Malate valves: old shuttles with new perspectives

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ABSTRACT
Malate valves act as powerful systems for balancing the ATP/NAD(P)H ratio required in various subcellular compartments in plant cells. As components of malate valves, isoforms of malate dehydrogenases (MDHs) and dicarboxylate translocators catalyse the reversible interconversion of malate and oxaloacetate and their transport. Depending on the co-enzyme specificity of the MDH isoforms, either NADH or NADPH can be transported indirectly. Arabidopsis thaliana possesses nine genes encoding MDH isoenzymes. Activities of NAD-dependent MDHs have been detected in mitochondria, peroxisomes, cytosol and plastids. In addition, chloroplasts possess a NADP-dependent MDH isoform. The NADP-MDH as part of the ‘light malate valve’ plays an important role as a poised mechanism to adjust the ATP/NADP ratio in the stroma. Its activity is strictly regulated by post-translational redox-modification mediated via the ferredoxin-thioredoxin system and fine control via the NADP+/NADP(H) ratio, thereby maintaining redox homeostasis under changing conditions. In contrast, the plastid NAD-MDH (‘dark malate valve’) is constitutively active and its lack leads to failure in early embryo development. While redox regulation of the main cytosolic MDH isoform has been shown, knowledge about regulation of the other two cytosolic MDHs as well as NAD-MDH isoforms from peroxisomes and mitochondria is still lacking. Knockout mutants lacking the isoforms from chloroplasts, mitochondria and peroxisomes have been characterised, but not much is known about cytosolic NAD-MDH isoforms and their role in planta. This review updates the current knowledge on MDH isoforms and the shuttle systems for intercompartmental dicarboxylate exchange, focusing on the various metabolic functions of these valves.

INTRODUCTION
The redox state provides information on the metabolic status of a plant cell, which is needed for fast adjustment of various metabolic activities in response to continuously changing environmental conditions and developmental requirements (Dietz 2003; Foyer & Noctor 2003; Scheibe et al. 2005). Since major imbalances in the redox state can cause severe damage, plant cells possess different redox sensors such as proteins with reversibly oxidisable thiols (e.g. cytosolic gyceraldehyde-3-phosphate dehydrogenase, cyclophilin 20–3) which detect deviations from redox homeostasis and activate poising mechanisms, thus avoiding the generation of excess reactive oxygen species (ROS; Scheibe & Dietz 2012; Park et al. 2013; Hildebrandt et al. 2015; Noctor & Foyer 2016).

Chloroplasts possess a light-dependent regulatory mechanism, namely the ferredoxin-thioredoxin system (Fd-Trx system), controlling the activities of various key enzymes involved in assimilatory and dissimilatory pathways (Buchanan 1984; Buchanan & Balmer 2005). Reduced Trx subsequently reduces regulatory disulfide bonds of various target enzymes, thereby altering their catalytic activities. Upon illumination, various Calvin-Benson cycle (CBC) enzymes are activated via the Fd-Trx system, whereas oxidative pentose phosphate pathway (OPPP) enzymes, such as glucose-6-phosphate dehydrogenase, become inactive. The light/dark modulation of various chloroplast proteins via the Fd-Trx system functions to control synthesis and degradation of carbohydrates to prevent futile cycling and waste of energy, by adjusting enzyme activities at the post-translational level.

The regulation of energy metabolism is particularly complex in plants. On the one hand, ATP and NAD(P)H are generated in a number of reactions that are localised in different subcellular compartments. During illumination, photosynthesis and respiration generate the energy carrier ATP and the reducing equivalents NADPH and NADH in chloroplasts, cytosol and mitochondria, respectively, in green parts of the plant, whereas glycolysis, respiration and the OPPP produce these energy molecules in the dark and in non-green tissues in various compartments. In turn, NAD(P)H and ATP are required for all energy-consuming reactions, such as assimilation of C, N, S and all other cellular activities, both during photoautotrophic and heterotrophic growth. However, the delivery of NAD(P)H and ATP for various energy-consuming reactions should occur at the required rates and in the specific compartments due to the rather limited pool sizes of these energy sources (Scheibe 2004).
Plant membranes are essentially impermeable to NAD(P)+ and NAD(P)H. Therefore, plant cells possess specific translocators enabling the direct and indirect exchange of reducing equivalents. On the one hand, NAD(P)+ can directly be shuttled between subcellular compartments via NAD+-carrier proteins localised in mitochondria as well as in plastids (Palmieri et al. 2009). However, these transporters exhibit a low affinity for NADH and NADPH (Palmieri et al. 2009; Maurino & Engqvist 2015). On the other hand, plant cells possess specific translocators for the exchange of dicarboxylates such as malate and oxaloacetate (malate/OAA shuttles; Fig. 1). Malate is a versatile compound in plant metabolism (Lance & Rustin 1984; Maurino & Engqvist 2015) that can easily be transported across subcellular membranes and can be used as a substrate for mitochondrial ATP production or provision of NADH to the cytosol (Fig. 1). Malate/OAA shuttles acting in combination with malate dehydrogenases (MDHs) as key enzymes (also termed malate valves) connect compartments. They are powerful systems for balancing metabolic fluxes by enabling indirect transfer of reducing equivalents, and therefore, they play a key role in plant metabolism.

A multigene family encodes malate dehydrogenases in *Arabidopsis*

The NAD-dependent MDH activities have been detected in chloroplasts, mitochondria and peroxisomes, as well as in the cytosol (Gietl 1992; Berkemeyer et al. 1998). Besides the non-redox regulated NAD-MDH, chloroplasts of higher plants and green algae additionally contain a redox-modulated NADP-dependent MDH (Scheibe & Anderson 1981).

All eukaryotic MDH isoforms can be traced back to two ancestral genes (Ocheretina & Scheibe 1997; Ocheretina et al. 2000). One of the two ancestral genes represents the origin of cytosolic NAD-dependent MDH isoenzymes (cyNAD-MDH 1–3) and the NADP-MDH in chloroplasts (Fig. 2). These isoforms arose through duplication of the ancestral gene. The second subfamily includes NAD-MDHs of mitochondria (mtNAD-MDH 1–2), plastids (plNAD-MDH) and peroxisomes (pNAD-MDH 1–2), which resulted from triplication of a pre-existing mitochondrial MDH gene and major changes within their target sequences (Fig. 2).

Within each subfamily the amino acid identity of the mature proteins is above 50%. Between the two subfamilies, amino acid identity lies at about 21–23%. However, MDHs possess...
similar tertiary and quaternary structures and almost identical catalytic properties (McAlister-Henn 1988). Each precursor protein of the organellar MDH isoforms possesses a characteristic N-terminal transit peptide (Gietl & Hock 1982). For instance, the plNAD-MDH from Arabidopsis was originally identified by its unique transit peptide of 80 amino acids despite the high identity with coding sequences of the cytosolic isoforms (Berkemeyer et al. 1998; Imsande et al. 2001).

The light malate valve in chloroplasts is strictly regulated

The NADP-MDH is strictly regulated by light through the Fd-Trx system. The NADP-MDH is present as a homodimer which possesses eight cysteine residues per subunit. Two cysteine pairs are located in the N- and C-terminal extensions, whereas the other four cysteine residues are present in the core of the protein (Crétin et al. 1990). The redox-sensitive cysteine pairs in the N- and C-terminal regions are involved in the light-dependent redox modulation of the NADP-MDH via the Fd-Trx system during photosynthesis; upon removal of one or the other sequence extension, the enzyme is constantly active (Heineke & Scheibe 2009). For instance, the activation of NADP-MDH is only enabled when NADPH increases because it is not consumed in the CBC, e.g. due to a lack of CO₂ or ATP. Under these conditions, the NADP-MDH uses the excess NADPH generated via the photosynthetic electron transport chain to convert OAA to malate, regenerating the electron acceptor NAD⁺. An increased level of NAD⁺ in turn acts as a negative effector for reductive activation of the NADP-MDH, in this way turning down its activity. This fine control guarantees that reducing equivalents are only exported to other subcellular compartments via the malate/OAA shuttle when they are in excess, and when they are not required in the chloroplast for assimilatory processes (Scheibe 1987, 1991; Backhausen et al. 2000). Upon a sudden increase in light intensity, NADP-MDH activity rapidly
increases within less than a minute and subsequently oscillates until the new steady-state is reached (Scheibe & Stitt 1988). Such hysteretic response is characteristic for this type of post-translational regulation.

Although NADP-MDH is known to function as a poising mechanism avoiding the generation of ROS under high light, nadp-mdh knockout mutants are indistinguishable from the wild type under various stress conditions when grown in soil. This is due to alternative systems such as the NADP-dependent thioredoxin reductase (NTRC)/2-Cys-peroxiredoxin system, as well as proline biosynthesis, photosynthesis and nitrate assimilation in nadp-mdh knock-out mutants contributing to the maintenance of redox homeostasis when malate valve capacities are lacking (Hebbelmann et al. 2012; Selinski & Scheibe 2014).

Upon sustained high-light intensity, an increase in the expression level of the nuclear encoded NADP-MDH points to a signal transfer from the chloroplast to the nucleus (Becker et al. 2006). The nature of such a signal is still not yet clarified, but a transient increase in H₂O₂ has been put forward as a potential signal since plants lacking NADP-MDH do not show such an increase (Heyno et al. 2014). Transfer of the signal might occur via oxidative modification of GapC (Hildebrandt et al. 2015). The glycolytic GapC, possibly in its ‘moonlighting’ function, had been found to bind to the NADP-MDH gene (Hameister et al. 2007).

It is conceivable that in vivo kinetic effects override the thermodynamic equilibrium of the NADP-MDH reaction. In particular, when fluctuating conditions give rise to immediate deviations from steady state. Then high pressure on the side of influx, e.g. light intensity, shifts the equilibrium towards efflux, namely the reduction of OAA. Literally, the excess electrons are then pushed through the malate valve. Malate is used for further reactions outside the chloroplasts where various options are possible, in the worst case, the dissipation of energy in the alternative oxidase pathway in the mitochondria. The flux of reductant through the valve will continue as long as the pressure from inside the chloroplast exists and keep it open. Such control is achieved by fine-tuning of NADP-MDH activation state by the NADP⁺/NADP(H) ratio in the chloroplast, with NADP⁺ hindering activation.

Factors that sustain a steady homeostatic flux through the CBC also include the controlled export of reducing equivalents from the chloroplast in the form of malate as required for balancing the ATP/NADPH ratio (Fridlyand et al. 1998; Fridlyand & Scheibe 1999). Due to the rapid responses of redox-regulated enzymes, such as NADP-GAPDH, NADP-MDH and others, to changing metabolite levels, homeostasis is maintained in a stable non-equilibrium state by thermodynamic buffering (Igamberdiev & Kleczkowski 2011). The system is kept in such a stable and, at the same time, flexible state as long as energy from light comes in and product is taken out. Even reactions close to the thermodynamic equilibrium are able to limit homeostatic fluxes when their total capacities are decreased in transgenic plants (Fridlyand & Scheibe 2000). Using the kinetic data available for the fine-control of NADP-MDH, a model has been developed that supports the function of this enzyme to control the indirect export of NADPH (Fridlyand et al. 1998).

The dark malate valve in plastids is constitutively active

In addition to the NADP-dependent MDH, chloroplasts contain a redox-independent activity of NADP-MDH functioning as part of the dark malate valve and playing an important role in dark metabolism. Non-green plastids also contain such plNAD-MDH. In these plastids and in chloroplasts during darkness, ATP and NADPH for anabolism are generated independently via plastidial glycolysis (ATP), and plastidial OPP (NADPH). The dark malate valve is here required to export the NADH that is formed during glycolysis at the substrate phosphorylation step catalysed by PGK and GAPDH. NADH production in this step occurs by either the bispecific CBC enzyme GapA/B in chloroplasts (Baalmann et al. 1995) or GapCp in plastids of non-green tissues (Backhausen et al. 1998; Muñoz-Bertomeu et al. 2009, 2010). Such oxidation of triose phosphates coupled with ATP generation requires a continuous supply of NAD⁺. The regeneration of NAD⁺ which allows for continued ATP production via glycolysis is possible due to the activity of plNAD-MDH for indirect export of NADH in the form of malate. In addition, ATP can be imported into plastids via ATP/ADP transporters of the NTT type (Kampfenkel et al. 1995; Neuhaus et al. 1997; Möhllmann et al. 1998; Tjaden et al. 1998; Reiser et al. 2004). However, although these ATP/ADP transporters exhibit high affinities for ATP and ADP, their capacities are low (Vmax for ATP (NTP1) = 24 nmol-mg protein⁻¹ h⁻¹; Vmax for ATP (NTP2) = 7 nmol-mg protein⁻¹ h⁻¹; Möhllmann et al. 1998). Therefore, an indirect mechanism for ATP import in plastids is more favourable, e.g. via shuttling of glycolytic intermediates such as glucose 6-phosphate (G-6-P) or phosphoenolpyruvate (PEP). These intermediates can be transported from the cytosol into plastids or vice versa via G-6-P/P, translocators or PEP/P, translocators (Facchinelli & Weber 2011). Homozygous plnad-mdh knock-out mutants do not exist. This is either due to compromised male or female gametophyte development (pollen or egg cell) in haploid stages of the plant or impaired embryo development and seed maturation in diploid stages. A total knock-out of plNAD-MDH that is present in 50% of the haploid pollen grains of heterozygous plnad-mdh/plNAD-MDH mutants causes impaired pollen tube growth in vitro, which can be overcome by adding substrates of NADH-dependent glutamine-2-oxoglutarate aminotransferase (GOGAT; Selinski et al. 2014). However, pollen tube growth does not appear to be affected in vivo. This indicates that the maternal tissue in the transducing tract of the style can supply the NADH-GOGAT substrates, in this way enabling pollen tube elongation. Although NADH-GOGAT was shown to be an alternative pathway maintaining redox homeostasis in heterozygous plnad-mdh/plNAD-MDH plants and plnad-mdh knock-out pollen when the capacity of the dark malate valve is lacking, NADH-GOGAT cannot compensate for the homozygous knock-out of plnad-mdh during embryogenesis leading to an embryo-lethal phenotype (Beeler et al. 2014; Selinski et al. 2014). Heterozygous plnad-mdh/plNAD-MDH mutants are able to grow, flower and set seed, but their siliques contain green as well as white seeds during the ripening process. Microscopic analysis of green and white seeds revealed that green seeds contain developed embryos at all developmental stages whereas white seeds exclusively contain embryos in the globular stage, independent of the developmental stage of the silique. These white seeds develop into tiny, shrunken and non-vital.
seeds that can easily be lost during seed cleaning procedures. Accordingly, the ratio of green to white seeds was determined as 3:1, which correlates with the theoretical ratio of 1:2:1 for wild types (green seeds) to heterozygotes (green seeds) to homozygotes (white seeds) after selfing.

The lack of chlorophyll in homozygous *plnad-mdh* knock-out seeds might be due to competition for glutamate, which is the precursor for chlorophyll biosynthesis as well as the substrate for the chloroplast 2-oxoglutarate (2-OG)/malate transporter AtOMT1 together with the dicarboxylate transporter DCT1. OM1T and DCT1 play important roles in both the malate valve and the 2-OG/malate shuttle for nitrate assimilation (Taniguchi et al. 2002; Kinoshita et al. 2011). Glutamine and 2-OG must be provided to the plastid in exchange for glutamate. Although an increased flux through the NADH-GOGAT pathway maintains redox homeostasis when substrate glutamate. Although an increased flux through the

As described above, excess reducing equivalents from the chloroplast are indirectly transferred as malate to the cytosol and to other subcellular compartments to avoid imbalances or lack of electron acceptors that would lead to oxidative stress (Scheibe 2004). The interconversion of malate to OAA with concomitant reduction of NAD⁺ to NADH can provide reducing equivalents into ATP or, in the worst case, energy dissipation as heat (Huang et al. 2018). Peroxisomal MDHs are involved in photorespiration and in fatty acid β-oxidation

Leaf peroxisomes and glyoxysomes in lipid-storing tissues are the two main types of microbodies in plant cells. Peroxisomes rely on reducing equivalents imported from mitochondria or chloroplasts by the malate-OAA shuttles, since they do not possess any metabolic pathways capable of delivering NADH at the high rates required *e.g.* for photorespiration. Reducing equivalents are imported into peroxisomes through the uptake of malate and its conversion to OAA by plNAD-MDH. In glyoxyxomes, the pNAD-MDH is essential for fatty acid β-oxidation in germinating *A. thaliana* seeds. In leaf peroxisomes, it is needed to maintain optimal rates of photorespiration by providing NADH required for the reduction of hydroxypyruvate to glycercate by peroxisomal hydroxypyruvate reductase (HPR; Cousins et al. 2008; Prucharoenwattana et al. 2010; Timm et al. 2011). Glycerate can be transported back to the chloroplast where it is phosphorylated to yield 3-phosphoglycerate, which can re-enter the CBC (Ogren 1984; Leegood et al. 1995; Boldt et al. 2005; Bartsch et al. 2010; Walker et al. 2016).

Mitochondrial MDHs play an important role in photorespiration and the provision of NADH for respiration

Photorespiration results in NADH formation in plant mitochondria due to the oxidation of glycine by the glycerate dehydrogenase complex (GDC). NADH can enter the respiratory electron transport chain, supporting ATP synthesis. Coupling of malate oxidation by mtNAD-MDH with GDC and the pyruvate dehydrogenase complex (PDC) reaction, respectively, in mitochondria helps to maintain high fluxes, apparently by recycling NAD⁺ required as electron acceptors for decarboxylating glycine and pyruvate oxidation in both complexes (Lindén et al. 2016). It has been discussed that mtNAD-MDH could play an important role in decreasing the concentration of NADH and relieving its inhibitory effect on PDC (Jambordev et al. 2014), or mtNAD-MDH could recycle the NADH from glycine oxidation to keep NADH concentrations low and thus prevent substrate inhibition of GDC (Bykova et al. 2014).

Furthermore, mtNAD-MDH was found to lower leaf respiration, probably based on cross-talk between mtNAD-MDH and the cytochrome *bc* complex (Wang et al. 2010). It also appears to alter the rate of photorespiration as well as plant development, demonstrating its important role in energy metabolism and redox homeostasis (Tomaz et al. 2010; Wang et al. 2010; Lindén et al. 2016). Proteomic analyses revealed that mtNAD-MDH 1 significantly accumulates during *Arabidopsis* seed germination (Fu et al. 2005), and transcript abundance of both mtNAD-MDH isoforms significantly increases in early stages of seed germination (Weitbrecht et al. 2011). In accordance with analyses of plNAD-MDH mutants, mtNAD-MDH was shown to play an important role in the earliest phases of the *Arabidopsis* life cycle (Beeler et al. 2014; Selinski et al. 2014; Sew et al. 2016). Recently, mtNAD-MDH 1 was shown to be insensitive to thiol-based redox control (*in vitro* and *in vivo*) but its activity is lowered by adenine nucleotides, especially ATP (Yoshida & Hisabori 2016). Mitochondrial NAD-MDH was demonstrated to play a significant role in buffering the redox state in the mitochondrial matrix due to its equilibrium properties (Hagedorn et al. 2004). For any calculation of *in vivo* concentrations of NADH, NAD⁺ and metabolite concentrations, it is essential to take into account the fact of ‘molecular crowding’ in the cell, resulting in binding of ‘small molecules’ to proteins and to changed concentrations of free
soluble compounds (Srere 1980). In an update on the current approaches for metabolic flux analyses, factors are compiled that determine the specific properties of a metabolic network as far as its stability and capability to maintain homeostasis is concerned (Nikolosi et al. 2015). In particular, the authors point out that more data are required so that the interrelation-ship of metabolite levels, fluxes and enzyme properties can be integrated into mathematical models.

**Plant cells possess various dicarboxylate transporters in chloroplasts and mitochondria**

The compartmentation of plant cells requires the transport of metabolites across intracellular membranes catalysed by specific membrane translocators. Especially, the inner membranes of chloroplasts and mitochondria represent the permeability barrier for metabolites and co-enzymes and are equipped with various transporters that are specific for the various substrates. The *Arabidopsis* genome encodes a number of dicarboxylate transporters catalysing the transfer of dicarboxylates across the chloroplastidic or mitochondrial membranes (Heineke et al. 1991; Weber et al. 2004; Weber & Fischer 2007; Linka & Weber 2010; Facchinelli & Weber 2011; Fischer 2011; Flügge et al. 2011; Haferkamp & Schmitz-Esser 2012; Lee & Millar 2016).

The two plastid-localised dicarboxylate translocators (DiTs) are involved in primary ammonium assimilation and the recycling of ammonium that is lost during photorespiration. DiT1 (also termed OMT1, 2-OG/malate-translocator) imports the precursor of ammonium assimilation, 2-OG, in exchange for malate. DiT2 (also termed DCT, glutamate/malate-translocato) exports glutamate in exchange for malate (Fig. 3; Weber et al. 1995; Weber & Flügge 2002; Renne et al. 2003; Schneider et al. 2006; Riebeseel et al. 2010). In addition to its high affinity for 2-OG and malate, DiT1 displays a high affinity for OAA and has been proposed as the OAA/malate valve (Taniuchi et al. 2002). The role as malate valve for DiT1 has been proven by mutant analyses, showing that cytosolic OAA is efficiently transported into chloroplasts mainly by DiT1 (Fig. 3; Kinoshita et al. 2011; Taniuchi & Miyake 2012).

In parallel to plastids, mitochondria contain various dicarboxylate translocators. The transport of specific dicarboxylates and tricarboxylates across the inner mitochondrial membrane is required for several metabolic processes, such as primary amino acid synthesis, export of reducing equivalents, fatty acid metabolism, gluconeogenesis and isoprenoid biosynthesis. The plant mitochondrial dicarboxylate/tricarboxylate carrier (DTC) mediates the transport of dicarboxylates (2-OG, OAA, malate and succinate) and tricarboxylates (citrate, isocitrate and aconitate) by counter-exchange (Fig. 3; Picault et al. 2002). Furthermore, plant mitochondria possess three dicarboxylate carriers (DIC1–3). These transporters facilitate the transfer of dicarboxylates, such as malate, OAA and succinate, as well as phosphate, sulphate and thiosulphate across the inner mitochondrial membrane. Accordingly, DICs may play an important role in the import of dicarboxylates into mitochondria in exchange for phosphate or sulphate to replenish the tricarboxylic acid cycle (TCAC). In addition, DICs might function as malate/OAA shuttles providing other cell compartments with reducing equivalents (Palmeri et al. 2008). Another dicarboxylate transporter, SFC, has been identified in plant mitochondria (Fig. 3). This carrier catalyses the transfer of succinate and fumarate across the inner mitochondrial membrane (Catoni et al. 2003). The main product of the glyoxylate pathway, succinate, is imported via SFC into mitochondria where it can enter the TCAC. In contrast, fumarate is exported to the cytosol in exchange for succinate entering gluconeogenesis after oxidation to malate (Catoni et al. 2003).

On both sides of the membranes carrying all of these dicarboxylate shuttle systems, the various MDH isoforms will convert OAA or malate as they arrive on the other side. Due to its equilibrium constant ($K_{eq} = 2.86 \times 10^{-5}$ at pH 7.0), MDH favours the reaction towards malate formation, i.e. the reverse of what is required to regenerate OAA in the mitochondria. Therefore, only removal of the products enables the correct direction for the TCAC. On the other hand, malate is formed in chloroplasts when excess NADPH is used by NADP-MDH for indirect export of reducing equivalents to the cytosol where it is either reconverted to OAA, when NADH is used for nitrate reduction, or transported to the mitochondria. In both examples, the direction of the enzyme reaction depends on input of substrate and removal of product in a steady-state situation, but not its equilibrium constant. The transporters generally function as exchange carriers in both directions, driven by the concentration gradient of the respective substrates on both sides. They strictly function as shuttles, however, without any restriction as far as regulation or energy requirements are concerned, only limited by their $V_{max}$ and the specificity towards the transported range of substrates. They help to connect the fluxes of energy-consuming and energy-converting pathways between the different cellular compartments. Together with the MDH isoforms in each compartment that are operating close to equilibrium, the dicarboxylate translocators support thermodynamic buffering of the cell.

**Assimilation of N is an important backup system maintaining redox homeostasis when plastidial malate valve capacities are lacking or limiting**

The removal of photorespiratory ammonium (NH$_4^+$), which is formed during the oxidation of glycine to serine, is essential for survival of a plant. The primary assimilation of NH$_4^+$ results from the collaborative activity of glutamine synthetase (GS) and GOGAT (GS/GOGAT cycle). GS catalyses the ATP-dependent assimilation of NH$_4^+$ into glutamate, using glutamate as a substrate. Subsequently, GOGAT catalyses the reductive transfer of the amido group from glutamine to 2-OG, resulting in the formation of two molecules of glutamate, one of them starting the assimilatory cycle again (Fig. 4). In chloroplasts of higher plants, two major classes of GOGAT enzymes are present, namely the Fd-dependent and the NADH-dependent GOGAT, which use either Fd or NADH as a reductant for the interconversion of glutamine and 2-OG to glutamate (Fig. 4). While NADH-GOGAT is crucial for NH$_4^+$ assimilation in heterotrophic tissues, Fd-GOGAT, representing the predominant form in leaves, contributes to the assimilation of NH$_4^+$ derived from photorespiration (Lancien et al. 2002; Konishi et al. 2014).

The lack of malate valve capacities leads to improved growth when nadp-mdh or pnah-mdh/pnAD-MDH *Arabidopsis* mutants are grown on nitrate or on ammonium, respectively, as the sole N source (Hebbelmann et al. 2012; Selinski et al. 2014). Under these conditions, Fd-GOGAT transcripts are
Fig. 3. Shuttles for dicarboxylate transport. Transport processes of dicarboxylates across the inner envelope of plastids and the inner mitochondrial membrane are depicted. 2-OG, 2-oxoglutarate; DCT (= DiT2), glutamate/malate translocator; DIC, decarboxylate carriers; DTC, dicarboxylate/tricarboxylate carrier; OAA, oxaloacetate; OMT (= DiT1), 2-OG/malate-translocator, malate/OAA translocator; SFC, succinate/fumarate carrier.

Fig. 4. Relationship between plastidial malate valves and NH₄⁺ assimilation. In the light, Fd-GOGAT represents an alternative electron acceptor for Fd, and therefore, counteracts accumulation of excess NADPH generated by the Fd-NADPH reductase (FNR). In the dark, NADH-GOGAT represents an alternative to pLNAD-MDH, since both are able to regenerate NAD⁺ to maintain continuous ATP production via plastidial glycolysis. G6P, glucose 6-phosphate; Glu, glutamate; Glu, glutamine; Glu, glutamate; GS, glutamine synthetase; mtETC, mitochondrial electron transport chain; NTT, plastidial ATP/ADP transporter; OMT, malate/OAA translocators; OPPP, oxidative pentose phosphate pathway.
increased in nadp-mdh knockout plants, and NADH-GOGAT transcripts are more abundant in heterozygous \textit{plnadh-mdh/plNAD-MDH} mutants (Selinski & Scheibe 2014). In the case of \textit{nadp-mdh} knockout mutants, nitrite reductase as part of nitrite assimilation and Fd-GOGAT which contributes to the assimilation of NH$_4^+$ derived from photorespiration, serve as alternative electron acceptors for reduced Fd and increase nitrite assimilation, thereby preventing, at the same time, the accumulation of excess NADPH (Hebelmann et al. 2012; Selinski & Scheibe 2014). Correspondingly, when the dark malate valve capacity is decreased, NADH-GOGAT, as a key enzyme of NH$_4^+$ assimilation, serves as an alternative mechanism maintaining redox homeostasis by removing NADH generated by GapCp during plastidial glycolysis (Fig. 4; Selinski et al. 2014; Selinski & Scheibe 2014).

Measurements of mitochondrial respiration in spinach leaves indicate that NADH formed inside mitochondria is oxidised by a malate/OAA shuttle to serve extra-mitochondrial processes, e.g. reduction of nitrate in the cytosol or of hydroxyypyruvate in the peroxisomes (Hanning & Heldt 1993). Furthermore, malate oxidation was shown to be one of the major sources of NADH for nitrate reduction in corn leaf blades (Neyra & Hageman 1976) that is catalysed by cytosolic NAD-MDH isoforms.

The malate/OAA shuttle, which itself is reversible depending on the concentrations of OAA and malate on both sides of the membrane, functions as a ‘thermodynamic buffer’ that maintains the chloroplast NADP$^+/NADPH$ ratio constant over a wide range of conditions. This corresponds to the concept put forward for the adenylate ratio for the first time by Stucki (1980). Malate valves are, therefore, important to communicate between cellular compartments and to optimise photosynthesis (Raghavendra & Padmasree 2003).

CONCLUSION

Malate valves, consisting of malate dehydrogenases and dicarboxylate transporters, are essential features for balancing energy metabolism and maintaining redox homeostasis in plants. In combination, they serve as thermodynamic buffers. The presence of different MDH isoforms in various subcellular compartments and their distinct roles enable plants to adapt to various stress conditions as well as to control growth and development. Although there has been much progress in unravelling roles and regulation of MDH isoforms in plants in recent years, knowledge about detailed mechanisms and controlled interplay between the systems at the various regulatory levels is still lacking in some cases.

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