-repeat receptor-like kinase in *Arabidopsis thaliana* that functions as a receptor for the toxic aluminum cations prevalent in acidic soils.

Aluminum (Al) toxicity is a major factor limiting crop production on acidic soils because even low concentrations of soluble aluminum cations (Al^{3+}) can inhibit root growth in important crops. Many species, and even genotypes within species, have evolved ways of coping with these harmful cations. Several of these resistance mechanisms have been reported, but one that has been confirmed in a diverse range of species relies on the Al^{3+}-dependent release of organic anions from root tips.[1] The most common anions released are malate and citrate and they protect the roots by binding the Al^{3+} cations in the apoplast, which prevents them from damaging the sensitive growing cells. This resistance mechanism was first characterized in wheat,[2] but subsequent studies in *Arabidopsis thaliana* have shown that the induction of Al^{3+}-resistance genes requires a transcription factor, SENSITIVE TO PROTON RHIZOTOXICITY1 (AtSTOP1).[3] When soil pH is high and soluble Al^{3+} concentrations are low, AtSTOP1 levels are kept low by post-transcriptional degradation involving the F-box protein REGULATION OF ALMT1 EXPRESSION 1 (RAE1).[4] When Al^{3+} is present, RAE1 function is reduced and the accumulation of AtSTOP1 allows Al^{3+}-resistance genes to be induced. The most important one of these in *Arabidopsis* is the *Al-activated malate transporter* (*AtALMT1*). *AtALMT1* encodes an anion channel that is activated by apoplastic Al^{3+} to facilitate malate release from roots.[5,6] AtSTOP1 also induces RAE1 expression thereby forming a negative feedback loop between AtSTOP1 and RAE1.[4]

Until now, no information has been available on the signaling pathways upstream of AtSTOP1 and RAE1. A notable gap in our knowledge has been how Al^{3+} is initially perceived by the root cells to trigger resistance. An important study by Ding et al.[7] fills this gap and reveals other valuable insights. The authors realized that receptor-like kinases (RLK) act as cell surface receptors for a wide range of substrates and they surmised that one of the > 600 members of this family in *Arabidopsis* might serve in the perception or signaling of toxic Al^{3+}. They screened a library of T-DNA insertional mutants and identified a leucine-rich-repeat RLK, named Al Resistance 1 (ALR1), which is critical for Al^{3+} resistance. The Al^{3+}-induced accumulation of the AtSTOP1 transcription factor was significantly reduced in *alr1* lines, which suggests ALR1 functions upstream of AtSTOP1. ALR1 was characterized previously as a receptor for phytosulfokine peptides named Phytosulfokine Receptor1 (PSKR1), so that its role in Al^{3+} resistance is a second function for PSKR1.

Ding et al.[7] propose that Al (and not other cations tested) binds to PSKR1/ALR1 and recruits a somatic embryogenesis receptor-like kinase (SERK) co-receptor named BAK1. The activated PSKR1/ALR1–BAK1 complex then phosphorylates a RbohD NADPH oxidase, which catalyzes the generation of reactive oxygen species (ROS) in the apoplast (probably superoxide that is rapidly dismutated to H_2O_2). ROS enters the cytosol and causes the degradation of RAE1 by oxidative modification of Cys364 residue. The reduction of RAE1 activity enables the AtSTOP1 transcription factor to accumulate and induce expression of *AtALMT1* and other Al^{3+}-resistance genes. Newly translated AtALMT1 proteins localize to the plasma membrane where they facilitate malate release from the root cells (Fig. 1).

The study by Ding et al.[7] provides a timely and welcome boost to our understanding, not only of Al^{3+} resistance in *Arabidopsis*, but also of the more general phenomenon of how cells perceive their environment. One surprising finding is that Al binds to the cytosolic domain of PSKR1/ALR1, and not to the extracellular domain where Al^{3+} concentrations are very much greater. Several lines of evidence supported this unexpected outcome. Four Cys residues on the cytosolic domain of PSKR1/ALR1 were found to be critical for Al^{3+} resistance and for PSKR1/ALR1 to bind sub-micromolar concentrations of Al. Substitution of those four residues prevented PSKR1/ALR1 from binding Al without disrupting its kinase activity while variants of PSKR1/ALR1, which do not bind extracellular phytosulfokines, maintained normal Al^{3+} resistance responses. Another intriguing finding was the proportional responses of the signaling pathway to Al^{3+} concentration. Both the inter-phosphorylation of PSKR1/ALR1–BAK1 and the phosphorylation of RbohD increased with greater Al^{3+} concentrations, which might partly explain the concentration-dependent release of malate from roots.

An important corollary to this work is that ROS produced by alternative means (e.g., chemical treatments, cellular metabolism, or other stresses) will not necessarily result in a wasteful release of malate from roots, even if those treatments induce *AtALMT1* expression. The reason for this is that maximum malate efflux only occurs when extracellular Al^{3+} is present to activate the AtALMT1 channel.[5,6]

Future studies can investigate the mechanism by which Al enters the cytosol and the chemical form of Al that binds PSKR1/

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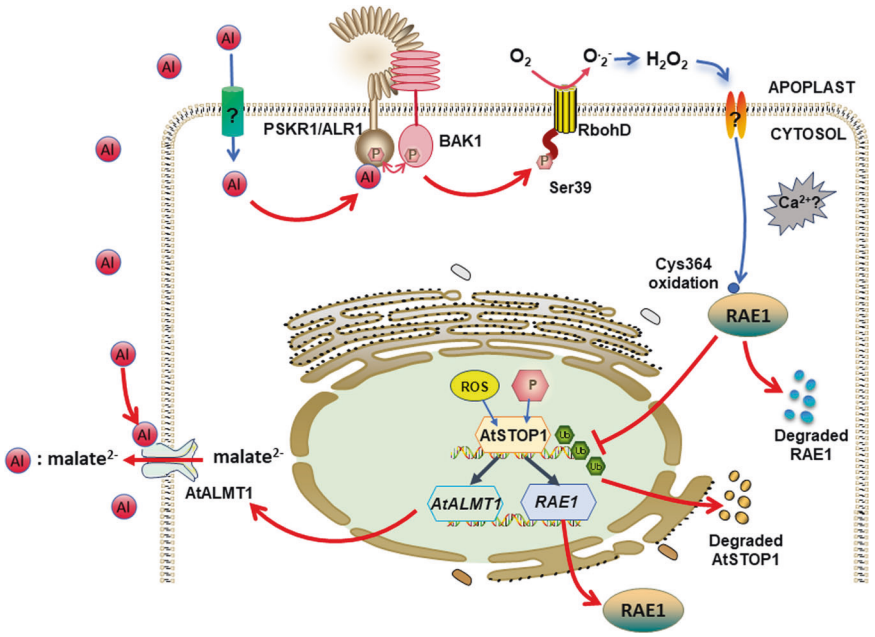

Fig. 1 The signaling pathway regulating the induction of Al^{3+}-resistance genes. Al enters the cytosol by an unknown mechanism and binds to the cytosolic domain of PSKR1/ALR1. The co-receptor BAK1 is recruited for inter-phosphorylation. The PSKR1/ALR1–BAK1 complex phosphorylates RbohD NADPH oxidase at Ser39 which contributes to ROS generation in the apoplast. ROS enters the cytosol (likely H_2O_2 via aquaporins) and modifies RAE1 at Cys364, which inhibits RAE1 activity and thus prevents the degradation of AtSTOP1. AtSTOP1 accumulation induces the expression of *AtALMT1* and other Al^{3+}-resistance genes. AtSTOP1 also induces *RAE1* expression, forming a negative feedback loop. Once the AtALMT1 channels localize to the plasma membrane, they are activated by Al^{3+} to release malate anions which chelate the toxic Al^{3+}. Recent studies conclude that AtSTOP1 can also be directly regulated by phosphorylation and oxidative modification. Apoplastic ROS might also trigger calcium signals (depicted in the figure as a burst of "Ca^{2+}?").

ALR1, as these were not explored by Ding et al.[7] Other regulatory pathways of AtALMT1 appear to be present; therefore it will be important to fully characterize these as well. For instance, Ding et al.[7] show that Al^{3+} can induce low, but measurable, levels of AtALMT1 in the *pskr1/alr1* mutant. Further, we know that treatments other than Al^{3+} can induce *AtALMT1* expression (e.g., low pH, phosphorus deprivation, hormones and flagellin22)[8] and that iron can enhance *AtSTOP1* and *AtALMT1* expression[9] without binding to PSKR1/ALR1.[7]

It will be important to determine whether the regulation of AtSTOP1 described by Ding et al.[7] overlaps with other studies showing direct regulation of AtSTOP1 by phosphorylation[10] and oxidation.[11] It will also be interesting to examine the possible involvement of Ca^{2+} signaling because apoplastic ROS can trigger Ca^{2+} channels in some cell types.[12] Collectively, these findings point to a complex network of signaling pathways regulating AtSTOP1 and AtALMT1 and it will be intriguing to determine which ones involve PSKR1/ALR1 or other novel receptors.

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ADDITIONAL INFORMATION

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