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Abstract

The dicarboxylic acid malate has long been thought to play important roles in plant physiology. In addition to being a major photosynthate in C4 and CAM plants and an intermediate of the tricarboxylic acid cycle it has been proposed to play essential roles in pH regulation and important roles in pathogen response, as a component of the root exudates and as a regulatory osmolyte affecting stomatal function. Recent years have seen the cloning and functional analysis of a wide range of enzymes and transporters associated with malate metabolism. Here we attempt to provide a synthesis of research in this field as well as re-evaluating the role of this metabolite in mediating guard cell function.
Molecules of Interest

Malate. Jack of all trades or master of a few?

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Abstract

The dicarboxylic acid malate has long been thought to play important roles in plant physiology. In addition to being a major photosynthate in C4 and CAM plants and an intermediate of the tricarboxylic acid cycle it has been proposed to play essential roles in pH regulation and important roles as a component of the root exudates and as a regulatory osmolyte affecting stomatal function. Recent years have seen the cloning and functional analysis of a wide range of enzymes and transporters associated with malate metabolism. Here we attempt to provide a synthesis of research in this field as well as re-evaluating the role of this metabolite in mediating guard cell function.
Graphical Abstract

Stomata movement

Root exudates

MALATE

Energy

Fatty acid

Reduction equivalents

CO₂ fixation
pH status
1.0 Introduction.

Malate is an important plant metabolite, it is likely present in all cell types and can accumulate to levels as high as 350 mM (Martonia and Rentsch, 1994). It has been proposed to exhibit multiplicity of functions, however, the evidence for some of these is much stronger than for others. Despite this, malate has clearly been defined to have roles as important photosynthate in CAM and C4 plants (Jia and Chollett, 1991), an essential storage carbon molecule (Martionoia and Rentsch, 1994) and intermediate of the TCA cycle in all plant species (Beevers, 1961; Fernie et al. 2004). It has, furthermore, been defined as a pH regulator (Mathieu et al., 1986) and to exhibit partial control over the efficacy of nutrient uptake (Weisskopf et al., 2006; Pellet et al., 1995) and over stomatal function (Lee et al., 2008; Hedrich and Martin, 1993a; b). The majority of these processes are well characterised at the molecular level, however, considerable recent developments have been made in the last two and we will thus focus our review of these advances. However, control of its intracellular transport has been purported to have two further functions. Firstly, it is believed to facilitate apoplastic NADH production which stimulates the production of the hydrogen peroxide needed to sustain lignin production (Gross et al., 1977). Secondly, the subcellular control of malate levels is used as a strategy to maintain the levels of stromal reductant to support efficient photosynthesis (Backhausen et al., 1994). In this model the chlorplastic NADP-malate dehydrogenase, serves as a redox valve by using excess NADPH to convert OAA to malate in order to regenerate the electron acceptor NADP. Importantly, NADPH activation is inhibited by its own product NADP and thereby switches off its own activity when NADPH is consumed for assimilatory processes in the chloroplast and as such prevents export of reducing equivalents in the form of malate (Scheibe, 1991). Given that ATP production is
maintained whilst electrons are transferred to malate, this valve is a useful “indirect export system” for reducing equivalents that provides an effective means to balance ATP/NADPH stoichiometry of the chloroplast (Backhausen et al., 1998). The existence of such a valve system has been strongly supported by the identification of plastial dicarboxylate translocators which could fulfil the malate-oxaloacetate shuttle function (Menzlaff and Flügge, 1993; Taniguchi et al., 2002; Renne et al., 2003). Moreover, given the fact that cytosolic malate has many potential cellular fates it can either (i) provide NADH for nitrate reduction, (ii) support photorespiration, (iii) utilized by the mitochondria for ATP generation or (iv) be stored in the vacuole, the identification and biochemical characterisation of proteins involved in its metabolism and transport is of vital importance to aid our understanding of the role of this key metabolite.

The recent cloning and characterisation of the vacuolar malate transporter (Emmerlich et al., 2003), as well as the previously mentioned plastidial translocators have facilitated the generation of tools to assess the relative importance of the various poolsizes with Arabidopsis knockout mutants of the vacuolar malate transporter revealing that this transporter is critical for mediating correct compartmentation of the dicarboxylates, with the mutant becoming effectively unable to store malate and that its absence severely compromises cellular pH status of the plant (Hurth et al., 2005; Emmerlich et al., 2003). The mutants, however, still contained a residual malate importing activity suggesting that this gene did not encode the only vacuolar malate transporter, coupled to its biochemical characteristics, it was therefore clear that another gene encoded the biochemically well characterised vacuolar malate channel (see Hafke et al., 2003; Martinoia et al., 1985; 1991; Pantoja and Smith, 2002; Pei et al., 1996). This channel has recently
been identified and characterised at the genetic level (Kovermann et al., 2007), however, deletion mutants of the channel displayed only mild phenotypes. When taken together the results of these studies suggest that the vacuolar malate transporter and malate channel can, at least partially, compensate for the loss of one another. In addition, three dicarboxylate and one dicarboxylate/tricarboxylate carrier of the mitochondrial carrier family, capable of transporting malate, have been identified in Arabidopsis to date (Palmieri et al., 2008; Picault et al., 2002), and recent physiological studies on the malate/oxaloacetate shuttle of isolated wheat mitochondria have indicated that this antiporter limits the rate of NADH oxidation particularly when the external NADH concentration is low (Pastore et al., 2003).

These studies as well as those aimed at assessing the individual functions of various enzymes participating in malate metabolism including the function of various isoforms of malate dehydrogenase (Nunes-Nesi et al., 2005; Cousins et al., 2008; Timm et al., 2008; Backhausen, 1998), malic enzyme (Fahenstich et al., 2007; Maurino et al., 2009) and fumarase (Nunes-Nesi et al., 2007), and further transporter proteins such as the proton dissipating uncoupling protein (Sweetlove et al., 2006), have recently both confirmed and enhanced our knowledge in this area. Important findings of these recent studies were (i) the confirmation of the role of the mitochondria in optimising the rate of photosynthesis, (ii) the demonstration that the peroxisomal malate dehydrogenase is not essential for photorespiration (Cousins et al., 2008; Timm et al., 2008), although it is required to ensure optimal rates of photosynthesis, (iii) plants deficient in the expression of the chloroplastic malate dehydrogenase as well as those deficient in the expression of the mitochondrial uncoupling protein UCP1 provide convincing, although circumstantial, support for the operation of the malate valve, (iv) that malate (and fumarate) are required in considerable amount as respiratory substrate to stave off dark induced senescence and finally (v) that plants
deficient in the expression of fumarase display altered stomatal function. This wealth of new data is allowing us a far more comprehensive idea of how malate regulates and is in turn regulated itself. That said some of the most exciting recent results concern malates extracellular role. In the next sections we review the role of malate in the root exudate and in the guard cell apoplast before providing concluding with a perspective of ongoing research on this enigmatic metabolite.

2.0 On the role of malate as a component of the root exudates

Roots excrete a large number of primary and secondary compounds (Bais et al. 2006; van der Merwe et al., 2009). Among the primary compounds malate is often a predominant metabolite. Malate excretion has attracted much interest in the agronomical field, since it has been observed that it is plays a role in aluminium tolerance, phosphate nutrition and in the establishment of microbial communities.

Aluminium toxicity is critical in acidic soils, which comprise about 50% of the agricultural soil worldwide, since below pH 5 the highly toxic Al$^{3+}$ cation is readily solubilized (Kochian et al. 2004). Al$^{3+}$ very rapidly inhibits root growth and results in reduced water uptake. It has been long observed that aluminium tolerance in plants correlated with the capacity to excrete organic acids such as malate and citrate (see Delhaize et al. 2007 and references therein). Excretion of carboxylates results in chelation of Al$^{3+}$, and consequently rhizodeposition, which prevents that the toxic Al$^{3+}$ cation is taken up by the plant. The first molecular identification of such a trait was achieved by comparing aluminium tolerant and sensitive wheat species (Sasaki et al. 2004). The gene identified encoded a malate channel (called ALMT for Aluminium-activated malate transporter) which is activated by aluminium from the apoplastic side. When the wheat gene was expressed in barley, it conferred Al$^{3+}$ tolerance to
this plant (Delhaize et al. 2004). The fact that the resistance is conferred just by expressing a malate channel, suggests that either cytosolic malate pools can be refilled very rapidly or that the vacuolar malate pool can be used to sustain malate excretion until the metabolism is adjusted to increase malate production. Recently, homologous genes to the wheat ALMT1 have been identified in Arabidopsis (Hoekenga et al. 2006), Brassica napus (Ligaba et al. 2006) and Secale (Fontecha et al. 2007). Interestingly in Arabidopsis ALMT1 is not only activated by Al\(^{3+}\) but the corresponding gene is also upregulated in response to Al\(^{3+}\). Detailed analysis of the wheat ALMT1 revealed that the channel is activated with a Km1/2 of 5\(\mu\)M and is strongly rectifying (Pineros et al. 2008). However, the exact binding site and mechanism of Al\(^{3+}\) channel activation awaits elucidation.

Phosphate is often tightly bound to soil particles or on rocks, however, its availability is increased in most plants by the formation of the mycorrhiza symbiosis (Bonfante and Genre, 2008). That said, some plants, such as Brassicaceae or most members of the Proteaceae and some other plants from diverse families do not form mycorrhiza. In order to improve their phosphate nutrition they excrete carboxylates. Some plants, mainly those of the Proteaceae family form specialized roots to excrete large amounts of carboxylates, so called cluster roots (Neumann and Martinoia, 2001, Shane and Lambers, 2005). These plants excrete predominantly citrate, but malate plays also an important role. The molecular nature of the malate release protein remains elusive, however, it is tempting to speculate that an ALMT is involved in this process. In white lupin, young roots excrete mainly malate, older roots and mature cluster roots, mainly citrate. Since it was observed that ATP-citrate lyase is highly expressed in young roots compared to older roots, it was postulated that this enzyme could be
responsible for the switch of root excretion from malate to citrate and have an impact of the nature of the carboxylate excreted (Langlade et al. 2002). Recently it has been shown that the excretion of malate attracts beneficial soil bacteria (Rudrappa et al. 2008). Leaves treated with the pathogen DC3000 induced root excretion of malate. This effect was much weaker when a Mock solution or a non-pathogen was used. DC3000 induced also malate excretion when applied to the roots, most probably through AtALMT1, since DC3000 had a similar effect on the induction of this ALMT as Al$^{3+}$. Malate excreted by the roots in turn attracted beneficial bacteria such as *Bacillus subtilis*.

### 3.0 On the role of malate in stomatal function

Similar to the situation described above for root exudation, malate has long been discussed as an important regulator of stomatal opening and is thought to be an integral part of the mechanism by which guard cells adjust their action in response to external CO$_2$ concentrations (Roêfsema et al., 2002, Van Kirk and Raschke, 1978; Hedrich and Marten, 1993a, b; Hedrich et al., 1994; Raschke et al., 2003). However, in contrast to root exudation malate is the only carboxylic acid proposed to play a major role in this process. That said both K$^+$ and Cl$^-$, as well as the phytohormone abscisic acid (ABA) and redox signals envoked by shifts in light quality, have additionally been demonstrated to be important in stomatal control (Raghavendra 1990, Schroeder and Hagiwara, 1989; Kearns and Assmann, 1993; Pei et al., 2000; Li et al., 2000; Chen and Gallie, 2004) and there was in fact considerable support for these factors having higher importance than malate. It should therefore be borne in mind that malate is only one of a range of cellular factors which is capable of regulating stomatal function. That said a series of recent papers have provided fresh support for the importance of malate in regard to the normal operation of stomata.
(Lee et al., 2008; Negi et al., 2008; Vahisalu et al., 2008). These studies focussed on either the ABC transporter AtABCB14 or the SLAC1 transporters both of which mediate transfer of malate between the apoplast and the cytosol. Plants lacking the ABCB14 transporter exhibited elevated closure on transition to elevated carbon dioxide levels in comparison to wild type controls. Moreover, in isolated epidermal strips that contained only guard cells, malate-dependent stomatal closure was faster in plants lacking the ABCB14 transporter and slower in plants overexpressing it indicating that this transporter catalyses the transport of apoplastic malate into the guard cells. Furthermore, heterologous expression of ABCB14 in E. coli and HeLa cells resulted in increases in malate transport confirming the role of the transporter (Lee et al., 2008). Similarly, the function of the SLAC1, slow S-type anion, channel was recently characterised (Negi et al., 2008; Vahisalu et al., 2008). This channel has been demonstrated to mediate CO2 sensitivity to gas exchange. The SLAC1 protein is a distant homolog of bacterial and fungal dicarboxylate transporters and is specifically localised to the plasma membrane of guard cells. A loss of function mutation in this protein was paralleled with an over accumulation of osmoregulatory anions in guard cell protoplasts, however, guard-cell specific expression of SLAC1 or its close homologs complemented the phenotype (Negi et al., 2008; Vahisalu et al., 2008). Intriguingly, mutations in SLAC1 were detailed to impair slow anion channel currents that are activated by cytosolic Ca2+ and ABA but do not affect either rapid anion channels or Ca2+ channel function (Vahisalu et al., 2008). Further, albeit indirect, evidence for the importance of malate in stomatal function was provide by the study of tomato plants deficient in the expression of fumarase, which catalyses its production in the mitochondrial. These plants exhibited a dramatic growth impediment and reduced rate of photosynthesis which could be attributed to a defective stomatal function with the rate of opening of the stomata being dramatically
compromised in the transgenic lines (Nunes-Nesi et al., 2007). Whilst in these plants the overall malate levels were not dramatically altered, the subcellular compartmentation of malate was not studied. When taken together, these findings identify the molecular mechanisms by which malate can affect guard cell functioning and likely represent the most convincing evidence, presented as yet, of its physiological role in plants.

4.0 Concluding remarks

Whilst the central importance of malate in plant metabolism and physiological processes has long been recognised, research in the post genomic era has allowed us far greater insight into mechanistic aspects of the biochemical and molecular mechanisms that underlie both the regulation of subcellular malate concentrations and the effects which it, in turn, has on diverse cellular processes. Due to space constraints we chose to focus on root exudation and stomatal function. However, there is also a wealth of important literature on its role in C4 and CAM metabolism as well as in fruit quality and development we refer the interested reader to (Kalt et al., 1990; Cushman and Bohnert, 1999; Brautigam et al., 2008; Carrari et al., 2006; Hawker, 1969) for further details. To summarize our article we think it is fair to say that great advances have been made in understanding the control of, and by, malate in the last few years. There remains, however, much still to be elucidated. Even in Arabidopsis there are many open questions concerning both cellular and whole plant partitioning of malate, precise details of its control of stomates and its function in delaying senescence as well as its contribution to cellular pH regulation. Despite its breadth of function we believe that malate can be described as a master tradesman in C3 plants. The release of the first genome sequence for a C4 plant (sorghum; Paterson et al., 2009), as well as the nascent plant sequencing projects for maize
and CAM species such as *Kalanchoe fedtschenko*
(http://www.liv.ac.uk/researchintelligence/issue35/pdfs/ri35pp4-5.pdf)
suggest that we will shortly be able to address similar questions in plant species
which utilize malate in a radically different manner. A more detailed knowledge about
root malate excretion should not only help to improve Al\(^{3+}\) tolerance and phosphate
nutrition but also to modulate the microflora in order to strengthen the plant.
Furthermore, since most nitrogen fixing bacteria prefer carboxylates as carbon
source malate excretion may also attract these beneficial bacteria and facilitate a
reduced nitrogen fertilizer input in agriculture.

References

acceptors in photosynthesis – regulation of the malate valve during CO2 fixation and
nitrite reduction. Photo Res 42, 75-86.

Backhausen, J.E., Emmerlich, A., Holtgreve, S., Horton, P., Nast, G., Roggers, J.J.M.,
Müller-Röber, B. (1998). Transgenic potato plants with altered expression levels of
chloroplast NADP-malate dehydrogenase: interactions between photosynthetic
electron transport and malate metabolism in leaves and in isolated intact
chloroplasts. Planta 207, 105-114.

exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant
Biol 57, 233-266.


evolutionary-developmental perspective.

of chloroplast envelopes from C3 and C4 plants reveals specific adaptations of the
plastid envelope to C4 photosynthesis and candidate proteins required for

Carrari, F., Baxter, C., Usadel, B., Urbanczyk-Wochniak, E., Zanor, M.I., Nunes-Nesi,
(2006). Integrated analysis of metabolite and transcript levels reveals the metabolic
shifts that underlie tomato fruit development and highlight regulatory aspects of metabolic network behaviour. Plant Physiol 142, 1380-1396.


Hedrich, R., Marten, I. (1993a). Malate-induced feedback regulation of anion channels could provide a CO2 sensor to guard cells. EMBO J 12, 897-901.


mitochondrial fumarase activity in tomato plants impairs photosynthesis via an effect on stomatal function. Plant J 50, 1093-1106.


