



**HAL**  
open science

## Physiological functions of malate shuttles in plants and algae

Ousmane Dao, Franziska Kuhnert, Andreas P.M. Weber, G. Peltier, Yonghua Li-Beisson

► **To cite this version:**

Ousmane Dao, Franziska Kuhnert, Andreas P.M. Weber, G. Peltier, Yonghua Li-Beisson. Physiological functions of malate shuttles in plants and algae. Trends in Plant Science, 2021, 10.1016/j.tplants.2021.11.007 . hal-03453418

**HAL Id: hal-03453418**

**<https://hal.science/hal-03453418>**

Submitted on 28 Nov 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

1 **Physiological functions of malate shuttles in plants and algae**

2

3 Ousmane Dao<sup>1</sup>, Franziska Kuhnert<sup>2</sup>, Andreas P. M. Weber<sup>2</sup>, Gilles Peltier<sup>1</sup>, Yonghua Li-  
4 Beisson<sup>1\*</sup>

5

6 <sup>1</sup>Aix Marseille Univ, CEA, CNRS, BIAM, Institut de Biosciences et Biotechnologies Aix-  
7 Marseille, CEA Cadarache, Saint Paul-Lez-Durance 13108, France

8 <sup>2</sup>Institute of Plant Biochemistry, Cluster of Excellence on Plant Science (CEPLAS), Heinrich  
9 Heine University, 40225 Düsseldorf, Germany

10

11

12 \*: Author for correspondence: Yonghua Li-Beisson (email: [yonghua.li@cea.fr](mailto:yonghua.li@cea.fr); Tel:  
13 +33(0)442254483)

14

15 **ORCID IDs:** 0000-0002-7040-5770 (O.D.); 0000-0002-0374-6671 (F.K.); 0000-0001-5545-  
16 7583 (A.P.M.W.); 0000-0002-2226-3931 (G.P.); 0000-0003-1064-1816 (Y.L.-B.)

17

18

19

20

21

22 **Key words:**

23 Dicarboxylate translocator; Redox Trafficking; Malate Dehydrogenase; CO<sub>2</sub> concentration  
24 mechanism; Photorespiration; Reactive Oxygen Species

25

26

## 1 **Glossary**

2 **CO<sub>2</sub> Concentrating Mechanism (CCM):** CCM, as the name implies, is a process of  
3 concentrating CO<sub>2</sub> to the active site of rubisco, the major protein of the carbon photo-  
4 reduction cycle (i.e. often called Calvin-Benson-Bassham cycle). It is therefore considered a  
5 mechanism to reduce the rate of photorespiration. Depending on species, CCM could refer to  
6 the dicarboxylic acid cycle in C<sub>4</sub> plants, CAM-based CCM in CAM plants, carboxysome  
7 based-CCM in cyanobacteria and the biophysical CCM occurring in algae (the conversion of  
8 CO<sub>2</sub> to bicarbonate and its transport to the pyrenoid).

9

10 **Cyclic Electron Flow (CEF):** CEF refers to the recycling of electron from the acceptor side  
11 of photosystem I through the cytb<sub>6</sub>/f complex, increasing the proton motive force (*pmf*). The  
12 *pmf* is then used to drive ATP synthesis. The CEF promote ATP synthesis without additional  
13 NADPH production and is therefore considered a way to increase the ATP/NADPH ratio in  
14 response to metabolic or environmental adaptations. Several pathways of CEF have been  
15 characterized: (1) the plant type I NADPH dehydrogenase complex (NDH); (2) the type II  
16 NDH (NDA2) unique to algae; and (3) the proton gradient regulator 5 (PGR5)/PGR like 1  
17 (PGRL1)-mediated CEF pathway, which is common between plants, algae and cyanobacteria.

18

19 **Fatty Acid  $\beta$ -Oxidation:** One of the major mechanisms of fatty acid degradation. Oxidation  
20 of fatty acids can occur either at the  $\alpha$ -,  $\beta$ - or  $\omega$ - carbon position of a given fatty acid, among  
21 which  $\beta$ -oxidation is found as a major pathway in plants and algae, therefore termed fatty acid  
22  $\beta$ -oxidation. This pathway ultimately breaks down fatty acids to acetyl-CoAs, which are  
23 further metabolized either for energy production through the mitochondrial electron transport  
24 chain, or for synthesis of sugars when coupled to glyoxylate and gluconeogenesis pathways.

25

26 **Glyoxylate Cycle:** An anabolic pathway occurring widely in plants, bacteria, fungi, and  
27 algae. It allows the conversion of acetyl-CoA to succinate for the synthesis of carbohydrates,  
28 therefore allowing microorganisms to utilize two carbon compounds as carbon source. For  
29 example, it plays an essential role in the heterotrophic growth of green algae on acetate. It  
30 shares several steps of tricarboxylic acid (TCA) cycle but bypasses the steps that release CO<sub>2</sub>.

31

32 **Malate Shuttle:** Malate shuttle refers to the means of redox trafficking from one subcellular  
33 compartment to another through malate transport. It is made of two major protein components  
34 i.e. malate dehydrogenases and di- or tri-carboxylate translocators.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15

**Photorespiration:** a metabolic pathway occurring in organisms performing oxygenic photosynthesis where the major enzyme rubisco can fix either CO<sub>2</sub> or O<sub>2</sub> depending on their stoichiometric ratio. The oxygenation reaction produces 2-phosphoglycolate, a toxic metabolite, which is detoxified by conversion to the CBC intermediate 3-phosphoglycerate. This conversion involves a set of reactions occurring in four subcellular compartments i.e. chloroplast, cytosol, peroxisome, and mitochondria, and it entails the loss of CO<sub>2</sub>, NH<sub>4</sub><sup>+</sup> and energy in the process.

**Pseudo-Cyclic Electron Flow (CEF):** Pseudo-CEF is a pathway where electrons generated from water splitting at PSII are used to reduce O<sub>2</sub> to H<sub>2</sub>O, thereby driving photosynthetic electron flow and generating a proton gradient, without NADPH production. In green algae, O<sub>2</sub>-photoreduction is mostly mediated by the flavodiiron (FLV) proteins.

1 **Abstract:**

2

3 Subcellular compartmentalization confers evolutionary advantage to eukaryotic cells but entails  
4 the need for efficient inter-organelle communication. Malate functions as redox carrier and  
5 metabolic intermediate. It can be shuttled across membranes through translocators. The  
6 interconversion of malate and oxaloacetate mediated by malate dehydrogenases requires  
7 oxidation/reduction of NAD(P)H/NAD(P)<sup>+</sup>, therefore malate trafficking serves to transport  
8 reducing equivalents, termed ‘malate shuttle’. Although the term “malate shuttle” has been  
9 coined more than 50 years ago, novel functions are still emerging. This review highlights recent  
10 findings on the functions of malate shuttles in photorespiration, fatty acid β-oxidation, inter-  
11 organelle signaling and its putative role in CO<sub>2</sub>-concentrating mechanisms. We compare and  
12 contrast knowledge in plants and algae, thereby providing an evolutionary perspective on redox  
13 trafficking in photosynthetic eukaryotes.

14

15 **Malate as a major cellular redox carrier**

16 Eukaryotic cells are compartmentalized, and distinct subcellular organelles house specific  
17 subsets of metabolic reactions that are physically separated from each other by biological  
18 membranes. Exchange of information and energy between compartments mostly mediated by  
19 metabolites are indispensable to achieve whole-cell homeostasis and ensure optimal growth  
20 [1,2]. While some of these exchanges occur through passive diffusion, most of them are  
21 mediated by transporters [3]. Understanding the complex interplay of energy and information  
22 between subcellular compartments is key towards the domestication of photosynthetic  
23 organisms for tailor-made production of valuable compounds.

24 Malate is a C4 dicarboxylic acid and is involved in a number of metabolic pathways,  
25 including the tricarboxylic acid (TCA), C4-dicarboxylic acid, and **glyoxylate cycles** in plants  
26 and algae [4–6]. Malate can be produced by malate synthase (MAS) and metabolized by two  
27 types of enzymatic reactions: (i) malic enzyme (ME), or (ii) malate dehydrogenase (MDH)  
28 (**Box 1**). Amongst these, only MDH catalyzes a reversible redox reaction that is coupled to the  
29 reduction/oxidation of NAD(P)<sup>+</sup>/NAD(P)H, while inter-converting malate and oxaloacetate  
30 (OAA). In contrast to the main cellular redox-couple, i.e. the pyridine nucleotides NAD(P)(H),  
31 malate can be efficiently transported across membranes through di- or tri-carboxylate  
32 translocators. Although specific transporters of NAD<sup>+</sup> are present in the membrane of  
33 chloroplast, mitochondria, or peroxisome [7,8], their activity is restricted in importing NAD<sup>+</sup>  
34 into organelles and probably important in de novo loading of organelles with NAD<sup>+</sup> pool. But

1 these transporters have shown a very low affinity for NAD(P)H [9], therefore probably not  
2 sufficient for redox exchange between compartments. Malate trafficking thus serves as an  
3 indirect but efficient way of transporting reducing equivalents.

4 Trafficking of malate between different subcellular compartments has been extensively  
5 studied in plants (**Box 2**) [10]. The inner membrane of chloroplast and mitochondria of plant  
6 and algal cells are equipped with several types of di- and tri-carboxylate translocators catalyzing  
7 malate transport in counter-exchange with, e.g., OAA, glutamate, or aspartate. It can function  
8 in either direction depending on the concentration gradient resulting from a metabolic demand  
9 [11,12]. Collectively, the trafficking of malate through specialized translocators from one  
10 compartment to another combined with the malate processing enzymes (MDHs) is termed  
11 “**malate shuttle**” [10].

12 Although the concept of “malate shuttle” has been coined over 50 years ago,  
13 components of this pathway remain hypothetical in microalgae, whereas considerable progress  
14 has been made in land plants [10]. Depending on the type of carboxylates being transported,  
15 several variations of “malate shuttle” occur, i.e. malate/OAA shuttle, malate/aspartate shuttle  
16 (**Box 3**), or malate/2-oxoglutarate shuttle. These various types of malate shuttles, their  
17 subcellular locations and involvement in different metabolic pathways are illustrated in **Figure**  
18 **1**.

19 Here, we review some of the most recent discoveries on the roles of malate shuttles in  
20 connecting carbon, nitrogen, and energy metabolism in plants and algae. While isoforms of  
21 MDHs and di-/tri-carboxylate translocators have been well characterized in land plants,  
22 evidence is just emerging regarding their functions in algae. We provide a comparative analysis  
23 of the corresponding proteins between the model plant arabidopsis (*Arabidopsis thaliana*) and  
24 the model green alga chlamydomonas (*Chlamydomonas reinhardtii*) (**Table 1**). We discuss the  
25 malate shuttle’s physiological importance in photosynthesis, **photorespiration**, and **fatty acid**  
26 **β-oxidation** and we point to its possible implication in algal **CO<sub>2</sub> concentrating mechanisms**  
27 (CCM). Finally, we highlight the role of malate shuttles in inter-organelle communication  
28 through interaction with reactive oxygen species (ROS)-mediated signal transduction.

### 30 **Malate shuttle as a valve for photosynthetic electron dissipation**

31 During the linear electron flow (LEF) of photosynthesis, light energy is converted by two  
32 photosystems (PSII and PSI) into chemical energy in the form of NADPH and ATP. ATP and  
33 NADPH are mostly used to drive metabolic reactions, particularly CO<sub>2</sub> fixation by Rubisco and  
34 its conversion into triose phosphates through the Calvin-Benson-Bassham (CBB) cycle [13].

1 However, the LEF is known to produce an insufficient amount of ATP (as compared to  
2 NADPH) than required for optimal CO<sub>2</sub> photo-reduction, leading to an imbalance [14]. The  
3 imbalance in ATP and NADPH could be further accentuated by changes in environmental  
4 conditions. For example, under CO<sub>2</sub> limiting conditions, photorespiration in plants or a CCM  
5 in algae requires additional ATP [14,15]. Excess reducing equivalents could potentially lead to  
6 ROS over-production [16]. Coordination between production and usage of ATP and NAD(P)H  
7 is therefore essential to cell fitness and survival in a changing environment.

8 ATP and NADPH balance can be achieved by increasing ATP production in the  
9 chloroplast or through import, or by lowering NADPH level through enhancing sink strength  
10 or export to other compartments (**Figure 2**). Several mechanisms are involved in the supply of  
11 extra-ATP, which include **cyclic electron flow** (CEF) around PSI, or the O<sub>2</sub>-photoreduction by  
12 flavodiiron proteins (FLV) also known as **pseudo-CEF**, or ATP import from mitochondria  
13 [17]. On the other hand, NADPH dissipation can occur either through enhanced metabolic  
14 demand (e.g. boosting de novo fatty acid synthesis which has high need for reducing  
15 equivalents), or through its export to other subcellular compartments by the “malate shuttle”  
16 [18].

17 Despite having been hypothesized for several decades, the role of malate shuttle in  
18 photosynthetic electron exportation (as a “malate valve”) has only recently been demonstrated.  
19 In arabidopsis, the knock-out mutants in plastidial NADP-MDH (*nadp-mdh*) possessed a more  
20 reduced plastoquinone pool but with only slight impairment in growth under high light (HL)  
21 [19,20]. Strong growth defects, however, were observed under fluctuating light in a mutant in  
22 which one of its regulatory redox switches at its C-terminus was deleted by genome editing  
23 [21]. The carboxylate translocator involved in the ‘malate valve’ (AtOMT1) has also been  
24 characterized in arabidopsis. AtOMT1 is a high-affinity OAA translocator, whose absence led  
25 to the hyper-accumulation of reducing power in the stroma, photoinhibition, and impaired  
26 growth under HL [22]. Taken together, above studies demonstrate the essential role of the  
27 malate valve in chloroplast energy homeostasis, which is particularly important to balance the  
28 chloroplast redox status during fluctuating conditions.

29 In algae, none of the components of the chloroplast ‘malate valve’ have been  
30 characterized at a physiological level. The chlamydomonas CrMDH5, the homolog of the  
31 arabidopsis NADP-MDH, has been studied in vitro [23]. The thioredoxin (TRX)-dependent  
32 redox regulation of the CrMDH5 activity is much faster (responding in an all-or-nothing way)  
33 than the plant homolog but the latter is fine-tuned due to the presence of an additional N-  
34 terminus disulfide regulatory domain. Even though its physiological function has not been

1 shown directly, it was proposed that the “malate valve” may operate in the chlamydomonas  
2 mutant lacking the proton gradient like 1 (PGRL1)-mediated CEF in conditions of strong  
3 ATP/NADPH imbalance [24]. It was hypothesized that the malate valve helps to evacuate  
4 excess reducing equivalents towards cytoplasm and mitochondria. Nevertheless, direct  
5 evidence on the operation of the malate valve in algae is yet to come.

6 It is worth bearing in mind that the “malate valve” in the chloroplast could also function  
7 in importing reducing power into the chloroplast. Responses of photosynthesis and chloroplast  
8 metabolism to changes in redox state in other subcellular compartments (mitochondria or  
9 peroxisome) seem to support such a possibility [25,26]. Our understanding of redox  
10 homeostasis in the chloroplast is further complicated by the presence of both NAD<sup>+</sup>- and  
11 NADP<sup>+</sup>-dependent MDHs and the co-existence of two pyridine nucleotide pools (NADH versus  
12 NADPH) (discussed in **Outstanding Questions**).

### 13 14 **The role of malate shuttle in plant photorespiration**

15 Photorespiration, initiated by the oxygenation reaction of Rubisco, is inevitable in an oxygen  
16 containing atmosphere. The rate of photorespiration is reduced in land plants performing C4  
17 photosynthesis and in algae having a CCM [27]. Photorespiration consists of multiple metabolic  
18 reactions distributed over four subcellular compartments: chloroplast, cytosol, peroxisome and  
19 mitochondria, and requires therefore intimate inter-organelle communication. Photorespiratory  
20 reactions have a strong impact on the cellular energetics, producing NADH in the mitochondria  
21 where two glycine molecules are converted to serine, but consuming NADH in the peroxisome  
22 where hydroxypyruvate is converted to glycerate (**Figure 1**). Therefore, NADH homeostasis in  
23 mitochondria and peroxisome is critical for optimal functioning of photorespiration. Further,  
24 changes in leaf internal CO<sub>2</sub> concentration, as a consequence of altered stomatal aperture, can  
25 impact redox status in the respective organelles through photorespiration. Indeed, in land plants,  
26 malate shuttle components (i.e. pNADP-MDH, pNAD-MDH, DiT1 and DiT2.1) are  
27 upregulated upon acclimation to low CO<sub>2</sub>, and downregulated under elevated CO<sub>2</sub> (where  
28 photorespiration is suppressed) suggesting their possible implication in the adaptation of  
29 cellular metabolism in response to fluctuating CO<sub>2</sub> availability [28,29].

30 The malate shuttle contributes to photorespiration at multiple levels (**Figure 1**). It is  
31 involved in the transamination of glyoxylate into glycine coupled with the conversion of  
32 glutamate to 2-oxoglutarate in the peroxisome catalyzed by the glutamate: glyoxylate  
33 aminotransferase (GGT). Plastidial malate/2-oxoglutarate shuttle provides glutamate from  
34 chloroplast that serves as a donor of NH<sub>3</sub> for the transamination of glyoxylate [30]. Arabidopsis



1 DiT2.1, but not DiT2.2, is a glutamate/malate translocator involved in photorespiration [31].  
2 And moreover, DiT2.1 and DiT1 (AtpOMT1) work in concert for the optimal functioning of  
3 photorespiration, and the lack of either DiT2.1 or DiT1 led to growth impairment under  
4 photorespiratory conditions [22,31,32].

5 The malate shuttle is further involved in the export of NADH produced during glycine  
6 decarboxylation in mitochondria, thus recycling NAD<sup>+</sup> and ensuring proper functioning of  
7 photorespiration. The decrease of mNAD-MDH activity in the *mmdh1* knockout mutant had a  
8 strong effect on photorespiratory flux, leading to a reduced growth under photorespiration-  
9 promoting conditions (i.e. very low-CO<sub>2</sub>) [33]. In fact, the *mmdh1* mutant displayed a high  
10 glycine/serine ratio indicative of impaired glycine decarboxylation due to the lack of the  
11 electron acceptor NAD<sup>+</sup> and an over-reduction in the mitochondria. The homeostasis of  
12 NAD<sup>+</sup>/NADH can be achieved either by increasing the consumption of NADH by  
13 mitochondrial reactions or by exporting the excess NADH to other compartments. A likely  
14 route for exporting NADH from glycine decarboxylation is through a malate/aspartate shuttle  
15 toward peroxisomes where NADH could be consumed by the hydroxypyruvate reductase  
16 (HPR), another key reaction of photorespiration (**Figure 1**). The aspartate/glutamate  
17 translocators involved in this malate/aspartate shuttle have been recently characterized in  
18 arabidopsis as belonging to the uncoupling protein (UCP) family [11]. Both AtUCP1 and  
19 AtUCP2 are able to efficiently counter-exchange aspartate and glutamate [11]. Similar to  
20 *mmdh1*, mutants of AtUCP1 are impaired in photorespiration with a reduced rate of glycine  
21 oxidation in the mitochondria [11]. Therefore, AtUCP1&2 and mNAD-MDH1&2 work  
22 together in photorespiration by forming the malate/aspartate shuttle that connects glycine  
23 decarboxylation in the mitochondria with hydroxypyruvate reduction in the peroxisome. We  
24 note that redox equivalents derived from photorespiratory glycine decarboxylation in  
25 mitochondria may not be necessarily shuttled to the peroxisome but could potentially fuel  
26 nitrate and/or hydroxypyruvate reduction in the cytoplasm [34,35]. Antisense repression of  
27 plastidial OMT1/DiT1 in tobacco however indicates a prominent role of the plastidial malate  
28 shuttle in supplying nitrate reduction with NADH [36]. Therefore, the contribution of  
29 mitochondria derived NADH in nitrate reduction in the cytosol could only be minor.

30 Finally, at the step of hydroxypyruvate reduction into glycerate by the peroxisomal  
31 HPR, pNAD-MDH can oxidize malate imported from the cytosol to provide additional reducing  
32 equivalents needed for HPR. Double mutants of pNAD-MDH1 & 2 were however viable under  
33 photorespiratory conditions (i.e. ambient CO<sub>2</sub>), but, the stoichiometry of photorespiratory CO<sub>2</sub>  
34 generated per Rubisco oxygenation was increased by 50% as compared to wild type [37]. This

1 was explained by an alternative pathway which was thought to be a non-enzymatic conversion  
2 of hydroxypyruvate to glycolate thereby releasing extra CO<sub>2</sub>. Timm et al [35] showed that  
3 *HPR2* encodes for a cytosolic hydroxypyruvate reductase activity (i.e., not peroxisomal) and  
4 that reduction of hydroxypyruvate can flexibly shift between peroxisomes and cytoplasm.  
5

### 6 **A putative role of malate shuttle in algal CCM**

7 To cope with the low CO<sub>2</sub> to O<sub>2</sub> ratio, unlike plants where photorespiration plays a significant  
8 role, microalgae frequently employ a CCM. The algal biophysical CCM is an energetic  
9 mechanism that pumps and sequesters atmospheric CO<sub>2</sub> into the pyrenoid close to the active  
10 site of Rubisco, key to algae's proliferation in their natural habitat where CO<sub>2</sub> level could be  
11 extremely low [27]. In the past 10 years, enzymes (carbonic anhydrases), transcription factors  
12 (CCM1) and also several inorganic carbon (Ci) transporters involved in CCM have been  
13 identified and characterized in chlamydomonas [38,39]. Known transporters include the HL  
14 activated 3 (HLA3) protein that is localized at the plasma membrane, a low-carbon inducible  
15 protein (LCIA) in the chloroplast envelope, and recently the thylakoid localized bestrophin-like  
16 (BST) transporters [40–43]. However, not until recently has the energization mechanism of the  
17 CCM begun to be unveiled.

18 By studying double mutants of chlamydomonas lacking both PGRL1 and FLV-B  
19 mediating the CEF and pseudo-CEF respectively, Burlacot et al [15] showed that double  
20 mutants are impaired in growth under air (i.e. low-CO<sub>2</sub> condition) as was the reduced affinity  
21 of photosynthesis of the double mutants for Ci. The authors concluded that the proton motive  
22 force produced through the combined action of CEF and pseudo-CEF mechanisms favors the  
23 conversion of bicarbonate anions transported by the thylakoid localized BSTs into CO<sub>2</sub> at the  
24 active site of the carboxylating enzyme Rubisco. By showing that inhibitors of mitochondrial  
25 respiration also decrease the affinity of photosynthesis for Ci, Burlacot et al [15] further  
26 proposed that mitochondrial respiration serves as an alternative source of energy powering  
27 distant transporters involved in CCM (located either at the plasma membrane or at the  
28 chloroplast envelope). Considering that CCM is a light driven process, it is thus highly plausible  
29 that the malate shuttle contributes to CCM through mediating redox exchange between  
30 chloroplast and mitochondria (**Figure 2**).

31 Furthermore, implication of the malate shuttle in CCM is supported by RNAseq-based  
32 transcriptome analyses, revealing the upregulation of putative chloroplastic 2-OG/malate  
33 translocators and MDHs in chlamydomonas and also in four phytoplankton species when  
34 transferred from high to low CO<sub>2</sub> condition [44–46]. Nevertheless, molecular actors and

1 experimental evidence for such a function is yet to come, e.i., by investigating the Ci affinity in  
2 mutants impaired in malate shuttle mediating the redox exchange between chloroplast and  
3 mitochondria.

#### 5 **Malate shuttle mediates inter-organelle signaling through ROS**

6 Inter-compartmental exchange of signals is a key for cellular homeostasis and the acclimation  
7 of photosynthetic organisms in fluctuating environments [47]. ROS signaling has been  
8 considered a powerful system for regulation of gene expression, leading to a cascade of  
9 physiological adjustments, such as induction of programmed cell death (PCD) [48,49]. ROS  
10 can be generated in four major subcellular compartments, i.e., chloroplast, peroxisome,  
11 mitochondria and cytosol. ROS are produced when O<sub>2</sub> serves as alternative electron acceptor  
12 during photosynthesis or mitochondrial respiration electron transfer and intimately linked to  
13 peroxisome-based fatty acid β-oxidation or photorespiration. ROS signaling has therefore been  
14 found to connect organelle redox state between chloroplast and mitochondria, and between  
15 chloroplast and peroxisome [19,49,50]. Below we discuss such examples.

16 ROS generation in the mitochondria was shown to trigger PCD in the arabidopsis mutant  
17 *mod1* (for *mosaic death 1*) affected in the chloroplast enoyl-ACP reductase (ENR) catalyzing  
18 the NADH-dependent enoyl-ACP reduction in the fatty acid synthase (FAS) complex [51]. A  
19 screen for *mod1* suppressors identified various components of the malate shuttle involved in  
20 chloroplast to mitochondrion communication, including pINAD-MDH, DiT1 and mNAD-  
21 MDH1 [50]. Fatty acid biosynthesis is a major sink for reductant in the chloroplast [52].  
22 Therefore, impairment in ENR leads to over-accumulation of NADH in the chloroplast. The  
23 pINAD-MDH in conjunction with DiT1 allow the export of reducing power in the form of  
24 malate. The translocator responsible of the malate import in mitochondria is still unknown.  
25 Nevertheless, once inside mitochondrion, malate oxidation by the mNAD-MDH1 generates  
26 NADH to fuel mitochondrial complex 1, ultimately resulting in the production of ROS [50].  
27 This mitochondria-generated ROS has been considered as the signaling molecule in triggering  
28 PCD in the *mod1* mutant cells [50].

29 Inter-organelle communication from chloroplast to peroxisome has also been proposed  
30 to play a role in retrograde signaling in arabidopsis [19]. Under conditions where the  
31 photosynthetic chain becomes over-reduced due to the limitations of the electron acceptor  
32 NADP<sup>+</sup>, molecular oxygen (O<sub>2</sub>) can be used to accept electron coming from PSI [53].  
33 Photoreduction of O<sub>2</sub> can occur via two routes, i.e. the non-enzymatic reaction (also known as  
34 Mehler reaction) [54,55] and the enzymatic reduction of O<sub>2</sub> [56,57]. In the Mehler reaction, the

1 transfer of electrons from PSI acceptor side to O<sub>2</sub> is associated with the generation of superoxide  
2 O<sub>2</sub><sup>-</sup> that could be converted to H<sub>2</sub>O<sub>2</sub> by superoxide dismutase (SOD) and then detoxified by the  
3 peroxidase located in the chloroplast such as ascorbate peroxidase (APX). H<sub>2</sub>O<sub>2</sub> can also diffuse  
4 out into other compartments where it can be detoxified, for instance, in the peroxisome by  
5 catalase (CAT) [58]. H<sub>2</sub>O<sub>2</sub> is a cellular messenger and has been shown to play an important role  
6 as signaling agent [59]. However, to take up such a role, the intracellular concentration of H<sub>2</sub>O<sub>2</sub>  
7 must be sufficient to induce the expression of H<sub>2</sub>O<sub>2</sub>-responsive nuclear genes. The reversible  
8 inactivation of CAT in the peroxisome, allowing a burst in H<sub>2</sub>O<sub>2</sub>, requires the import of  
9 reducing equivalents originating from the chloroplast through malate shuttle to peroxisome  
10 [19,60]. This reversible inactivation of CAT was abolished in the arabidopsis *nadp-mdh*  
11 mutants [19], indicating the important role of the light malate valve (i.e. plNADP-MDH) in the  
12 regulation of CAT activity by transmitting the redox state of the chloroplast to the peroxisome.

13 A H<sub>2</sub>O<sub>2</sub>-based signaling pathway from peroxisome to chloroplast has also been  
14 proposed recently in chlamydomonas. The chlamydomonas mutants deficient in the  
15 peroxisomal MDH2 exhibited a higher rate of photosynthesis, starch, and *de novo* fatty acid  
16 biosynthesis in the chloroplast during photoautotrophic nitrogen deprivation [25].  
17 Communication between the two organelles is proposed to be mediated by a H<sub>2</sub>O<sub>2</sub>/malate-  
18 dependent signaling pathway as supported by an elevated amount of H<sub>2</sub>O<sub>2</sub> and malate in the  
19 *mdh2* knockout mutants. Exogenously supplied H<sub>2</sub>O<sub>2</sub> has a strong positive effect on the  
20 expression of starch- and lipid- related genes in wild-type cells of chlamydomonas [61],  
21 corroborating that the increase in starch and lipid in the *mdh2* mutants resulted from the increase  
22 in H<sub>2</sub>O<sub>2</sub> intracellular concentration [25]. Collectively, the malate shuttle represents a redox-  
23 poise system communicating the redox-state between compartments involving H<sub>2</sub>O<sub>2</sub>-mediated  
24 signaling pathway.

25

### 26 **Malate shuttle connects fatty acid catabolism to chloroplast metabolism**

27 Fatty acid β-oxidation, photorespiration, and glyoxylate cycle occur either totally or partially  
28 in the peroxisome, making it a third subcellular compartment involved in energy metabolism  
29 after chloroplast and mitochondrion [62–64]. In addition, peroxisomes house reactions that  
30 produce H<sub>2</sub>O<sub>2</sub>. Fatty acid degradation and glyoxylate cycle generate reducing equivalents as  
31 NADH inside the peroxisome, whereas photorespiration consume it. Therefore, CO<sub>2</sub>  
32 availability, by modulating the activity of photorespiration, can potentially impact these  
33 metabolic pathways. Plants and algae have therefore evolved several mechanisms that regulate  
34 NADH homeostasis in the peroxisome. This could be achieved either indirectly through the use

1 of metabolite translocators involving the malate shuttle or directly through the import of NAD<sup>+</sup>  
2 via the NAD<sup>+</sup> carrier or through NAD<sup>+</sup> regeneration by local antioxidant system [7,65,66]. In  
3 germinating castor bean, the transfer of NADH generated by fatty acid  $\beta$ -oxidation from  
4 peroxisome to the mitochondria was postulated to occur through a malate/aspartate shuttle [67].  
5 In arabidopsis, both direct or indirect routes occur, which include a peroxisomal NAD<sup>+</sup> carrier  
6 (PXN) belonging to the mitochondria carrier family (MCF) [7], the two peroxisomal MDHs  
7 (pMDH1, pMDH2) [68], the photorespiratory HPR [69], or the components of the ascorbate  
8 peroxidase/monodehydroascorbate reductase (APX/MDAR) electron transfer system [70]. The  
9 occurrence of these multiple mechanisms suggests the importance of the redox balance control  
10 in peroxisomes. Indeed, mutants deficient in either of these pathways have difficulty in seed  
11 germination, seedling rigor or optimal growth.

12 The occurrence of plant-like peroxisomes has initially been debated in chlamydomonas,  
13 and this was mostly built on two observations i) the lack of a crystalloid core under electron  
14 microscope, typically observed in plant- or animal-type of peroxisomes; and ii) the likely  
15 location of catalase in mitochondria based on organelle purification studies [71]. But recent  
16 data have firmly established that chlamydomonas does have classical peroxisomes, and  
17 evidence are i) peroxisomes have been observed in chlamydomonas both under confocal as well  
18 as by electron microscopy [72,73]; ii) the typical peroxisomal targeting signal (PTS1/2) is  
19 functional in Chlamydomonas [73]; iii) the subcellular localization studies for enzymes of  
20 glyoxylate cycle [4]; iv) the identification of a H<sub>2</sub>O<sub>2</sub>-producing activity i.e. acyl-CoA oxidase  
21 in peroxisome and its role in fatty acid  $\beta$ -oxidation [63]; v) catalase 1 is found to be peroxisomal  
22 in chlamydomonas [74]; and finally genes encoding homologs of the arabidopsis proteins  
23 involved in redox homeostasis (MDH, PXN, HPR, and MDAR) or in fatty acid  $\beta$ -oxidation are  
24 present [75].

25 That said, except for MDH2, experimental evidence for the function of the other proteins  
26 is still lacking. The chlamydomonas *mdh2* mutants, similar to its arabidopsis counterpart (the  
27 *pmdh1pmdh2* mutants), were compromised in oil remobilization during nitrogen recovery  
28 following a period of N deprivation [25], firmly establishing its contribution to fatty acid  $\beta$ -  
29 oxidation in green algae. Interestingly, in addition to peroxisome metabolism, metabolic  
30 responses have also been observed in the chloroplast, i.e., enhanced photosynthetic activity,  
31 and enhanced starch and *de novo* fatty acid biosynthesis under high CO<sub>2</sub> condition (2% of CO<sub>2</sub>  
32 supplementation to the air). It is worth mentioning here that the phenotypes of the  
33 chlamydomonas *mdh2* mutants were suppressed under atmospheric CO<sub>2</sub> level (photorespiratory  
34 condition), pointing to the complex contribution of diverse pathways to redox management and

1 homeostasis. Nevertheless, the exact route or the molecular identity of the peroxisomal  
2 translocators involved remain to be identified (see **Outstanding Questions**).

3

#### 4 **Concluding remarks and future perspectives**

5 Because variations in environmental parameters may differentially affect the different cellular  
6 functions involved in bioenergetics of photosynthetic cells, which are located in different  
7 subcellular compartments, plants and algae have evolved efficient trafficking of reducing  
8 equivalents between subcellular compartments to maintain redox homeostasis. Among these  
9 mechanisms, the “malate shuttle” enables efficient transport of reducing equivalents between  
10 chloroplasts, cytosol, mitochondria and peroxisomes. Research in the past decades has shed  
11 light on the role of malate shuttle in key subcellular pathways including photosynthesis,  
12 photorespiration, fatty acid  $\beta$ -oxidation, nitrogen assimilation, and inter-organelle signaling,  
13 but has also raised new questions (detailed in **Outstanding Questions**). Beyond physiology  
14 and energy homeostasis, emerging literature suggests a critical role for the malate shuttle in the  
15 CO<sub>2</sub> concentration mechanism [15] and carbon storage [25].

16 Future investigations towards detailed understanding of malate shuttle mechanism in  
17 plants and algae should involve the combination of cutting-edge molecular genetics tools for  
18 creating combinatory mutations in different genes, overexpressing multiple isoforms combined  
19 with the use of <sup>13</sup>C labeling techniques to monitor changes in metabolic fluxes. We envision  
20 that emerging genetically encoded biosensors to monitor real-time changes in subcellular redox  
21 and ATP status will be valuable to monitor in real time redox status in subcellular compartments  
22 [76,77]. Taken together, by connecting energy homeostasis to carbon and nitrogen metabolism,  
23 the malate shuttle represents an exciting target for engineering the cellular energetic status for  
24 better climate resilience and for improved production of plant and algal biomass for food, fuel  
25 and biomaterials, key to a sustainable and greener future.

26

#### 27 **Acknowledgements:**

28 O. Dao thanks CEA for a PhD scholarship. G. Peltier and Y. Li-Beisson thanks the continuous  
29 financial support of CEA (LD-power, CO2Storage) for supporting research in her laboratory.  
30 F. Kuhnert and A.P.M. Weber acknowledge funding by the Deutsche Forschungsgemeinschaft  
31 (DFG, German Research Foundation) – Project-ID 267205415 – CRC 1208 and under  
32 Germany’s Excellence Strategy EXC-2048/1, Project ID 390686111.

33

1 **Table 1. Malate shuttle main components in arabidopsis and chlamydomonas<sup>a</sup>**

Protein family	Chlamydomonas	Phytozome ID	Arabidopsis	TAIR ID	Localization	Refs.
Malate dehydrogenases	CrMDH1	Cre03.g194850	cpNAD-MDH	AT3G47520	Chloroplast (arabidopsis) Peroxisome* (chlamydomonas)	[4,50,78–80]
	CrMDH2	Cre10.g423250	pMDH1	AT2G22780	Peroxisome	[25,37,68]
			pMDH2	AT5G09660		
	CrMDH3	Cre02.g145800	cyNAD-MDH1	AT1G04410	Cytosol	[81,82]
			cyNAD-MDH2	AT5G43330		
			cyNAD-MDH3	AT5G56720		
	CrMDH4	Cre12.g483950	mMDH1	AT1G53240	Mitochondria	[33,83,84]
mMDH2			AT3G15020			
CrMDH5	Cre09.g410700	cpNADP-MDH	AT5G58330	Chloroplast	[19,20,23]	
Malate translocators	CrOMT1	Cre17.g713350	DiT1 (OMT1)	AT5G12860	Chloroplast	[22,32,50]
	CrOMT2	Cre17.g713200				
	CrLCI20	Cre06.g260450	DiT2.1 (DCT1)	AT5G64290		
			DiT2.2 (DCT2)	AT5G64280		
	CrMiTC14	Cre16.g672650	DIC1	AT2G22500	Mitochondria	[11,85,86]
			DIC2	AT4G24570		
			DIC3	AT5G09470		
DTC			AT5G19760			

2 Abbreviations: Cr, *Chlamydomonas reinhardtii*; ‘pl’, plastidial; ‘p’, peroxisome; ‘cy’, cytosol  
3 and ‘m’, mitochondria. \* Refers to a putative localization of CrMDH1 in the peroxisome  
4 based on fusion of N-terminus transit peptide with fluorescent tag.

5 <sup>a</sup>Homology between chlamydomonas and arabidopsis proteins were identified through  
6 reversible BlastP search of TAIR (for arabidopsis) and Phytozome v13 (for chlamydomonas)  
7 proteins. Arabidopsis protein sequences were used first as baits. Note that the function  
8 assigned to mitochondrial malate translocators in arabidopsis is only based on in vitro  
9 experiments except for DIC2 which has recently been characterized in vivo [87]. Other  
10 players in the malate shuttle can be found in the **Box 2 and 3**.

11  
12  
13  
14  
15  
16  
17

1

## 2 **Box 1. Malate metabolism in photosynthetic cells**

3 Malate is a versatile compound and their cellular metabolism involve malate synthase (MAS),  
4 malic enzyme (ME), or malate dehydrogenase (MDH) (**Figure I**). MAS catalyzes the formation  
5 of malate from acetyl-CoA and glyoxylate, which is a key step in the glyoxylate cycle [4,88].  
6 The glyoxylate cycle allows cells to utilize two-carbon compounds (C2) such as acetate, and it  
7 is essential for the heterotrophic growth of some algae such as chlamydomonas [89]. The  
8 concerted action of MAS and isocitrate lyse (ICL) in the glyoxylate cycle forms succinate that  
9 enters the mitochondrial TCA cycle, bypassing the steps that result in carbon loss [4,64].  
10 ME catalyzes the decarboxylation of malate to pyruvate, resulting in a production of NAD(P)H  
11 and CO<sub>2</sub>. This reaction has been shown as an important source of reducing equivalents for de  
12 novo fatty acid synthesis as well as desaturation in fungi [90], plants [91] and more lately in  
13 algae [92]. It is also a crucial reaction in C<sub>4</sub> and CAM (Crassulacean acid metabolism)  
14 photosynthetic metabolism [93].

15 MDHs are the only enzymes catalyzing a reversible reaction, important for its function  
16 in modulating redox status. In arabidopsis, nine genes encoding MDHs are present in the  
17 genome and activities of MDH could be detected in most subcellular compartments including  
18 mitochondrion (mNAD-MDH1, 2), peroxisome (pNAD-MDH1, 2), cytosol (cyNAD-MDH1,  
19 2, 3) and plastid (pNAD-MDH and pNADP-MDH) [10]. In contrast to land plants, five  
20 isoforms of MDH (MDH1-5) are encoded in the genome of Chamydomonas [94]. CrMDH5  
21 (the only NADP<sup>+</sup> requiring MDH) is predicted to be chloroplastic, CrMDH2 peroxisomal  
22 [4,63], CrMDH3 is considered cytosolic and CrMDH4 mitochondrial [95]. The situation is  
23 more complex with CrMDH1 which is predicted as chloroplast-based on PredAlgo [95], but  
24 found peroxisomal when its 25 aa of its N-terminus is fused to the yellow fluorescent protein  
25 (YFP) [4]. Therefore, subcellular localization(s) of CrMDH1 using full-length protein fusion  
26 or immunogold labeling would be needed to clarify its location.

27

## 28 **Box 2, Di- and tri-carboxylate translocators**

29 In addition to MDHs, the malate shuttle requires translocators of malate/or other di- or  
30 tricarboxylates from one compartment to another. Different translocators have been predicted  
31 and experimentally verified in arabidopsis chloroplasts and mitochondria [12,96]. The transfer  
32 of malate through the chloroplast envelope membrane can be mediated by at least three different  
33 translocators. The 2-oxoglutarate (OG)/malate-translocator 1 (OMT1 or DiT1) catalyzes the  
34 export of malate in exchange for 2-OG [31]. AtOMT1 was also shown to have high affinity for



1 OAA in reconstituted liposomes [22]. The dicarboxylate translocators 1 and 2 (DCT1 & 2 also  
2 named sometimes as DiT2.1 & 2.2) are glutamate/malate-translocators importing malate in  
3 counter-exchange with glutamate [31]. Very likely also malate uniporters exist in the  
4 chloroplast envelope membrane. However, the corresponding genes have not yet been  
5 identified.

6 The transfer of malate across the mitochondrial inner membrane is mediated by at least  
7 three dicarboxylate carriers (DIC1, DIC2, DIC3) and one dicarboxylate-tricarboxylate carrier  
8 (DTC) belonging to the mitochondrial carrier family (MCF) [85,86]. The recombinant DICs,  
9 when introduced into liposomes, catalyze the transport of a wide range of dicarboxylates such  
10 as malate, OAA, succinate but with low affinity for 2-OG. These DICs were proposed to be  
11 involved in mitochondrial malate-OAA shuttle [86]. Very recently, the physiological function  
12 of DIC2 has been demonstrated in arabidopsis as being a high affinity malate–citrate antiporter  
13 in the mitochondria, through evidence from the reverse genetic approach in combination with  
14 comprehensive *in vitro*, *in organello*, and *in vivo* analyses [87]. On the other hand, in addition  
15 to dicarboxylates (malate, OAA, 2-OG and succinate), DTC was shown to transport also  
16 tricarboxylates (such as citrate or isocitrate) [85]. Therefore, DTC can serve as a malate/2-OG  
17 translocator. A malate/aspartate shuttle has been proposed by [67] connecting mitochondria to  
18 peroxisome, which requires coordinated functions of two types of translocators i.e. malate/2-  
19 OG carrier (likely DTC) and a glutamate/aspartate carrier.

20 Genes encoding putative proteins homologous to the plant di- or tri-carboxylate  
21 translocators are present in the chlamydomonas genome (**Table 1**). Three putative plastidial 2-  
22 OG/malate translocators (annotated as OMT1, OMT2 and low carbon inducible20 - LCI20),  
23 and one mitochondrial translocator known as MiTC14 and belonging to the MCF family, have  
24 been identified [94]. The protein sequence of LCI20 shares 55% and 60% of similarity with  
25 DiT2.1 and DiT2.2 respectively whereas AtDiT1 shares 63% of similarity with both CrOMT1  
26 and CrOMT2 suggesting that they are evolutionary related dicarboxylate translocators.

27

### 28 **Box 3, Other players in the malate shuttle**

29 Apart from MDH and the malate translocators listed in Table 1, other enzymes could also be  
30 involved in functioning of the variants of malate shuttle. One notable example is the malate-  
31 aspartate shuttle, which require aspartate aminotransferases (AspAT), sometimes named as  
32 glutamate-oxaloacetate transaminases (GOT). AspATs are ubiquitous enzymes encoded by a  
33 multigenic family catalyzing the reversible interconversion between aspartate and 2-  
34 oxoglutarate with glutamate and OAA. In arabidopsis, five isoforms of eukaryotic type AspAT

1 are present i.e. AspAT1 (mitochondrial), AspAT2 and AspAT4 (cytosolic), AspAT3  
2 (peroxisomal), AspAT5 (plastidial) and one prokaryotic type AspAT (PTA) localized in the  
3 chloroplast [97,98]. Activity of AspAT was also detected in chlamydomonas [99] and five  
4 genes *AST1-AST5* (phytozome v13) encode for AspAT proteins [94] respectively. AST1 and  
5 AST3 (mitochondrial), AST2 (chloroplastic), AST4 (mitochondrial by homology) and AST5  
6 (cytosolic). The plant peroxisomal and mitochondrial isoforms of AspAT have been proposed,  
7 in conjunction with MDHs, to play a role in shuttling reducing equivalents between both  
8 organelles through malate-aspartate shuttle during photorespiration [100], but none of the algal  
9 proteins has been studied.

10

### 11 **Figure Legends:**

12

#### 13 **Figure 1. Known and putative steps of malate trafficking in a subcellular context.**

14 The known di-/tri-carboxylate translocators based on either in vivo or in vitro characterization  
15 are reported in **table 1**. Transporter name in white refers to arabidopsis proteins and black refers  
16 to the chlamydomonas putative transporters (\*) based on homology without any functional data.  
17 “?” represent the putative translocators involved in the malate shuttle. Dashed lines indicate  
18 reactions with several transformation steps. Created with BioRender.com.

19 **Abbreviations:** ADP, adenosine diphosphate; ATP, adenosine triphosphate; Asp, aspartate;  
20 AspAT, aspartate aminotransferase (**Box 3**);  $\beta$ -Ox.,  $\beta$ -Oxidation; CBBC, Calvin-Benson-  
21 Bassham cycle; DIC/DTC, mitochondrial dicarboxylate/ dicarboxylate-tricarboxylate carrier;  
22 DiT1/2, dicarboxylate translocator 1/2; FA, fatty acid; FdGOGAT, ferredoxin-dependent  
23 glutamine 2-oxoglutarate aminotransferase; G3P, glyceraldehyde-3-phosphate; GDC, glycine  
24 decarboxylase; GGAT, glutamate-glyoxylate aminotransferase; Gln, glutamine; Glu,  
25 glutamate; GS, glutamine synthase; HPR, hydroxypyruvate reductase; Mal, malate; MiTC14,  
26 mitochondrial substrate carrier protein 14; MDH, malate dehydrogenase; mETC, mitochondrial  
27 electron transport chain; NAD(P)H, nicotinamide adenine dinucleotide (phosphate); OAA,  
28 oxaloacetate; 2-OG, 2-oxoglutarate; OH-Pyr, hydroxypyruvate; OMT1/2, 2-  
29 oxoglutarate/malate translocator; OPPP, oxidative pentose phosphate pathway; 2-PG, 2-  
30 phosphoglycolate; 3-PGA, 3-phosphoglycerate; PS, photosystems; RuBisCO, ribulose-1,5-  
31 biphosphate carboxylase/oxygenase; TCA, tricarboxylic acid; UCP, uncoupling protein.

32

#### 33 **Figure 2. Photosynthetic redox production, management and carbon metabolism in algae.**

1 The photosynthetic linear electron flow (LEF) occurring in the thylakoid membrane produces  
2 ATP and NADPH for the fixation of CO<sub>2</sub> in the Calvin Benson Bassham (CBB) cycle to yield  
3 carbohydrates. The G3P, ATP and NAD(P)H are then used for starch and de novo fatty acid  
4 biosynthesis (blue arrows). Alternative electron pathways are represented by cyclic electron  
5 flow (CEF; orange arrows) and pseudo-CEF (PCEF; orange arrows). Chloroplast to  
6 mitochondrial communication (CTM) is represented in dark green arrows. Excess NAD(P)H is  
7 used by plNAD(P)-MDHs to reduce OAA to malate that is shuttled through chloroplastic  
8 malate translocators to fuel the mitochondrial electron transport chain (mETC) for the synthesis  
9 of ATP. The CCM is represented in the chloroplast where atmospheric CO<sub>2</sub> is pumped then  
10 sequestered into the pyrenoid. Part of energy needed for CCM comes from the proton motive  
11 force generated by photochemical reactions and another part is likely to be derived from mETC  
12 (dashed black arrow). Note that most of the reactions shown in this figure are also present in  
13 higher plants except the algal CCM. Created with BioRender.com.

14 **Abbreviations:** ACCase, acetyl-CoA carboxylase; ADP, adenosine diphosphate; AGPase,  
15 ADP-glucose pyrophosphorylase; ATP, adenosine triphosphate; CBB, Calvin Benson  
16 Bassham; CCM, CO<sub>2</sub>-concentrating mechanism; CEF, cyclic electron flow; Cyt *b<sub>6</sub>f*,  
17 cytochrome *b<sub>6</sub>f* complex; ER, enoyl-acyl carrier protein (ACP) reductase; FA, fatty acid; FAS,  
18 fatty acid synthase; Fd, ferredoxin; FLV, flavodiiron protein; FNR, ferredoxin-NADP<sup>+</sup>  
19 reductase; G1P, glucose-1-phosphate; G3P, glucose-3-phosphate; G1P, glucose-1-phosphate;  
20 KAR, ketoacyl-ACP Reductase; Mal, malate; MDH, malate dehydrogenase; mETC,  
21 mitochondrial electron transport chain; NAD(P)H, nicotinamide adenine dinucleotide  
22 (phosphate); NDA2, NAD(P)H dehydrogenase 2; OAA, oxaloacetate; PC, plastocyanin; PCEF,  
23 pseudo-CEF; PETC, photosynthetic electron transport chain; PGR5, proton gradient regulation  
24 5; PGRL1, proton gradient like 1; PQ/PQH<sub>2</sub>, plastoquinone/plastoquinol; PTOX, plastoquinone  
25 terminal oxidase; PS, photosystem; Q<sub>A</sub>, quinone A; ROS, reactive oxygen species; TAG,  
26 triacylglycerol; TCA, tricarboxylic acid.

27

28 **Figure I, in Box1: Metabolic reactions that involve malate.**

29 MDH, ME and MAS enzymes are found in higher plants and algae. MAS catalyzes the  
30 biosynthesis of malate by condensing glyoxylate and acetyl-CoA. This reaction is part of the  
31 glyoxylate cycle. ME uses either NADP<sup>+</sup> or NAD<sup>+</sup> to decarboxylate malate into pyruvate  
32 releasing CO<sub>2</sub> and present in several subcellular compartments. MDH catalyzes the reversible  
33 interconversion between malate and oxaloacetate using either NAD(P)<sup>+</sup> or NAD(P)H as  
34 cofactor. This reaction occurs in the plastid, cytosol, peroxisome, and mitochondria.

1 **Abbreviations:** CoA, Co-enzyme A; MDH, malate dehydrogenase; ME, malic enzyme;  
2 NAD(P)<sup>+</sup>, nicotinamide adenine dinucleotide (phosphate).

3 \* Denote that MDH reaction is reversible.

#### 4 **References:**

- 5 1 Raghavendra, A.S. and Padmasree, K. (2003) Beneficial interactions of mitochondrial metabolism  
6 with photosynthetic carbon assimilation. *Trends Plant Sci.* 8, 546–553
- 7 2 Weber, A.P.M. and Linka, N. (2011) Connecting the Plastid: Transporters of the Plastid Envelope  
8 and Their Role in Linking Plastidial with Cytosolic Metabolism. *Annu. Rev. Plant Biol.* 62, 53–77
- 9 3 Neuhaus, H.E. and Wagner, R. (2000) Solute pores, ion channels, and metabolite transporters in  
10 the outer and inner envelope membranes of higher plant plastids. *Biochimica et Biophysica Acta*  
11 *(BBA) - Biomembranes* 1465, 307–323
- 12 4 Lauersen, K.J. *et al.* (2016) Peroxisomal microbodies are at the crossroads of acetate assimilation  
13 in the green microalga *Chlamydomonas reinhardtii*. *Algal Research* 16, 266–274
- 14 5 Ludwig, M. (2016) The Roles of Organic Acids in C<sub>4</sub> Photosynthesis. *Front. Plant Sci.* 7,  
15 6 Eisenhut, M. *et al.* (2019) Mechanistic understanding of photorespiration paves the way to a new  
16 green revolution. *New Phytologist* 223, 1762–1769
- 17 7 van Roermund, C.W.T. *et al.* (2016) The Peroxisomal NAD Carrier from Arabidopsis Imports NAD  
18 in Exchange with AMP1[OPEN]. *Plant Physiol* 171, 2127–2139
- 19 8 Hashida, S. and Kawai-Yamada, M. (2019) Inter-Organelle NAD Metabolism Underpinning Light  
20 Responsive NADP Dynamics in Plants. *Front. Plant Sci.* 10,  
21 9 Palmieri, F. *et al.* (2009) Molecular Identification and Functional Characterization of Arabidopsis  
22 thaliana Mitochondrial and Chloroplastic NAD<sup>+</sup> Carrier Proteins \*. *Journal of Biological Chemistry*  
23 284, 31249–31259
- 24 10 Selinski, J. and Scheibe, R. (2019) Malate valves: old shuttles with new perspectives. *Plant Biol*  
25 *(Stuttg)* 21, 21–30
- 26 11 Monné, M. *et al.* (2018) Uncoupling proteins 1 and 2 (UCP1 and UCP2) from Arabidopsis thaliana  
27 are mitochondrial transporters of aspartate, glutamate, and dicarboxylates. *Journal of Biological*  
28 *Chemistry* 293, 4213–4227
- 29 12 Lee, C.P. and Millar, A.H. (2016) The Plant Mitochondrial Transportome: Balancing Metabolic  
30 Demands with Energetic Constraints. *Trends in Plant Science* 21, 662–676
- 31 13 Allen, J.F. (2002) Photosynthesis of ATP—Electrons, Proton Pumps, Rotors, and Poise. *Cell* 110,  
32 273–276
- 33 14 Kramer, D.M. and Evans, J.R. (2011) The Importance of Energy Balance in Improving  
34 Photosynthetic Productivity<sup>1</sup>[W]. *Plant Physiol* 155, 70–78
- 35 15 Burlacot, A. *et al.* (2021) Alternative electron pathways of photosynthesis drive the algal CO<sub>2</sub>  
36 concentrating mechanism. *bioRxiv* DOI: 10.1101/2021.02.25.432959
- 37 16 Erickson, E. *et al.* (2015) Light stress and photoprotection in *Chlamydomonas reinhardtii*. *The Plant*  
38 *Journal* 82, 449–465
- 39 17 Alric, J. and Johnson, X. (2017) Alternative electron transport pathways in photosynthesis: a  
40 confluence of regulation. *Current Opinion in Plant Biology* 37, 78–86
- 41 18 Burlacot, A. *et al.* (2019) Subcellular Energetics and Carbon Storage in *Chlamydomonas*. *Cells* 8,  
42 1154
- 43 19 Heyno, E. *et al.* (2014) Putative role of the malate valve enzyme NADP–malate dehydrogenase in  
44 H<sub>2</sub>O<sub>2</sub> signalling in Arabidopsis. *Philosophical Transactions of the Royal Society B: Biological*  
45 *Sciences* 369, 20130228
- 46 20 Hebbelmann, I. *et al.* (2012) Multiple strategies to prevent oxidative stress in Arabidopsis plants  
47 lacking the malate valve enzyme NADP-malate dehydrogenase. *J Exp Bot* 63, 1445–1459
- 48 21 Yokochi, Y. *et al.* (2021) Redox regulation of NADP-malate dehydrogenase is vital for land plants  
49 under fluctuating light environment. *PNAS* 118,

- 1 22 Kinoshita, H. *et al.* (2011) The chloroplastic 2-oxoglutarate/malate transporter has dual function  
2 as the malate valve and in carbon/nitrogen metabolism. *The Plant Journal* 65, 15–26
- 3 23 Lemaire, S.D. *et al.* (2005) NADP-Malate Dehydrogenase from Unicellular Green Alga  
4 *Chlamydomonas reinhardtii*. A First Step toward Redox Regulation? *Plant Physiology* 137, 514–521
- 5 24 Dang, K.-V. *et al.* (2014) Combined Increases in Mitochondrial Cooperation and Oxygen  
6 Photoreduction Compensate for Deficiency in Cyclic Electron Flow in *Chlamydomonas reinhardtii*.  
7 *The Plant Cell* 26, 3036–3050
- 8 25 Kong, F. *et al.* (2018) Interorganelle Communication: Peroxisomal MALATE DEHYDROGENASE2  
9 Connects Lipid Catabolism to Photosynthesis through Redox Coupling in *Chlamydomonas*. *The*  
10 *Plant Cell* 30, 1824–1847
- 11 26 Massoz, S. *et al.* (2015) Isolation of *Chlamydomonas reinhardtii* mutants with altered  
12 mitochondrial respiration by chlorophyll fluorescence measurement. *J Biotechnol* 215, 27–34
- 13 27 Wang, Y. *et al.* (2015) The CO<sub>2</sub> concentrating mechanism and photosynthetic carbon assimilation  
14 in limiting CO<sub>2</sub>: how *Chlamydomonas* works against the gradient. *The Plant Journal* 82, 429–448
- 15 28 Backhausen, J.E. and Scheibe, R. (1999) Adaptation of tobacco plants to elevated CO<sub>2</sub>: influence  
16 of leaf age on changes in physiology, redox states and NADP-malate dehydrogenase activity.  
17 *Journal of Experimental Botany* 50, 665–675
- 18 29 Li, Y. *et al.* (2014) Was low CO<sub>2</sub> a driving force of C<sub>4</sub> evolution: Arabidopsis responses to long-term  
19 low CO<sub>2</sub> stress. *Journal of Experimental Botany* 65, 3657–3667
- 20 30 Takahashi, S. *et al.* (2007) Impairment of the Photorespiratory Pathway Accelerates  
21 Photoinhibition of Photosystem II by Suppression of Repair But Not Acceleration of Damage  
22 Processes in Arabidopsis. *Plant Physiology* 144, 487–494
- 23 31 Renné, P. *et al.* (2003) The Arabidopsis mutant *dct* is deficient in the plastidic glutamate/malate  
24 translocator DiT2. *The Plant Journal* 35, 316–331
- 25 32 Taniguchi, M. *et al.* (2002) Identifying and Characterizing Plastidic 2-Oxoglutarate/Malate and  
26 Dicarboxylate Transporters in Arabidopsis thaliana. *Plant Cell Physiol* 43, 706–717
- 27 33 Lindén, P. *et al.* (2016) Reduced mitochondrial malate dehydrogenase activity has a strong effect  
28 on photorespiratory metabolism as revealed by <sup>13</sup>C labelling. *Journal of Experimental Botany* 67,  
29 3123–3135
- 30 34 Bloom, A.J. *et al.* (2010) Carbon Dioxide Enrichment Inhibits Nitrate Assimilation in Wheat and  
31 Arabidopsis. *Science* 328, 899–903
- 32 35 Timm, S. *et al.* (2008) A Cytosolic Pathway for the Conversion of Hydroxypyruvate to Glycerate  
33 during Photorespiration in Arabidopsis. *The Plant Cell* 20, 2848–2859
- 34 36 Schneidereit, J. *et al.* (2006) Antisense repression reveals a crucial role of the plastidic 2-  
35 oxoglutarate/malate translocator DiT1 at the interface between carbon and nitrogen metabolism.  
36 *The Plant Journal* 45, 206–224
- 37 37 Cousins, A.B. *et al.* (2008) Peroxisomal Malate Dehydrogenase Is Not Essential for Photorespiration  
38 in Arabidopsis But Its Absence Causes an Increase in the Stoichiometry of Photorespiratory CO<sub>2</sub>  
39 Release. *Plant Physiol* 148, 786–795
- 40 38 Moroney, J.V. *et al.* (2011) The carbonic anhydrase isoforms of *Chlamydomonas reinhardtii*:  
41 intracellular location, expression, and physiological roles. *Photosynth Res* 109, 133–149
- 42 39 Mackinder, L.C.M. *et al.* (2017) A Spatial Interactome Reveals the Protein Organization of the Algal  
43 CO<sub>2</sub>-Concentrating Mechanism. *Cell* 171, 133-147.e14
- 44 40 Duanmu, D. *et al.* (2009) Knockdown of limiting-CO<sub>2</sub>-induced gene HLA3 decreases HCO<sub>3</sub>-  
45 transport and photosynthetic C<sub>i</sub> affinity in *Chlamydomonas reinhardtii*. *PNAS* 106, 5990–5995
- 46 41 Wang, Y. and Spalding, M.H. (2014) Acclimation to Very Low CO<sub>2</sub>: Contribution of Limiting CO<sub>2</sub>  
47 Inducible Proteins, LCIB and LCIA, to Inorganic Carbon Uptake in *Chlamydomonas reinhardtii*. *Plant*  
48 *Physiology* 166, 2040–2050
- 49 42 Mukherjee, A. *et al.* (2019) Thylakoid localized bestrophin-like proteins are essential for the CO<sub>2</sub>  
50 concentrating mechanism of *Chlamydomonas reinhardtii*. *Proc Natl Acad Sci USA* 116, 16915–  
51 16920

- 1 43 Kono, A. and Spalding, M.H. (2020) LCI1, a Chlamydomonas reinhardtii plasma membrane protein,  
2 functions in active CO<sub>2</sub> uptake under low CO<sub>2</sub>. *The Plant Journal* 102, 1127–1141
- 3 44 Hennon, G.M.M. *et al.* (2017) Diverse CO<sub>2</sub>-Induced Responses in Physiology and Gene Expression  
4 among Eukaryotic Phytoplankton. *Front. Microbiol.* 8,
- 5 45 Yamano, T. *et al.* (2008) Expression Analysis of Genes Associated with the Induction of the Carbon-  
6 Concentrating Mechanism in Chlamydomonas reinhardtii. *Plant Physiol* 147, 340–354
- 7 46 Miura, K. *et al.* (2004) Expression Profiling-Based Identification of CO<sub>2</sub>-Responsive Genes  
8 Regulated by CCM1 Controlling a Carbon-Concentrating Mechanism in Chlamydomonas  
9 reinhardtii. *Plant Physiol* 135, 1595–1607
- 10 47 Czarnocka, W. and Karpiński, S. (2018) Friend or foe? Reactive oxygen species production,  
11 scavenging and signaling in plant response to environmental stresses. *Free Radical Biology and*  
12 *Medicine* 122, 4–20
- 13 48 Dietz, K.-J. *et al.* (2016) Redox- and Reactive Oxygen Species-Dependent Signaling into and out of  
14 the Photosynthesizing Chloroplast. *Plant Physiology* 171, 1541–1550
- 15 49 Zhao, Y. *et al.* (2020) Malate Circulation: Linking Chloroplast Metabolism to Mitochondrial ROS.  
16 *Trends in Plant Science* 25, 446–454
- 17 50 Zhao, Y. *et al.* (2018) Malate transported from chloroplast to mitochondrion triggers production  
18 of ROS and PCD in Arabidopsis thaliana. *Cell Research* 28, 448–461
- 19 51 Mou, Z. *et al.* (2000) Deficiency in Fatty Acid Synthase Leads to Premature Cell Death and Dramatic  
20 Alterations in Plant Morphology. *The Plant Cell* 12, 405–417
- 21 52 Li-Beisson, Y. *et al.* (2019) The lipid biochemistry of eukaryotic algae. *Progress in Lipid Research* 74,  
22 31–68
- 23 53 Allen, J.F. (1975) Oxygen reduction and optimum production of ATP in photosynthesis. *Nature* 256,  
24 599–600
- 25 54 Mehler, A.H. (1951) Studies on reactions of illuminated chloroplasts: I. Mechanism of the  
26 reduction of oxygen and other hill reagents. *Archives of Biochemistry and Biophysics* 33, 65–77
- 27 55 Curien, G. *et al.* (2016) The Water to Water Cycles in Microalgae. *Plant and Cell Physiology* 57,  
28 1354–1363
- 29 56 Chaux, F. *et al.* (2017) Flavodiiron Proteins Promote Fast and Transient O<sub>2</sub> Photoreduction in  
30 Chlamydomonas. *Plant Physiology* 174, 1825–1836
- 31 57 Gerotto, C. *et al.* (2016) Flavodiiron proteins act as safety valve for electrons in Physcomitrella  
32 patens. *PNAS* 113, 12322–12327
- 33 58 Mubarakshina, M.M. *et al.* (2010) Production and diffusion of chloroplastic H<sub>2</sub>O<sub>2</sub> and its  
34 implication to signalling. *Journal of Experimental Botany* 61, 3577–3587
- 35 59 Gadjev, I. *et al.* (2006) Transcriptomic Footprints Disclose Specificity of Reactive Oxygen Species  
36 Signaling in Arabidopsis. *Plant Physiology* 141, 436–445
- 37 60 Michelet, L. *et al.* (2013) Down-regulation of catalase activity allows transient accumulation of a  
38 hydrogen peroxide signal in Chlamydomonas reinhardtii. *Plant, Cell & Environment* 36, 1204–1213
- 39 61 Blaby, I.K. *et al.* (2015) Genome-wide analysis on Chlamydomonas reinhardtii reveals the impact  
40 of hydrogen peroxide on protein stress responses and overlap with other stress transcriptomes.  
41 *The Plant Journal* 84, 974–988
- 42 62 Lazarow, P.B. (2016) Peroxisomes. In *Encyclopedia of Cell Biology* (Bradshaw, R. A. and Stahl, P.  
43 D., eds), pp. 248–272, Academic Press
- 44 63 Kong, F. *et al.* (2017) Chlamydomonas carries out fatty acid  $\beta$ -oxidation in ancestral peroxisomes  
45 using a bona fide acyl-CoA oxidase. *The Plant Journal* 90, 358–371
- 46 64 Plancke, C. *et al.* (2014) Lack of isocitrate lyase in Chlamydomonas leads to changes in carbon  
47 metabolism and in the response to oxidative stress under mixotrophic growth. *The Plant Journal*  
48 77, 404–417
- 49 65 Gakière, B. *et al.* (2018) NAD<sup>+</sup> Biosynthesis and Signaling in Plants. *Critical Reviews in Plant*  
50 *Sciences* 37, 259–307
- 51 66 Charton, L. *et al.* (2019) Plant peroxisomal solute transporter proteins. *Journal of Integrative Plant*  
52 *Biology* 61, 817–835

- 1 67 Mettler, I.J. and Beevers, H. (1980) Oxidation of NADH in Glyoxysomes by a Malate-Aspartate  
2 Shuttle. *Plant Physiology* 66, 555–560
- 3 68 Pracharoenwattana, I. *et al.* (2007) Arabidopsis peroxisomal malate dehydrogenase functions in  
4  $\beta$ -oxidation but not in the glyoxylate cycle. *The Plant Journal* 50, 381–390
- 5 69 Pracharoenwattana, I. *et al.* (2009) Fatty acid beta-oxidation in germinating Arabidopsis seeds is  
6 supported by peroxisomal hydroxypyruvate reductase when malate dehydrogenase is absent.  
7 *Plant Mol Biol* 72, 101
- 8 70 Eastmond, P.J. (2007) MONODEHYROASCORBATE REDUCTASE4 Is Required for Seed Storage Oil  
9 Hydrolysis and Postgerminative Growth in Arabidopsis. *The Plant Cell* 19, 1376–1387
- 10 71 Kato, J. *et al.* (1997) Characterization of catalase from green algae *Chlamydomonas reinhardtii*.  
11 *Journal of Plant Physiology* 151, 262–268
- 12 72 Hayashi, Y. *et al.* (2015) Increase in peroxisome number and the gene expression of putative  
13 glyoxysomal enzymes in *Chlamydomonas* cells supplemented with acetate. *J Plant Res* 128, 177–  
14 185
- 15 73 Hayashi, Y. and Shinozaki, A. (2012) Visualization of microbodies in *Chlamydomonas reinhardtii*. *J.*  
16 *Plant Res.* 125, 579–586
- 17 74 Kato, N. *et al.* (2021) Subcellular Localizations of Catalase and Exogenously Added Fatty Acid in  
18 *Chlamydomonas reinhardtii*. *Cells* 10, 1940
- 19 75 Kong, F. *et al.* (2018) Lipid catabolism in microalgae. *New Phytologist* 218, 1340–1348
- 20 76 Voon, C.P. *et al.* (2018) ATP compartmentation in plastids and cytosol of Arabidopsis thaliana  
21 revealed by fluorescent protein sensing. *PNAS* 115, E10778–E10787
- 22 77 Elsässer, M. *et al.* (2020) Photosynthetic activity triggers pH and NAD redox signatures across  
23 different plant cell compartments. *bioRxiv* DOI: 10.1101/2020.10.31.363051
- 24 78 Beeler, S. *et al.* (2014) Plastidial NAD-Dependent Malate Dehydrogenase Is Critical for Embryo  
25 Development and Heterotrophic Metabolism in Arabidopsis. *Plant Physiology* 164, 1175–1190
- 26 79 An, Y. *et al.* (2016) Purification and characterization of the plastid-localized NAD-dependent  
27 malate dehydrogenase from Arabidopsis thaliana. *Biotechnology and Applied Biochemistry* 63,  
28 490–496
- 29 80 Schreier, T.B. *et al.* (2018) Plastidial NAD-Dependent Malate Dehydrogenase: A Moonlighting  
30 Protein Involved in Early Chloroplast Development through Its Interaction with an FtsH12-FtsHi  
31 Protease Complex. *The Plant Cell* 30, 1745–1769
- 32 81 Huang, J. *et al.* (2018) Self-protection of cytosolic malate dehydrogenase against oxidative stress  
33 in Arabidopsis. *Journal of Experimental Botany* 69, 3491–3505
- 34 82 Liszka, A. *et al.* (2020) Three cytosolic NAD-malate dehydrogenase isoforms of Arabidopsis  
35 thaliana: on the crossroad between energy fluxes and redox signaling. *Biochemical Journal* 477,  
36 3673–3693
- 37 83 Tomaz, T. *et al.* (2010) Mitochondrial Malate Dehydrogenase Lowers Leaf Respiration and Alters  
38 Photorespiration and Plant Growth in Arabidopsis[W][OA]. *Plant Physiol* 154, 1143–1157
- 39 84 Sew, Y.S. *et al.* (2016) Loss of Mitochondrial Malate Dehydrogenase Activity Alters Seed  
40 Metabolism Impairing Seed Maturation and Post-Germination Growth in Arabidopsis1[OPEN].  
41 *Plant Physiol* 171, 849–863
- 42 85 Picault, N. *et al.* (2002) Identification of a Novel Transporter for Dicarboxylates and Tricarboxylates  
43 in Plant Mitochondria: BACTERIAL EXPRESSION, RECONSTITUTION, FUNCTIONAL  
44 CHARACTERIZATION, AND TISSUE DISTRIBUTION \*. *Journal of Biological Chemistry* 277, 24204–  
45 24211
- 46 86 Palmieri, L. *et al.* (2008) Molecular identification of three Arabidopsis thaliana mitochondrial  
47 dicarboxylate carrier isoforms: organ distribution, bacterial expression, reconstitution into  
48 liposomes and functional characterization. *Biochemical Journal* 410, 621–629
- 49 87 Lee, C.P. *et al.* (2021) The versatility of plant organic acid metabolism in leaves is underpinned by  
50 mitochondrial malate–citrate exchange. *The Plant Cell* DOI: 10.1093/plcell/koab223
- 51 88 Nogales, J. *et al.* (2004) Functional analysis and regulation of the malate synthase from  
52 *Chlamydomonas reinhardtii*. *Planta* 219, 325–331

- 1 89 Bogaert, K.A. *et al.* (2019) Metabolic, Physiological, and Transcriptomics Analysis of Batch Cultures  
2 of the Green Microalga *Chlamydomonas* Grown on Different Acetate Concentrations. *Cells* 8, 1367  
3 90 Wynn, J.P. and Ratledge, C. (1997) Malic Enzyme is a Major Source of NADPH for Lipid  
4 Accumulation by *Aspergillus Nidulans*. *Microbiology* 143, 253–257  
5 91 Zell, M.B. *et al.* (2010) Analysis of Arabidopsis with Highly Reduced Levels of Malate and Fumarate  
6 Sheds Light on the Role of These Organic Acids as Storage Carbon Molecules. *Plant Physiology* 152,  
7 1251–1262  
8 92 Zhu, B.-H. *et al.* (2018) The Role of Malic Enzyme on Promoting Total Lipid and Fatty Acid  
9 Production in *Phaeodactylum tricornutum*. *Front. Plant Sci.* 9,  
10 93 Schlüter, U. and Weber, A.P.M. (2020) Regulation and Evolution of C4 Photosynthesis. *Annual*  
11 *Review of Plant Biology* 71, 183–215  
12 94 Merchant, S.S. *et al.* (2007) The *Chlamydomonas* genome reveals the evolution of key animal and  
13 plant functions. *Science* 318, 245–250  
14 95 Tardif, M. *et al.* (2012) PredAlgo: A New Subcellular Localization Prediction Tool Dedicated to  
15 Green Algae. *Mol Biol Evol* 29, 3625–3639  
16 96 Weber, A.P.M. and Linka, N. (2011) Connecting the Plastid: Transporters of the Plastid Envelope  
17 and Their Role in Linking Plastidial with Cytosolic Metabolism. *Annu. Rev. Plant Biol.* 62, 53–77  
18 97 Torre, F. de la *et al.* (2006) Identification and functional analysis of a prokaryotic-type aspartate  
19 aminotransferase: implications for plant amino acid metabolism. *The Plant Journal* 46, 414–425  
20 98 Schultz, C.J. and Coruzzi, G.M. (1995) The aspartate aminotransferase gene family of Arabidopsis  
21 encodes isoenzymes localized to three distinct subcellular compartments. *The Plant Journal* 7, 61–  
22 75  
23 99 Muñoz-Blanco, J. *et al.* (1988) Characterization of an L-aspartate aminotransferase activity in  
24 *Chlamydomonas reinhardtii*. *Physiologia Plantarum* 74, 433–439  
25 100 Journet, E.-P. *et al.* (1981) Role of Glutamate-oxaloacetate Transaminase and Malate  
26 Dehydrogenase in the Regeneration of NAD<sup>+</sup> for Glycine Oxidation by Spinach leaf Mitochondria  
27 1. *Plant Physiology* 67, 467–469  
28  
29  
30

## 31 Outstanding Questions

- 32 • *Does redox exchange occur between NADH and NADPH pools in the chloroplast?* Both  
33 plant and algal chloroplasts contain two pools of pyridine nucleotides (NADH versus  
34 NADPH). Possible interaction between the two redox pools (NADH versus NADPH)  
35 in the chloroplast is not known but likely mediated by the NAD(P)<sup>+</sup> transhydrogenase.  
36 • *Do Chlamydomonas chloroplasts contain a NAD-MDH?* CrMDH1 is found  
37 peroxisomal based on fusion of the first 25 amino acids at the N-terminus to the yellow  
38 fluorescent protein, but it is predicted to be chloroplastial by PredAlgo. A key question  
39 is the subcellular location(s) of CrMDH1. The location of this enzyme could have  
40 implications for our understanding of metabolism and redox homeostasis not only for  
41 the chloroplast but also for the peroxisome.  
42 • *Is there any connection between the enzymatic and non-enzymatic function of the*  
43 *plastidial NAD-dependent MDH?* It was recently shown that the NAD-dependent MDH



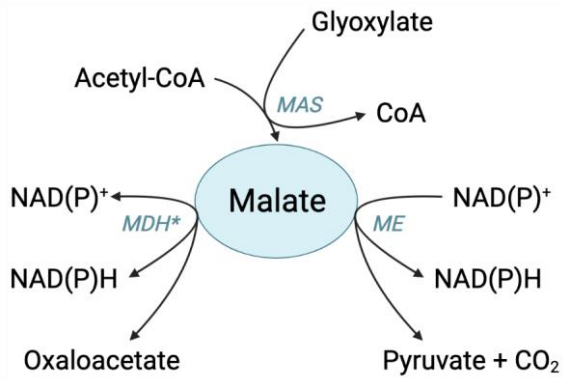
1 plays a moonlighting function in chloroplast metabolism and biogenesis. The  
2 moonlighting function is an interesting feature of some proteins to have more than one  
3 function in the cell. In addition to its enzymatic function as malate dehydrogenase, the  
4 plastidial NAD-dependent MDH plays a central role in chloroplast biogenesis through  
5 its physical interaction with the FtsH12-FtsHi protease complex. However, it is still not  
6 clear whether these two functions are connected or what is the biological significance  
7 of grouping these two functions (enzymatic and structural) in one widespread protein  
8 such as MDH?

- 9 • *What is the identity of peroxisomal malate translocator?* This is probably one of the  
10 most elusive components of peroxisome redox metabolism. Putative candidates are  
11 emerging with modern proteomics analysis of isolated peroxisomes.
- 12 • *What is the interaction between redox trafficking through malate shuttle and carbon  
13 storage in microalgae?* Starch and fatty acid biosynthesis are major sink of cellular  
14 carbon and energy. Their synthesis requires a carbon source, ATP and NAD(P)H but at  
15 different ratio. Alterations of redox trafficking and cell energetic metabolism could have  
16 direct consequences for biomass productivity and composition.
- 17 • *What is the nature of the malate uniport mechanism in C4 plants?* Using isolated  
18 chloroplasts from a range of C4 plants two types of malate uniporters have been found,  
19 one coupled to sodium symport (in most C4 species tested), the other coupled to proton  
20 symport (in maize, sorghum, and sugarcane). In contrast to the metabolite translocators,  
21 this type of malate transporter would allow for net malate transport for, e.g., the malic  
22 enzyme reaction.

23  
24  
25  
26  
27  
28  
29  
30  
31  
32

1 Figure in Box 1:

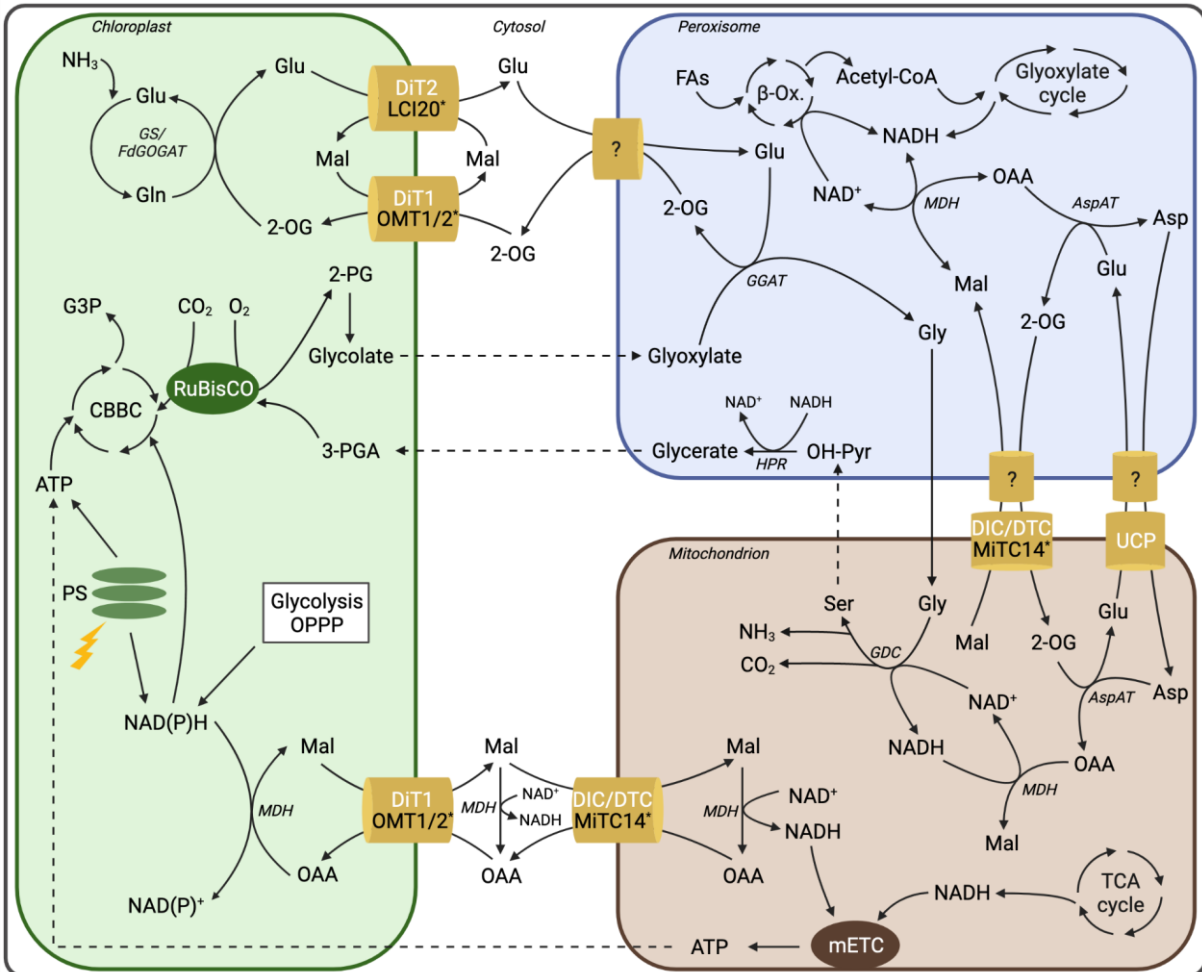
2



3

4

5 **Figure 1:**



6

7

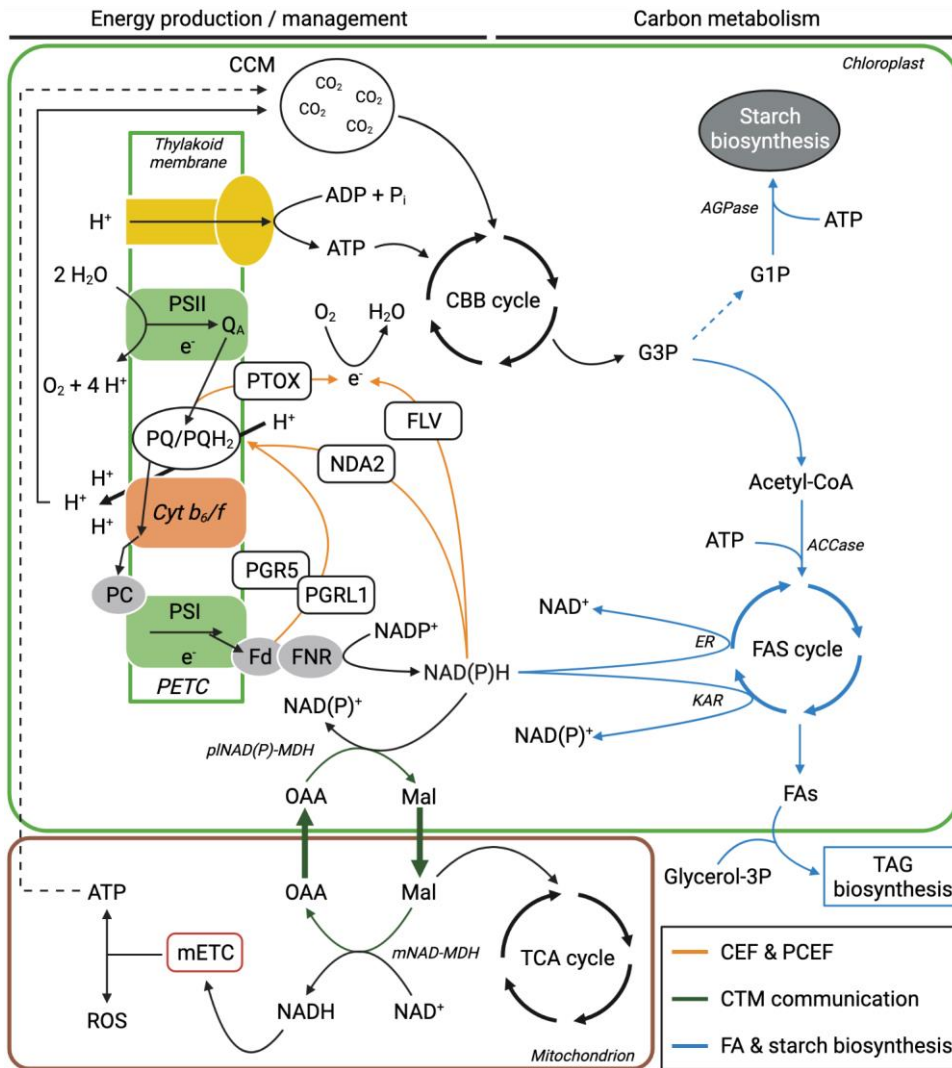
8

9

1

2 **Figure 2:**

3



4