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The role of malate in plant homeostasis

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Abstract

Malate is a central metabolite of the plant cell with important roles in plant physiology and metabolism. Here, we summarize the most recent advances in our understanding of malate homeostasis in central metabolism, guard cell functioning, and root exudation.

Introduction and context

The dicarboxylic acid malate has a multitude of functions in plant metabolism and homeostasis. These range from its most prominent roles in the mitochondrial tricarboxylic acid (TCA) cycle and in CAM and C4 metabolism, to its roles as an osmoticum, as a regulator of pH homeostasis, as a reducing equivalent that is shuttled between subcellular compartments, and as an important root exudate [1]. Although the various functions of malate in plant metabolism have been known for many years, the recent identification of diverse malate transport proteins in various tissues and compartments and the analysis of mutant and transgenic plants with altered malate metabolism have shed new light on its broader importance for cellular functions.

Major recent advances

Perturbation of malate metabolism in the TCA cycle has unexpected biochemical consequences

As an intermediate of the TCA cycle, malate is intimately associated with mitochondrial energy metabolism and is also the origin of carbon skeletons exported from the mitochondrion in support of amino acid biosynthesis [2]. However, recent reverse genetic experiments targeting TCA cycle enzymes suggest that malate has a metabolic influence well beyond the standard textbook associations. In particular, antisense suppression of mitochondrial malate dehydrogenase in tomato plants leads to an unexpected increase in the rate of photosynthesis [3]. While the precise role of the TCA cycle in leaves is still the subject of debate [4], one nevertheless would anticipate that inhibition of TCA cycle flux would have a negative rather than a positive effect on photosynthesis. The mechanism for this effect is not due simply to inhibition of the TCA cycle; the suppression of other TCA cycle enzymes does not necessarily have the same consequence [5]. In addition, disruption of malate metabolism has a specific effect on root growth that is independent of alterations in leaf metabolism [6]. Again, the mechanism for this is not completely clear, but inhibition of root respiration and consequent changes in root metabolism, including altered giberellic acid levels, may be responsible. One aspect that may help explain the different biochemical phenotypes resulting from inhibition of different steps in the TCA cycle is the presence of different flux modes within the cycle [7,8]. Ultimately, detailed metabolic network flux analysis will be required to fully understand the manifold metabolic roles of malate.

AtABCB14 and SLAC1 regulate malate homeostasis involved in stomatal movement of guard cells

The transport of malate across the plasma membrane of guard cells is an important process in the regulation of guard cell turgor pressure, mediating guard cell opening and closure. Stomata respond to changes in external CO_2 concentrations, and increased apoplastic malate levels can be observed at high CO_2 levels when stomata are closed [9]. Moreover, it has been demonstrated that the presence of floating leaves on a malate solution leads to stomatal closure [9,10]. Although the existence of a malate-sensitive guard cell anion channel was reported

by Hedrich and Marten [11] more than 15 years ago, the identity of this channel was unknown until recently. A major breakthrough was the independent identification by two groups [12,13] of the slow anion channel-associated 1 (SLAC1) protein, which possesses features of a C4-dicarboxylate transporter. This channel was shown to affect slow anion current channel function and thus malate ion homeostasis in guard cells. SLAC1 loss-of-function mutants showed a constitutive stomatal opening phenotype with a complete lack of high CO_2-induced stomatal closure, and an overaccumulation of malate, fumarate, and potassium guard cell protoplasts was observed [12]. In addition, the first plasma membrane ABC transporter (AtABCB14) responsible for uptake of malate from the apoplast was identified recently [10]. Malate uptake into guard cells leads to stomatal opening through increased guard cell turgor. Hence, *AtABCB14* knockout plants showed more pronounced stomatal closure at high CO_2 concentrations, as the cells were still able to release malate but not to take it up again. In line with this, overexpressors of *AtABCB14* showed a highly decreased stomatal closure response to high CO_2. Thus, it was demonstrated that AtABCB14 plays an important role in the CO_2-mediated guard cell response. Furthermore, transgenic tomato plants with suppressed expression of fumarase (the TCA cycle enzyme that produces malate) were also reported to have defects in stomatal opening leading to decreased photosynthetic rates and plant growth [14]. Interestingly, total levels of leaf malate were increased rather than decreased in these lines as one might expect. However, there may be guard cell-specific effects on malate homeostasis in the fumarase-deficient lines and these effects might be different from those seen in whole tissues.

The molecular mechanisms that regulate malate exudation in roots conferring aluminium tolerance have been identified

The excretion of organic acids, including malate, by plant roots is a key factor in the tolerance of plants toward aluminium (Al) toxicity by chelation of Al in the soil (reviewed in [15]). Al toxicity is a problem that affects agricultural crop growth worldwide, and resolving the mechanism of Al tolerance in plants is of great economic importance. *Arabidopsis* plants, as well as wheat, excrete high levels of malate when the root is subjected to Al^{3+} stress, and the level of Al tolerance correlates with the amount of excreted malate in different ecotypes of these species [16,17]. Recently, the gene responsible for Al-activated malate efflux, *ALMT1* (Al-activated malate transporter 1), was identified in wheat and *Arabidopsis* [18,19] and the physiological properties of Al^{3+}-activated malate transport were studied in *Xenopus* oocytes [20]. Moreover, overexpression of wheat *ALMT1* in barley resulted in enhanced exudation of malate by roots following Al^{3+} treatment and increased Al tolerance of the normally Al-sensitive barley [21]. These experiments proved that the synthesis of malate is not the rate-limiting step for its efflux from roots, although overexpression of a nodule-enhanced form of malate dehydrogenase was also able to considerably enhance the exudation of malate in an Al-sensitive alfalfa cultivar [22]. Recently, an important step toward the understanding of the regulatory mechanisms of malate excretion was made. In a forward genetic screen, the putative Cys_2His_2-type zinc finger transcription factor, STOP1 (sensitive to proton rhizotoxicity 1), which is responsible for the strong induction of *ALMT1* transcripts after Al treatment, was identified [19,23]. Plants with defects in STOP1 did not show induced expression of *ALMT1* and malate release after Al treatment and exhibited an Al-hypersensitive phenotype [23]. Interestingly, Rudrappa *et al.* [24] uncovered another important role for ALMT1 in root exudation of malate. *Arabidopsis* seedlings that were infected with the foliar pathogen *Pseudomonas syringae* exudated malate to recruit the beneficial rhizobacterium *Bacillus subtilis* in a dose-dependent manner, reducing its susceptibility to pathogen attack.

Future directions

Besides the already known roles and functions of malate in plant metabolism, there still seems to be more to uncover. The role of malate as a root exudate determining interactions of plant microbia and resistance to acidic soils may have broad impacts on biodiversity and the distribution of plant communities. One interesting issue will be to decipher the signalling pathways that lead to increased exudation of malate into the apoplast and soil, which also seems to involve root-to-shoot communications. The identification of the guard cell-specific malate-sensitive anion channel raises the possibility that similar proteins might be uncovered, which would help us to understand in more detail how malate homeostasis is regulated at the cellular level and may have important roles in signalling.

Abbreviations

Al, aluminium; ALMT1, aluminium-activated malate transporter 1; SLAC1, slow anion channel-associated 1 protein; STOP1, sensitive to proton rhizotoxicity 1; TCA, tricarboxylic acid.

Competing interests

The authors declare that they have no competing interests.

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