Function and compensatory mechanisms among the components of the chloroplastic redox network

Paulo V.L. Souza[1], Yugo Lima-Melo[1,2], Fabricio E. Carvalho[1], Jean-Philippe Reichheld[3], Alisdair R. Fernie[4], Joaquim A.G. Silveira[1], Danilo M. Daloso[1*]

[1] Laboratório de Metabolismo de Plantas, Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Ceará. Fortaleza/CE, Brasil.
[2] Molecular Plant Biology, Department of Biochemistry, University of Turku. Turku, Finland.
[3] Laboratoire Génome et Développement des Plantes, Unité Mixte de Recherche 5096, Centre National de la Recherche Scientifique, Université de Perpignan Via Domitia, 66860 Perpignan, France.
[4] Max-Planck-Institute of Molecular Plant Physiology, 14476 Potsdam-Golm, Germany.

*Corresponding author

Tel: +55 85 33669821

E-mail address: daloso@ufc.br

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Contents

Summary. Life on earth depends on the presence of photoautotrophic organisms that are able to input carbon into the ecosystems through the process of photosynthesis which, with a few specialized exceptions, takes place within the chloroplast. This organelle contains the most complex redox system in plants being composed of numerous players including thiol reductases, peroxidases, and glutathione-related enzymes. It seems likely that these proteins act together to adjust redox metabolism enabling plants to grow efficiently under both normal and stressed conditions. However, our knowledge concerning how these proteins interact and if they can compensate one another is relatively limited. This is in part due to the failure of considering these components from a systemic perspective. Here we provide a systemic view of the chloroplastic-redox network highlighting how it operates and how its components co-operate to maintain efficient chloroplastic function. We further explore the cross talk between chloroplastic-redox metabolism and that of other subcellular compartments. Given the complexity of plant redox metabolism and the compensatory role played by different redox systems, we argue that a unique possibility to understand this system is afforded by systems biology approaches and by characterizing mutants for multiple genes. Taking this into account, we highlight how gene co-expression and protein-protein network analyses coupled with different reverse genetic strategies could be used to reveal the function, potential redundancies and complementarities among the components of the chloroplastic redox network.

Keywords: chloroplast, peroxidases, redox network, redox metabolism, systems biology, thioredoxins.

I. Introduction

By contrast to animals, plants are sessile organisms. As such they require a higher phenotypic plasticity in the face of prevailing environmental changes. They accomplish this, in part, by the increased number of gene duplications and thereby protein isoforms found in plants, which are distributed in the three genomes located at the nucleus, chloroplasts, and mitochondria (Arabidopsis Genome Initiative, 2000). These genomes encode proteins localized in different plant cell organelles which interact with one another following endogenous and environmental signals and induce transcriptional and post translational alterations, ultimately regulating metabolic fluxes and their associated physiological response (Geigenberger and Fernie, 2014). Many enzymes of plant cell metabolism are subjected to a post translational regulation including acetylation, carboxylation, sumoylation, phosphorylation, and redox regulation (Friso and van Wijk, 2015). Plants possess an unprecedentedly complex redox regulation system, in which the presence of different redoxins and peroxidases are of pivotal importance to maintain growth and development (Reichheld *et al.*, 2010; Foyer and Noctor, 2011; Geigenberger *et al.*, 2017). Despite their important role in the redox regulation of metabolism, it has been shown that several of these enzymes are not essential for plants, meaning that plants individually lacking some of these proteins present no apparent phenotype and can survive even under stress conditions. This suggests that the components of the redox network may be able to compensate one another in order to maintain homeostasis. However, precise mechanistic details of the complementarity between different components of the redox system and how they interact with one another remains elusive -especially so for those components associated with the chloroplast.

Life on earth depends on the process of photosynthesis which in most species takes place in the chloroplast. This organelle contains the most complex redox system found in plants (Buchanan, 2016b). The chloroplastic redox system is involved in the regulation of different important physiological processes including the light-dependent redox activation of Calvin-Benson cycle enzymes and the avoidance of reactive oxygen species (ROS) overaccumulation (Buchanan, 2016a; Noctor *et al.*, 2018). Thus, the chloroplastic redox system contributes not only to activate the process of CO_2 fixation but also to avoid oxidative stress caused by ROS overaccumulation. It seems likely that CO_2 fixation is mainly regulated by the chloroplastic thioredoxin system,

which is composed by different thioredoxins (TRX) and TRX-reductases such as ferredoxin reductase (FTR) and NADPH-dependent thioredoxin reductase C (NTRC) (Table 1; Figure 1). The TRX system is capable to regulate the redox status of thiols by reversibly controlling the formation or degradation of the disulfide bridge formed between two Cys residues in the target protein. This leads to alteration in the structure of the protein that leads to loss or gain of function (Meyer *et al.*, 2009). Given the high number of proteins that are redox regulated, it is reasonable to conclude that the redox status of Cys residues is of pivotal importance for the regulation of metabolism. Furthermore, recent evidence suggests that enzymes of, or associated to, the tricarboxylic acid (TCA) cycle that are redox regulated contain at least two Cys residues conserved between microorganisms, animals, and plants (Daloso *et al.*, 2015). This suggests that redox regulation of metabolism is, in itself, a conserved mechanism, although it assumes a higher degree of complexity in plants given the presence of additional isoforms and the need to cope with constant adverse environmental conditions.

A wide range of different environmental stress conditions leads to oxidative stress within plant cells, which is mainly due the overaccumulation of different ROS such as singlet oxygen ($^1O_2$), superoxide (O_2^-), and hydrogen peroxide (H_2O_2). Although ROS accumulation can be due to the reactions occurring in other compartments such as mitochondria, cytosol and peroxisomes, the chloroplast has been documented as one of the most important sources of ROS in plants (Mittler, 2017). Given its higher stability among the ROS, H_2O_2 has been suggested to be the key molecule involved in retrograde signalling and in the oxidation of thiols of redox-regulated proteins (Noctor *et al.*, 2017). H_2O_2 assumes therefore a pivotal importance in the interorganellar communication and in the deactivation of redox regulated enzymes, being a counter-point of the TRX system (Pesaresi *et al.*, 2007; Farnese *et al.*, 2016). However, it is important to highlight that plants must avoid overaccumulation of H_2O_2 and other ROS given the harmful potential of these molecules. For this, plants possess innumerous antioxidant enzymes such as ascorbate peroxidase (APX), glutathione peroxidase (GPX), superoxide dismutase (SOD), and peroxiredoxin (PRX) (Table 1) that are not only capable of removing the excess of ROS but also regulate the balance of reduced and oxidized forms of ascorbate (ASC and DHA) and glutathione (GSH and GSSG) (Figure 1).

It is interesting to note that whilst animal GPXs exclusively use GSH as electron donor (Passaia and Margis-Pinheiro, 2015), plant GPXs display a considerably higher affinity to use TRXs as electron donor (Herbette *et al.*, 2002; Jung *et al.*, 2002; Iqbal *et al.*, 2006). Additionally, different PRXs, 2-Cys PRXs and methionine sulfoxide reductase (MSR) have also been demonstrated to be regulated by TRX (Figure 1). This indicates that ROS metabolism, ROS scavenging and the thioredoxin systems are tightly interconnected. However, unfortunately these systems have largely been studied and reviewed separately. Furthermore, despite the advances obtained in unraveling key points of metabolic regulation by adopting systems biology approaches; they have surprisingly not been often adopted in the study of plant redox metabolism. Thus, an integrative view of these systems is urgently needed to unravel the connection and the complementarity between these systems in plants. Here we first briefly review the function and the knowledge accumulated of each component of the chloroplastic redox system and then discuss how these systems work co-operatively to maintain the redox status of the cell under both normal and adverse conditions. The outstanding questions that should be addressed in this field in order to improve our understanding on how the plant redox system works and regulates the photosynthetic process are highlighted throughout the text. We finally provide a perspective concerning how the characterization of plants lacking multiple components of the chloroplastic redox network and the adoption of systems biology approaches can be used to reach these goals.

II. Roles of the different components of the chloroplastic redox network

A. The versatility of the chloroplastic thioredoxin system

TRXs comprise an ancient family of proteins found in both prokaryote and eukaryote organisms that regulate the redox status of target proteins (Figure 2) (Buchanan *et al.*, 2012). TRXs contain a redox-active dithiol cysteine residue which allows these small polypeptides to catalyze the reduction of disulfide bonds, regulating the function and structure of target proteins (Meyer *et al.*, 2012). Chloroplastic TRXs are mostly reduced by FTR and to a lesser extent by NTRC (Figure 2) (Lemaire *et al.*, 2007; Tan *et al.*, 2010). Unlike the situation observed in other organisms, plant TRXs are encoded by a large gene family. For instance, the model plant *Arabidopsis thaliana* L. contains more than 20 TRX isoforms of which TRXs *f1-2*, *m1-4*, *x*, *y1-2*, and *z* are

located at the chloroplast, TRX *o1-2* in the mitochondrion, and the proteins of the TRX *h* family (TRX *h1-9*) are distributed in cytosol, nucleus, mitochondria, plasma membrane and endoplasmic reticulum (Belin *et al.*, 2015; Delorme-Hinoux *et al.*, 2016). In addition to the canonical chloroplastic TRXs, additional TRX-like proteins such as the chloroplast drought-induced stress protein of 32 kDa (CDSP32) (Broin and Rey, 2003), four ACHT proteins (Dangoor *et al.*, 2009) and other TRX-like proteins are also found in the chloroplasts (Meyer *et al.*, 2009). Given their wide distribution within plant cells, TRXs have been involved in the regulation of several key processes over the entire plant life cycle, especially those associated with chloroplast function (Geigenberger *et al.*, 2017).

It has been shown that TRXs *m* and *f* regulate several proteins of the Calvin-Benson cycle (CBC), chlorophyll biosynthesis, starch synthesis, oxidative pentose phosphate pathway, malate metabolism, and ATP synthesis (Collin *et al.*, 2003; Courteille *et al.*, 2013; Laugier *et al.*, 2013; Thormählen *et al.*, 2013; Wang *et al.*, 2013; Okegawa and Motohashi, 2015; Yoshida *et al.*, 2015). By contrast, TRXs *x*, *y*, and *z* are less studied and their function has been limited to the regulation of stress-related proteins such as 2-Cys PRXs, thiol-peroxidases and methionine sulfoxide reductase (MSR) (Geigenberger *et al.*, 2017). Whilst the TRX-target proteins were revealed by proteomic studies, the physiological function of TRXs has been demonstrated mainly by the characterization of TRX mutants. For instance, the characterization of *trxm* mutants has revealed the involvement of the TRXs *m3* and *m4* in the regulation of cyclic electron transport (Benitez-Alfonso *et al.*, 2009; Courteille *et al.*, 2013). Furthermore, although single or combined mutation in TRXs *m1* and *m2* revealed no changes in photosynthetic parameters under normal growth conditions (Laugier *et al.*, 2013; Okegawa and Motohashi, 2015), these proteins proved to be important under fluctuating light and high light conditions (Thormählen *et al.*, 2017), most probably due to their capability to regulate the chloroplastic malate valve and ATP synthesis (Wolosiuk *et al.*, 1977; Carrillo *et al.*, 2016; Nikkanen *et al.*, 2016; Yoshida and Hisabori, 2016b; Thormählen *et al.*, 2017). Similarly to *trxm1* and *trxm2* single mutants, the single *trxf1* mutant and the double *trxf1f2* mutant showed no apparent phenotype despite a deficiency and a delay in light activation in the CBC-fructose-1,6-biphosphatase (FBPase) enzyme and in the starch synthesis-related enzyme ADP glucose pyrophosphorylase (AGPase) (Thormählen *et al.*, 2013; Yoshida *et al.*, 2015; Naranjo *et al.*, 2016). More

importantly, triple *trxm1-m2-m4* mutant plants displayed a visible phenotype alteration being characterized by a pale-green color and a 50% reduction in their photosynthetic rate (Wang and Vanlerberghe, 2013), highlighting the compensatory role between *m* type TRXs. Taken together, these studies were essential in demonstrating the role of these TRXs *in vivo* and suggest that TRXs *f* and *m* are key regulators of the carbon assimilation photosynthetic pathway. Furthermore, the fact that the observed phenotype is more severe when more than one TRX is mutated demonstrates the capacity of the chloroplastic TRX system in adjusting its main actors in order to maintain chloroplastic redox homeostasis. Beyond this cooperation between chloroplastic TRXs, it is known that both chloroplastic TRX reductase systems also work co-operatively (Yoshida and Hisabori, 2016b). Thus, it seems likely that the NTRC-TRX and Fdx-TRX systems form an interconnected redox network which enables plants to respond according to the light condition (Pérez-Ruiz *et al.*, 2017).

B. The critical but non-essential role of the NADPH-dependent thioredoxin reductase C in chloroplasts

Chloroplastic TRXs are mainly reduced by FTR and to a minor extent by NTRC. TRXs from other compartments are mainly reduced by NTRA and NTRB, although a compensatory role of the NADPH/GR/GSH/GRXs system in reducing TRXs has also been observed (Reichheld *et al.*, 2007). NTRC is exclusively found in plastids whilst NTRA and NTRB are both simultaneously located in the cytosol and mitochondria, which potentiates redundancy amongst these proteins and explains the absence of phenotype apparent in the *ntra* and *ntrb* single mutants (Reichheld *et al.*, 2005, 2007). Whilst NTRA and NTRB present only TRX reductase activity, NTRC contains an unusual TRX domain which allows this protein to act simultaneously as a TRX and TRX reductase. First described in *Oryza sativa* (Serrato *et al.*, 2004), NTRC was initially pointed out as an alternative protein involved with redox regulation of chloroplastic metabolism (Perez-Ruiz *et al*., 2006). However, biochemical studies and reverse genetic characterization of rice and Arabidopsis *ntrc* mutants demonstrated the importance of this protein and led to its inclusion as a major player in chloroplastic redox metabolism (Serrato *et al.*, 2004; Perez-Ruiz *et al*., 2006; Thormählen *et al.*, 2015). The Arabidopsis *ntrc* knockout mutant presented severely impaired photosynthesis rate and reduced growth rate (Serrato *et al.*, 2004; Perez-Ruiz *et al*., 2006; Thormählen *et al.*, 2015). However, despite its extreme importance for plant growth, it is noteworthy that this system is not essential given that the single *ntrc* mutant is

still viable. This viability is abolished in the *ftrb ntrc* double mutant (Yoshida and Hisabori, 2016b; Pérez-Ruiz *et al.*, 2017), indicating that FTR can compensate the role performed by NTRC and that a complete TRX reductase system is indispensable for plant growth and development.

The function of NTRC differs from that of the Fdx/TRX system, which fundamentally uses electrons from photosystem I (PSI), while NTRC uses NADPH as electron donor. The source of NADPH for NTRC can be from the photosynthetic electron transport chain in the light or the oxidative pentose phosphate pathway (OPPP) which also works under dark conditions (Montrichard *et al.*, 2009). This implies that NTRC can also act in non-green tissues such as root amyloplasts (Michalska *et al.*, 2009). In fact, the Arabidopsis *ntrc* mutant is hypersensitive to darkness (Pérez-Ruiz *et al.*, 2017). However, the phenotype of the *ntrc* mutant is more severe when plants were grown under a short day photoperiod or under fluctuating light conditions (Thormählen *et al.*, 2015, 2017). The function of NTRC during light exposure is mainly linked to the regulation of stromal enzymes that are involved in either the CBC or H_2O_2 detoxification (Perez-Ruiz *et al.*, 2006). The NTR domain of NTRC is able to reduce TRXs such as *f1*, *m1*, *m3*, *x*, and *y1 in vitro* even when its TRX domain is inactivated (Yoshida and Hisabori, 2016). Furthermore, NTRC is involved in starch biosynthesis by activating AGPase (Michalska *et al.*, 2009; Lepistö *et al.*, 2013), ATP synthesis by activating the CF1γ subunit of ATP synthase (Nikkanen *et al.*, 2016), and tetrapyrrole biosynthesis by activating two key enzymes of this pathway, the magnesium chelatase and magnesium protoporphyrin methyltransferase (Richter *et al.*, 2013; Pérez-Ruiz *et al.*, 2014).

Interestingly, the NTRC-mediated CF1γ activation seems to be independent of the TRXs *f1* and *f2* given that the *trxf1-f2* double mutant did not show impairment in light-dependent reduction of CF1γ (Yoshida *et al.*, 2015), despite the interaction observed between TRX *f1* and CF1γ *in vivo* (Nikkanen *et al.*, 2016). It has been suggested that the NTRC-mediated CF1γ activation occurs mainly under low light condition whilst the FTR-TRX *f1* system is able to compensate NTRC under a high light environment (Carrillo *et al.*, 2016; Nikkanen *et al.*, 2016). Among the CBC enzymes, FBPase is the most clearly described enzyme that it is regulated by NTRC *in vitro* and *in vivo* (Thormählen *et al.*, 2015). Beyond the deficiency in activating FBPase, which reduces the capacity of the plant to regenerate ribulose-1,5-bisphosphate in the CBC, the reduced growth phenotype of *ntrc* is also due to an impairment in photochemical quenching, as demonstrated by the lower values of the effective

quantum yield of PSII (Thormählen *et al.*, 2015). As a consequence, *ntrc* deficiency leads to decreased photosynthetic efficiency in different light growth conditions (Carrillo *et al.*, 2016; Naranjo *et al.*, 2016; Yoshida and Hisabori, 2016b). On the other hand, the overexpression of NTRC and TRX *f1* leads to increased biomass accumulation and starch biosynthesis in tobacco (Sanz-Barrio *et al.*, 2013). This highlights the importance of the chloroplast NTR/TRX system for plant growth. Furthermore, given the importance of enzymes of the antioxidant system for plant stress tolerance and based in the fact that several proteins of this system are redox regulated by TRXs, it seems reasonable to assume that the chloroplast NTR/TRX system may contribute to the plant stress acclimation by modulating the activity of antioxidant enzymes. In the next sections, we will briefly describe the functions of important enzymes of the antioxidant system such as PRXs, SRXs, APXs, GPXs, and other glutathione-related enzymes in plant cells and will also discuss how these proteins interact with FTR, NTRC and TRXs in the regulation of chloroplast metabolism.

C. The interplay between thioredoxins, peroxiredoxins, and sulfiredoxins in chloroplasts

Peroxiredoxins (PRXs) and sulfiredoxins (SRXs) are important components of the chloroplastic antioxidant defense system (Dietz, 2011). SRX is conserved in eukaryotes yet absent in prokaryotes, with the exception of cyanobacteria (Rouhier *et al.*, 2006). Like the TRXs, PRXs are also small proteins (with their apparent molecular masses ranging from 17 to 22 kDa) and are widely distributed within plant subcellular compartments (Dietz *et al.*, 2006; Sevilla *et al.*, 2015). The 2-Cys PRXs are the most abundant PRX in plants. SRX and 2-Cys PRXs form an interconnected network with the TRX system, given that 2-Cys PRXs reduction depends on the activity of TRX and/or SRX. Moreover, evidence suggests that the presence of NTRC is important for 2-Cys PRX reduction (Puerto-Galán *et al.*, 2015; Sevilla *et al.*, 2015). The oxidation state of the Cys residues of 2-Cys PRXs determines whether the reduction is performed by SRX or by the NTR/TRX system (Figure 1). This is based on the fact that one of the two Cys residues of 2-Cys PRX can be oxidized by H_2O_2 to form sulfenic acid (SOH) which can be subsequently further oxidized by H_2O_2 to form sulfinic acid (SO_2H). The overoxidized SO_2H is reduced by SRX to form SOH, which can then be reduced to SH by TRX (Biteau *et al.*, 2003; Jeong *et al.*, 2006; Puerto-Galán *et al.*, 2015). It has been proposed that the interconversion between reduced, oxidized and overoxidized status of 2-Cys PRX have an important role in the homeostasis of

H_2O_2 content by acting as ROS sensors (Liebthal *et al.*, 2017), including this enzyme as another component of the ROS-scavenging system. This idea is based on the fact that two molecules of H_2O_2 are consumed for the overoxidation of 2-Cys PRX. In this respect, SRX proteins are of pivotal importance to reduce the overoxidized form of the 2-Cys PRX, which can then be reduced by the TRX system, enabling the restart of the H_2O_2 consuming cycle (Figure 1).

The known function(s) of SRX have until recently been limited to specifically reduce sulfinic acid of 2-Cys PRXs, given that *srx* mutant present increased level of overoxidized 2-Cys PRX (Puerto-Galán *et al.*, 2015) and based in the fact that SRX is not capable to reduce the same oxidized Cys residue in other PRXs or in glyceraldyhyde-3-phosphate dehydrogenase (Hyun *et al.*, 2005). However, interesting results from a recent 2-Cys PRX interactome study revealed that this protein interacts with several other chloroplastic proteins related to photosynthesis, carbon, nitrogen and sulfur metabolisms, antioxidant defense, and the TRX system (Cerveau *et al.*, 2016). What remains unclear is how the PRX-SRX cycle interacts with the FTR/NTRC-TRX system (Liebthal *et al.*, 2017). Furthermore, it was shown that 2-Cys PRX interacts with important enzymes of the antioxidant system such as SOD, GR and PRXQ, raising the question whether these proteins act in concert in the regulation of ROS homeostasis. In the next sections we review the role of other chloroplastic ROS-scavenging enzymes such as APX and GPX as well as the glutathione metabolism in the regulation of chloroplastic redox metabolism.

D. Chloroplastic APX and GPX: more than ROS scavenging enzymes

APXs and GPXs are other important enzymes of the antioxidant defence system involved in ROS scavenging in plant cells. APX and GPX isoforms are present in almost all plant cell compartments including cytosol, chloroplast, peroxisome and mitochondria (Teixeira *et al.*, 2006; Margis *et al.*, 2008). Most plant species harbour two different chloroplastic APX isoforms (chlAPX), one targeted to the stroma (sAPX) and the other to thylakoid membranes (tAPX) (Table 1) (Teixeira *et al.*, 2006). The most remarkable evidence for the biological role presented by chlAPXs is provided by the characterization of plants lacking sAPX and/or tAPX (Danna, 2003; Giacomelli *et al.*, 2007; Miller *et al.*, 2007; Kangasjärvi *et al.*, 2008; Maruta *et al.*, 2010; Caverzan *et al.*, 2014). Except in wheat (Danna, 2003), plants deficient in both chlAPX isoforms are capable of

tolerating excessive light (Giacomelli *et al.*, 2007; Kangasjärvi *et al.*, 2008; Maruta *et al.*, 2010; Caverzan *et al.*, 2014). For instance, Arabidopsis mutants and rice antisense transgenic plants for both chlAPXs presented no phenotypic differences compared to WT plants even under high light conditions (Giacomelli *et al.*, 2007; Caverzan *et al.*, 2014). A similar study reported that tAPX is important in the first hours of high light exposure, but not in long term light exposure (Maruta *et al.*, 2010). These results suggest that these proteins are not essential for plant stress responses but may rather be mainly related to the mediation of H_2O_2 retrograde signalling.

In contrast to cytosolic isoforms that actively work to prevent excessive ROS accumulation, chlAPX isoforms likely display an important role at the onset of photooxidative stress, controlling H_2O_2-induced retrograde signalling and activating other antioxidant mechanisms (Maruta *et al.*, 2016). Indeed, recent works have reported that H_2O_2 generated in chloroplasts closely associated to nucleus are involved in the signalling for different antioxidative defense mechanisms and that this process is dependent on the inactivation of chlAPX isoforms (Exposito-Rodriguez *et al.*, 2017). The fact that these enzymes are not essential for photooxidative stress responses may be explained by the fact that they are promptly inactivated in the presence of H_2O_2 and should be completely dispensable or replaceable in detoxification mechanisms related to plant defence against excessive light. This idea is strengthened by the increased level of 2-Cys PRXs found in the *tapx sapx* double mutant under high light, possibly as an effective compensatory mechanism (Kangasjärvi *et al.*, 2008). However, whether other enzymes such as GPXs can also compensate the absence of chlAPXs remain to be determined.

GPX catalyzes the reduction of H_2O_2 and organic peroxides into water and the corresponding alcohols using reducing power provided by different reductants (Figure 1) (Herbette *et al.*, 2007; Selles *et al.*, 2012). The GPX family is found in virtually all kingdoms of life and has been increasingly studied in plants (Margis *et al.*, 2008; Passaia and Margis-Pinheiro, 2015). This enzyme family is part of the heme-free thiolperoxidase class which can use glutathione, TRX and other reducing agents as substrates (Herbette *et al.*, 2002). The class-4 GPX, also named phospho-lipid GPX (commonly called PHGPX) can utilize organic hydroperoxide/H_2O_2 and GSH as oxidant and reductant, respectively, for its activity (Ursini *et al.*, 1985; Margis *et al.*, 2008). However, plant GPX isoforms exhibit higher affinity to use TRXs as electron donors (Herbette *et al.*, 2002; Cha *et al.*,

2008; Wang *et al.*, 2017) and the utilization of GSH as reducing agent by this enzyme has been questioned under *in vivo* conditions (Herbette *et al.*, 2002; Jung *et al.*, 2002; Iqbal *et al.*, 2006; Navrot *et al.*, 2006). Chloroplast GPX isoforms have been specially reported as important players for plant development and to cope against different environmental stresses in several species (Rodriguez Milla *et al.*, 2003; Kim *et al.*, 2009; Passaia *et al.*, 2013; Wang *et al.*, 2017), while non-chloroplast GPX isoforms play important role in photosynthesis (Lima-Melo *et al.*, 2016). Additionally, single GPX proteins or the balance between reduced (GSH) and oxidized (GSSG) glutathione seems to act as redox sensors in plant cells (Miao *et al.*, 2006; Passaia and Margis-Pinheiro, 2015). As such the balance between GSH/GSSG and the activity of glutathione-related enzymes could regulate the chloroplastic redox metabolism.

E. The role of glutathione-related enzymes in the control of the redox metabolism

Given that glutathione is one of the major players in plant redox regulation, glutathione-related enzymes such as GPX, glutathione synthetase (GS), glutathione transferase (GST), and glutathione reductase (GR) are therefore important redox players in addition to the previously mentioned members of the plant antioxidant defense system. The role of glutathione-related enzymes has been well described regarding their roles in antioxidative mechanisms, especially under abiotic stresses (Su *et al.*, 2016; Harshavardhan *et al.*, 2017). These proteins are directly involved in the balance between reduced (GSH) and oxidized (GSSG) glutathione concentration, controlling thus the GSH redox state (Foyer and Noctor, 2013; Burritt, 2017). More than that, some of these enzymes are also important for the redox metabolism because they can interplay directly with other proteins such as DHAR, GR, APX, TRX, and PRX (Noctor *et al.*, 2012; Passaia and Margis-Pinheiro, 2015; Burritt, 2017).

Plant GSTs are found in virtually all subcellular locations, including mitochondria, peroxisomes, nucleus, and mainly chloroplasts and cytosol (Dixon *et al.*, 2009; Lallement *et al.*, 2014). GSTs catalyze the conjugation of GSH to electrophilic sites on a wide range of hydrophobic compounds (Chronopoulou *et al.*, 2014; Labrou *et al.*, 2015). They can be sub-divided into 17 distinct classes (Nianiou-Obeidat *et al.*, 2017) and can play a great variety of reactions, which include transferase, glutathionylation, peroxidase, isomerase, dehalogenation, and deglutathionylation activities (Lallement *et al.*, 2014; Su *et al.*, 2016). The diversity of

GSTs function is high and a special role of these proteins in the regulation of secondary metabolism has been postulated (Dixon and Edwards, 2010). In chloroplasts and cytosol, the most studied GST is dehydroascorbate reductase (DHAR), which is involved in the ascorbate regeneration from the Foyer-Halliwell-Asada cycle (Foyer and Halliwell, 1976; Asada, 1999).

The GR1 and GR2 enzymes belong to a NADPH-dependent oxidoreductase group of flavoproteins. GR1 is found in the cytosol whilst GR2 is found in both mitochondrion and chloroplast (Chew *et al.*, 2003). These enzymes play an essential role in the maintenance of the GSH pool by catalyzing the reduction of one molar equivalent of GSSG to two molar equivalents of GSH. Taking into account that the balance of GSH/GSSG is an important indicator of the redox state of the cell, it was previously thought that GR activity could be of pivotal importance for controlling the redox potential of plant cells (Delorme-Hinoux *et al.*, 2016), especially in chloroplasts where the input of energy by light absorption leads to a highly dynamic and ROS-enriched environment (Wu *et al.*, 2015; Ding *et al.*, 2016a). However, plants lacking GR1 showed increased content of GSSG and this did not lead to higher stress sensitivity (Marty *et al.*, 2009). This suggests that another system can compensate the absence of GR1. In fact, it has been shown that Arabidopsis NTRA/TRX *h3* system exhibit functional redundancy with cytosolic GR1 (Reichheld *et al.*, 2007; Marty *et al.*, 2009). On the other hand, it was demonstrated that the function of GR1 in day length-dependent redox signalling cannot be replaced by the chloroplastic/mitochondrial isoform GR2 or by the TRX system (Mhamdi *et al.*, 2010). In contrast to *gr1* mutants, the knockout of GR2 is lethal in Arabidopsis (Tzafrir *et al.*, 2004), whilst *gr2* knockdown plants presented early leaf senescence, defective root development, and higher sensitivity to high light stress than WT, which was associates to PSII damage caused by ROS overaccumulation and by an altered GSH/GSSG redox status (Yu *et al.*, 2013; Ding *et al.*, 2016a, 2016b). It seems likely that GR1 acts in synchrony with the cytosolic NTR/TRX system while the effects of chloroplastic GR2 knockdown cannot be compensated by the chloroplastic NTR/TRX systems.

III. Crosstalk between chloroplast and other subcellular compartments

In the previous section we have provided a brief overview regarding the function of the main players of the chloroplastic redox network. It is clear that the activity of the enzymes of this redox system is important to maintain the chloroplast metabolism in perfect harmony. However, it is important to highlight that the plant organelles work in concert, with several molecules being responsible for the communication between different subcellular compartments. Thus, the function of the different chloroplastic redox players is not limited to this organelle. In the following sections we highlight which chloroplastic molecules contribute to the interorganellar communication and provide a perspective concerning how signals from and to chloroplast can coordinate the entire plant cell redox metabolism.

A. Chloroplast ROS-mediated signalling

Most redox components are highly active under light conditions. Light-dependent chloroplast reactions provide electrons via the PSI to ferredoxin which reduces either TRX via FTR or NADPH via FNR (Meyer *et al.*, 2009). The thiol reductase system is responsible for the regulation of a wide range of proteins and thus plays a pivotal role in the redox regulation of the plant cell. However, in parallel to the light-induced energy excitation, oxygen evolution in chloroplasts can lead to the formation of different ROS which are extremely harmful in high concentrations. Therefore, the ROS scavenging system which is mainly comprised of APX, GPX, SOD and PRX must work concurrently with ~~to~~ the NTR/FTR-TRX system in order to maintain the balance of ROS and thereby avoid overoxidation of the chloroplast (Figure 1). Alternatively, ROS can be transported to the cytosol where another antioxidant system takes place to eliminate the excess of these harmful molecules. Thus, the transport of ROS out of the chloroplast might act as a signal that connects the different compartments of the plant cell (Mittler, 2017).

Among the different ROS, H_2O_2 has been credited as the most potent signaling molecule. This concept relies on some important characteristics presented by H_2O_2, including relatively low reactivity and higher half-life, as compared with other ROS (Polle, 2001; Møller *et al.*, 2007). The recent discovery of a sub-population of chloroplasts closely associated with nuclei that are able to induce nuclear accumulation of H_2O_2 during the onset of light stress indicates a possible pathway for crosstalk and retrograde signaling mechanisms connecting

photosynthesis and nuclear gene expression regulation (Exposito-Rodriguez *et al.*, 2017). This idea is strengthened by the fact that some yeast redox-sensitive thiol-disulphide transcription factors are activated by TRXs and peroxidases and deactivated by H_2O_2-mediated oxidation (Fomenko *et al.*, 2011). It has been proposed that this redox-relay mediation mechanism should be also conserved in plants (Exposito-Rodriguez *et al.*, 2017). For instance, the redox-responsive transcription factors from the RAP2.4 family, which control the 2-Cys PRX and chlAPX expression in plants, presents cysteine residues that might be oxidized by H_2O_2 (Shaikhali *et al.*, 2008; Rudnik *et al.*, 2017). This suggests that chloroplast signals are important to regulate nuclear gene expression which seems to be subjected to a H_2O_2-mediated redox regulation.

Beyond H_2O_2, it is noteworthy that other signals can also act as intermediates of interorganellar communication. For instance, the transport of malate from the chloroplast to the cytosol and subsequently to mitochondria has been proposed as a possible mechanism that connects these subcellular compartments (Heyno *et al.*, 2014; Geigenberger *et al.*, 2017). In the next section we discuss the role of malate metabolism in the interorganellar communication under different light/dark conditions.

B. The role of the circulating malate to both NAD(P)(H) metabolism and interorganellar communication under dark and light conditions

Malate metabolism regulates a wide range of physiological processes such as respiration, stomatal movements, fruit ripening, photorespiration, and seed maturation (Zhang and Fernie, 2018). Malate is found in different cell compartments and it seems likely that its function depends on the concentration and the location where it is found (Fernie and Martinoia, 2009). For instance, it has been shown that malate act as a signalling molecule, respiratory substrate and a regulator of stomatal movements when located in cytosol, mitochondria, and apoplastic space, respectively (Araújo *et al.*, 2011; De Angeli *et al.*, 2013; Medeiros *et al.*, 2016, 2017). Thus, it is not surprising that malate metabolism is finely regulated among plant cell organelles. In this context, the chloroplastic malate valve has long been thought to have implications for the communication between chloroplast and other organelles. This idea is based on the fact that the chloroplastic NADP-dependent malate dehydrogenase (MDH) activity is strictly dependent on light and TRX activation and that malate is a circulating form of reducing power throughout plant cell (Michelet *et al.*, 2013). It therefore seems likely that the transport

and accumulation of malate in different subcellular compartments closely follow different environmental signals. Malate is thus a possible redox sensor that links different plant cell organelles according to the light/dark condition (Figure 3).

In the light, mitochondria respiration is inhibited (Tcherkez *et al.*, 2012) whilst both chloroplastic and cytosolic MDH are active. Thus, the amount of the circulating malate in the light is mainly due the activity of the non-mitochondrial MDH enzymes and the export of previously night stored organic acids from the vacuole (Figure 3). An active NADP-MDH is highly important to consume the excess of NADPH generated by the photosynthetic electron transport chain and to regenerate the electron acceptor $NADP^+$ (Hara *et al.*, 2006; Huang *et al.*, 2018). Intriguingly, both cytosolic and chloroplastic MDH are clearly redox regulated by TRX (Wolosiuk *et al.*, 1977; Hara *et al.*, 2006; Yoshida *et al.*, 2015; Thormählen *et al.*, 2017), while the mitochondrial MDH is irresponsive to TRX (Daloso *et al.*, 2015; Yoshida and Hisabori, 2016a; Huang *et al.*, 2018). Perhaps more challenging is the fact that fumarase (FUM) is activated by TRX *h2* and deactivated by TRX *o1 in vitro* (Daloso *et al.*, 2015), suggesting that FUM can be positively and negatively regulated by different TRXs (Figure 3). However, given the absence of data from *trxh2* mutants and the dual location of TRX *h2*, which is found in both cytosol and mitochondria, it is still unclear whether the TRX *h2*-mediated FUM regulation also occurs *in vivo* and if so in which subcellular compartment this occurs. Another outstanding question is why FUM and cytosolic MDH (cMDH) would be simultaneously activated by TRX *h2*. Could the TRX-mediated activation of both FUM and cMDH act as a mechanism for the synthesis of fumarate in the cytosol? This idea is supported by the fact that the carbon fluxes to the TCA cycle are light-inhibited, which thus compromise the synthesis of organic acids in mitochondria, and also by the fact that the massive accumulation of fumarate in the light depends on the cytosolic FUM (FUM2) activity (Pracharoenwattana *et al.*, 2010), which is higher in the direction of fumarate synthesis and activated by Gln, Asn and OAA (Figure 3) (Zubimendi *et al.*, 2018). The questions raised here can be solved by the analysis of mutants lacking different TRX isoforms (discussed latter in the section 5) and by non-aqueous fractionation metabolic analysis that can determine the accumulation of malate and fumarate in different subcellular compartments (Krueger *et al.*, 2014; Medeiros *et al.*, 2017). Thus, further experiments in this direction must assume a paramount importance to

elucidate the role of malate and malate valve for the cross-talk between chloroplasts and mitochondria in leaves exposed to light.

By contrast to the accumulated knowledge regarding light/dark reactions in the chloroplasts, information concerning light/dark regulation of mitochondrial enzymes remains very limited (Nietzel *et al.*, 2017). It is reasonable to assume that chloroplastic malate valve may only have minor impact on the redox regulation of plant cells in the dark given that the input of energy through photosynthesis is absent and NADP-MDH is inactive (Figure 3). However, another redox mechanism mediated by NTRs (NTRA and NTRB) and TRXs (TRX *h* family) can activate cytosolic MDH in the cytosol (Hara *et al.*, 2006; Huang *et al.*, 2018) In this case the source of NADPH for NTRs comes from the oxidative pentose phosphate pathway (OPPP). Mitochondria also contain both NTRA and NTRB enzymes and different TRXs (TRX *o1*, *o2*, *h2*). In this organelle, NADPH can be supplied by the activity of a NADP-dependent isocitrate dehydrogenase (ICDH) and alternatively by a presumable mitochondrial NAD(H) kinase (NADk) (Figure 3) (Møller and Rasmusson, 1998; Møller, 2001; Rasmusson *et al.*, 2004; Geigenberger *et al.*, 2017). Interestingly, ICDH-dependent NADPH production seems to be redox regulated by TRX (Yoshida and Hisabori, 2014). Beyond ICDH, other TCA cycle-related enzymes such as citrate synthase (CS), FUM, and succinate dehydrogenase (SDH) as well as the alternative oxidase (AOX) have already been suggested to be TRX-regulated (Gelhaye *et al.*, 2004a; Schmidtmann *et al.*, 2014; Yoshida and Hisabori, 2014; Daloso *et al.*, 2015). Recent evidence indicated that TRX *o1* controls the flux of C to the TCA cycle in the light by deactivating both FUM and SDH (Daloso *et al.*, 2015). This result, together with the light inhibition of pyruvate dehydrogenase (PDH) (Tovar-Méndez *et al.*, 2003) and the role of mitochondrial MDH, which seems to act preferentially in the synthesis of malate to be exported to cytosol (Tomaz *et al.*, 2010) (Figure 3), could explain why respiration is inhibited in the light. Furthermore, the light inhibition of FUM1, SDH and PDH also helps to explain previous predictions from a genome scale metabolic model (Cheung *et al.*, 2014) and recent results from a nuclear magnetic resonance-based metabolic flux study that indicate a non-cyclic mode of operation of the TCA cycle in the light (Abadie *et al.*, 2017). The results from these studies suggest that the TCA cycle works in a non-cyclic mode to sustain glutamate (Glu) and glutamine (Gln) biosynthesis in the light, in which TRXs would be key regulators by activating CS and ICDH

and deactivating FUM and SDH (Figure 3). In turn, Gln biosynthesis would activates FUM2 leading to the synthesis of fumarate in the cytosol. However, whether these reactions also occur in the dark remain to be seen.

In summary, it seems likely that both NAD(P)(H) and malate metabolism, especially the malate valve and the activities of MDH and FUM, are important players for the crosstalk between chloroplasts and other organelles. It has been shown that alteration of key chloroplastic, mitochondrial, or peroxissomal enzymes alter not only the processes that take place autonomously within each organelle but also have high impact in processes occurring in other organelles. This suggests that the different plant cell organelles are tightly regulated. In the next section we explore the mechanisms and players for this connection between plant subcellular compartments.

C. Chloroplastic and mitochondrial metabolism are tightly regulated

The characterization of plants lacking or displaying reduced activity of key enzymes of redox metabolism has demonstrated that the perturbation of some chloroplastic enzymes affects different processes in other subcellular locations. Similarly, perturbation in mitochondrial enzymes has also been demonstrated to alter chloroplastic metabolism. The cross-regulation between chloroplast and mitochondria is probably the most studied interorganellar system in plants (Blanco *et al.*, 2014; Mignolet-Spruyt *et al.*, 2016; Uhmeyer *et al.*, 2017; Noctor *et al.*, 2018). Special attention has been given to the mitochondrial enzyme AOX, given its importance for both mitochondrial respiration and in maintaining chloroplast redox homeostasis and photosynthetic rates (Vishwakarma *et al.*, 2014; Florez-Sarasa *et al.*, 2016a; Welchen and Gonzalez, 2016; Dahal and Vanlerberghe, 2017; Del-saz *et al.*, 2018). Several other mitochondrial proteins involved with the TCA cycle and mitochondrial electron transport chain have additionally been shown to regulate chloroplast metabolism (Sweetlove *et al.*, 2006; Timm *et al.*, 2012a, 2018; Pires *et al.*, 2016). However, little is known about the importance of enzymes of the redox network such as APXs, GPXs, and TRXs among others in this interorganellar regulation. Recent evidence showed that the silencing of a mitochondrial glutathione peroxidase (OsGPX1) penalizes photosynthetic assimilation and growth rates in rice by a mechanism involving changes in cell H_2O_2 and GSH contents (Lima-Melo *et al.*, 2016). Similarly, plants lacking the mitochondrial TRX *o1* presented high activities of enzymes of redox metabolism such as SOD and catalase under salt stress (Calderón

et al., 2018) beyond up regulation of AOX (Del-Saz *et al.*, 2016). Taken together, these studies suggest the perturbation of mitochondrially located GPX or TRX had consequences for plant cell redox metabolism in general. On the other hand, mutants lacking specific chloroplast proteins have been used to investigate the consequences on mitochondrial metabolism. For example, it was demonstrated that Arabidopsis mutants lacking proteins related to cyclic electron flow display higher AOX activity and changes in NAD(P)/NAD(P)H ratios under high light (Florez-Sarasa *et al.*, 2016a). Therefore, it seems that both chloroplast and mitochondrial metabolism are tightly regulated, by a mechanism which may involve the transmission of signals such as ROS, nitric oxide and calcium as well as the accumulation of shared metabolites such as malate and fumarate (Fernie and Martinoia, 2009; Araújo *et al.*, 2011a; Florez-Sarasa *et al.*, 2016b; Del-Saz *et al.*, 2018). Further studies will likely prove important in unraveling the connections between these organelles as well as the importance of this communication for plant stress tolerance.

D. Cytosol as a redox buffer and an interorganellar communication mediator

Experimental evidence has accumulated that the plant cytosol might represent a crucial cellular compartment for metabolic regulation of the whole cell, acting as a redox buffer and an important crossroad for retrograde signaling pathways. Indeed, the cytosolic APX and ASC-GSH cycle display a central role in the scavenging of excessive H_2O_2 produced by other organelles such as peroxisomes, chloroplasts and mitochondria (Davletova *et al*., 2005). The cytosol contains the majority of the antioxidant defense protein isoforms, comprising a dynamic system involved in ROS production and scavenging in a manner that might affect both local and systemic responses (Munne-Bosch *et al.*, 2013). Furthermore, cytosol also contains a complete NTR/TRX system composed by NTRA/NTRB and different TRX *h* proteins. The buffering capacity of cytosol is evidenced by the fact that the excess of cytosolic H_2O_2 generated by APX deficiency in Arabidopsis inhibited photosynthesis by inducing oxidative stress in chloroplasts (Davletova *et al*., 2005). The excess ROS can cause denaturation of crucial Calvin-Benson cycle proteins by carbonylation, including Rubisco (Davletova *et al*., 2005), and even delays in the PSII repair process (Murata and Nishiyama, 2018). Together, these damages can induce a strong restriction of CO_2 assimilation and photoinhibition of PSII, contributing to a general impairment in photosynthetic capacity and plant growth (Foyer *et al.*, 2012). Recently, we have demonstrated that

accumulation of peroxisomal H_2O_2 induced by CAT inhibition and APX knockdown in rice plants also deeply affected the cytosolic antioxidant protein synthesis, especially in those enzymes involved in ASC-GSH cycle (unpublished data). Besides cytosolic H_2O_2, other signaling molecules produced by several different metabolic pathways such as GSH might trigger retrograde signaling, which in turn may alter several redox responses in the distinct plant cell compartments (König *et al.*, 2018). In this vein, the photorespiratory pathway is ~~consists in~~ an important route for the generation of exchangeable signaling molecules, especially for connecting chloroplasts, cytosol, peroxisomes and mitochondria (Timm *et al.*, 2013).

E. Beyond a wasteful pathway: the important role of photorespiratory metabolism to photosynthesis and for the maintenance of plant cell redox state

In addition to chloroplasts and mitochondria, the peroxisome is also an important organelle involved in ROS-related signaling (Del Río *et al.*, 2003; Dietz, 2015; Noctor and Foyer, 2016). Chloroplasts and peroxisomes are connected by the process of photorespiration, in which the oxygenase activity of the chloroplastic enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) induces the photorespiratory pathway that also involves mitochondria (Bauwe *et al.*, 2010). Furthermore, peroxisomes have been described as the main source of ROS in plant cells, especially in C3 leaves exposed to light (Foyer and Noctor, 2003). A study with Arabidopsis mutant plants overexpressing glycolate oxidase in the chloroplast and plants deficient in peroxisomal catalase showed that the H_2O_2-dependent signal is different when this ROS is generated in chloroplast from that generated in peroxisomes (Sewelam *et al.*, 2014). Additionally, it was shown that plant cells have an integrated signal network that works independently of the subcellular site of H_2O_2 production (Sewelam *et al.*, 2014). These reports show the importance of understanding redox and ROS signaling pathways originating from different subcellular compartments, especially those from peroxisomes. In this context, RNAi suppression of both peroxisomal rice APX isoforms coupled with a pharmacological inhibition of catalase triggered favorable antioxidant and compensatory mechanisms to cope with oxidative burst conditions, most probably due to a priming mechanism induced by peroxisomal H_2O_2 signalling (Sousa *et al.*, 2015). These results suggest that peroxisomal H_2O_2-mediated signalling has a pivotal role for the maintenance of redox state of the whole plant cell.

Photorespiration has long been described as a wasteful pathway that competes with the carboxylase activity of RubisCO and thus reduces the yield in C3 plants. With this idea in mind, several research groups have engineered plants to improve plant yield by suppressing photorespiration or by increasing the carboxylation-to-oxygenation ratio of RubisCO. However, recent reports showed that the overexpression of some photorespiratory enzymes leads to increased photosynthesis and plant growth (Timm *et al.*, 2012b, 2015) and that photorespiration is crucial for the photosynthetic and stomatal responses to CO_2 availability (Eisenhut *et al.*, 2017). These findings indicate that the photorespiratory metabolism is important for the control of photosynthesis. Photorespiration may contribute to increased photosynthetic rate by eliminating toxic intermediates, recycling substrates for RubisCO carboxylation, and providing substrates for other metabolic pathways (Wingler *et al.*, 2000). Moreover, it has been shown that the activity of Calvin-Benson cycle enzymes are regulated by the accumulation of the photorespiratory metabolite 2-phosphoglycolate (Flügel *et al.*, 2017). Thus, given that photorespiration encompasses transport of metabolites between chloroplasts, peroxisomes, cytosol and mitochondria and involves the production of H_2O_2 in peroxisomes and NADH in mitochondria, it is reasonable to attribute a great importance to this pathway in the regulation of the entire plant cell redox network (Geigenberger and Fernie, 2014; Obata *et al.*, 2016; Timm *et al.*, 2016). It is therefore clear that the function of each enzyme of the plant cell redox system is not specific to the organelle where that reaction takes place, suggesting that the entire redox system acts in synchrony. Evidence for this comes from studies that have revealed several compensatory and redundant roles among the redox players, which has been mainly discovered by the characterization of plants lacking multiple isoforms of a redox system. In the next section, we review these findings and provide a perspective on which hypothesis should be tested in the near future to improve our knowledge concerning how the different redox components interact and compensate to each other.

IV. Reverse genetic strategies to unravel redundant and compensatory mechanisms between redox network components

The redundancy and the compensatory role observed among the players of the redox metabolism is mainly perceptible by studies that use specific inhibitors coupled with reverse genetic approaches (Rizhsky *et*

al. 2002; Sousa *et al.* 2015; Bonifacio *et al.* 2016; Rahantaniaina et al., 2017) or by the characterization of mutants that lack the activity of more than one protein of the redox network (Reichheld *et al.*, 2007; Bashandy *et al.*, 2009; Marty *et al.*, 2009; Daloso *et al.*, 2015; Thormählen *et al.*, 2015; Yoshida and Hisabori, 2016b; Pérez-Ruiz *et al.*, 2017). Recent reports have used these strategies and were able to provide considerable information as to how the different components of the chloroplastic redox network can compensate for one another. For instance, it is known that the reduced growth rate of both the *ntrc* mutant and the *ntrc trxf1 trxf2* triple mutant is due the overaccumulation of oxidized 2-Cys PRX which supposedly acts as a sink for reducing power from the chloroplastic TRXs (Pérez-Ruiz *et al.*, 2017). Furthermore, despite the essentiality of NTR for mammalian systems (Conrad *et al.*, 2004), the *ntrc* mutant and the *ntra ntrb* double mutant are still viable, most probably due a compensatory role of FTR system in chloroplasts and GR and glutathione metabolism in cytosol and mitochondria (Reichheld *et al.*, 2007; Bashandy *et al.*, 2009; Marty *et al.*, 2009; Daloso *et al.*, 2015; Yoshida and Hisabori, 2016b). It seems clear therefore that different redox components of each organelle could act in concert to maintain its redox state in perfect harmony. However, how the redox systems from different compartments interact with one another remains unclear. For instance, it remains to be investigated as to whether plants are still viable with the knockout of the entire plant NTR system (Table 2).

Despite recent advances, relatively few studies have simultaneously disrupted chloroplastic and non-chloroplastic redox components, a fact which hampers our understanding on how these systems compensate to each other. We propose here that the establishment and characterization of a number of mutants lacking different components of the plant cell redox network will certainly provide great information for the plant redox scientific community. Given that the compensation of components of the chloroplastic redox network might be achieved by non-chloroplastic redox components, different combinations of mutations should be carried out (Table 2). For instance, assuming that TRX *o2* is mitochondrially located, the characterization of the *trxo1 trxo2 trxh2* triple mutant, which will supposedly present no mitochondrial TRX activity, may reveal the possible redundancy between these TRXs and enable the investigation whether other enzymes such as mitochondrial GRXs can compensate the absence of these TRXs. Furthermore, given the already described role of TRX *h* proteins in the activation of cMDH and FUM2, it is reasonable to assume that the characterization of plants

lacking multiple TRX *h* proteins may provide important information regarding how these TRXs regulate malate metabolism and whether GRXs can compensate the absence of these proteins (Table 2).

In the case of chloroplastic peroxidases, few studies have investigated double or triple mutants. Thus, different combinations to knockout sAPX, tAPX, and GPXs would be anticipated to provide important insights. All other possible combinations which include plants lacking or deficient in more than one enzyme of the chloroplastic reductase system (NTRC, FTRA, FTRB, and GR2) are described in detail in the Table 2. After the establishment of plants lacking different enzymes of the redox network, it is important to highlight that the processes regulated by these enzymes are better understandable by adopting systems biology approaches. In the next section we provide a perspective with two practical examples on how systems biology approaches can be used to improve our understanding on the function and interactions among redox-related enzymes.

V. Using systems biology approaches to unravel interaction and cross- regulation among enzymes of the chloroplastic redox network

The emergence of omic platforms in the last decades has produced vast quantities of data which are currently available for bioinformatics and modelling analysis. Plant biology thus became a data-enriched, multidisciplinary field in which the application of mathematics, physics, and computational biology concepts are proving to be essential for integrating and understanding the experimental data acquired. This multidisciplinary scientific field is based on the ideas from the general systems theory (von Bertalanffy, 1968) and it is commonly referred to as systems biology (Friboulet and Thomas, 2005). The aim of systems biology is to understand the dynamic of the interactions between the different networks that compose a complex organism such as plants (Barabási and Oltvai, 2004; Sheth and Thaker, 2014). For this, different systemic approaches can be adopted in order to provide a holistic view of plant responses by contrast of the reductionism approaches that are mainly focused in looking at the parts (Fernie, 2012; Souza *et al.*, 2016). Systems biology approaches have been successfully applied to the study of gene expression and metabolic networks in plants (Toubiana *et al.*, 2013), which resulted in several mathematical models able to predict plant metabolic responses (Williams *et al.*, 2010; Hills *et al.*, 2012; Nikoloski *et al.*, 2015; Robaina-Estévez *et al.*, 2017). However, the application of such

systems biology approaches has been surprisingly neglected in the study of redox metabolism. In this section we carried out different gene co-expression (Figure 4) and protein-protein interaction network (Figure 5) analysis and discuss the main findings from these analyses as well as how these approaches can help to understand the interaction between the components of the chloroplastic redox metabolism. Although these approaches have been widely used in plant biology, it is important to highlight the limitation of gene expression and specially protein-protein interaction databases, which are mostly limited to model plants, in particular Arabidopsis, beyond the intrinsic limitation of *in silico* analysis that needs further experimental validation. In this context, Arabidopsis protein-protein interaction databases are still fragmentary and thus caution should be taken when evaluating the results coming out from this analysis. Among the problems faced by this analysis it is noteworthy the presence of false positives interactions as well as the absence of protein-protein interactions not detected by the Arabidopsis interactome study.

A. Gene co-expression analysis reveals a highly connected network among the components of the chloroplastic redox system

Gene co-expression and protein-protein interaction network analyses were carried out using all chloroplastic isoforms of TRXs, PRXs, GRXs, GR, NTR, FTRs, APXs, and GPXs, which is hereafter referred to as chloroplastic redox network. Interestingly, the gene co-expression analysis revealed that these genes are highly co-expressed, leading to a highly dense, inter-connected network (Figure 4). Surprisingly, both tAPX and sAPX are not co-expressed to each other and slightly co-expressed with other genes of the chloroplastic redox network. For instance, tAPX is only co-expressed with PRXQ, NTRC, GR2, and both 2-Cys PRX. Moreover, the Arabidopsis interactome database (Arabidopsis Interactome Mapping Consortium, 2011) does not show any interaction for sAPX isoform, suggesting that whether sAPX isoform is regulated by mechanisms linked to the chloroplastic redox network this occurs in an indirect manner.

B. Is plant gene expression redox-regulated?

In silico analyses revealed that GRX and GPX are highly connected nodes in the co-expression network. At the protein level, GRXS14 seems to be a hub-like node, i.e. a node (enzymes) with high number of

links (interaction) (Barabási and Oltvai, 2004). GRXS14 directly interacts with five proteins, including plastidial transcription factor 1 (PTF1), a chloroplastic transcription factor (TF) (Baba *et al.*, 2001), which was linked to different other important proteins of the chloroplastic redox network such as NTRC and TRX *y1* (Figure 6). This suggests that PTF1 is potentially redox regulated by different components of the chloroplastic redox network. This has considerable consequences for chloroplast function given the extremely high number of protein-protein interactions detected for PTF1 (Figure 6). It has been shown that TRXs can directly activate or deactivate redox-sensitive TFs in mammalian cells (Schenk *et al.*, 1994; Sun and Oberley, 1996; Powis and Montfort, 2001). However, this post-translational control of TF activity remains relatively less studied in plants (Farnese *et al.*, 2016). It is known that HD-Zip proteins and Class I TCP TF are oxidized by H_2O_2, GSSG and other oxidants and reduced by the NTR/TRX system (Comelli and Gonzalez, 2007; Viola *et al.*, 2013). Similarly, several TFs are suggested to be redox-regulated (Shaikhali and Wingsle, 2017). Thus, it seems that plants resemble animals in the redox regulation of transcription through a TRX-mediated (de)activation of TFs. Our *in silico* analysis provides new insights into transcription regulation in plants by suggesting TFs which interact with NTRC, TRXs, and GRXs and thus might be subjected to a redox regulation. Efforts to confirm this hypothesis assume a paramount importance for future investigation.

C. TRX *h3* is a putative regulator of different cytosolic proteins

Proteins from the TRX *h* family (TRX *h1-9*) have been extensively studied in seeds (Shahpiri *et al.*, 2009; Hägglund *et al.*, 2016). It has been proposed that the majority of TRX *h* proteins are located in the cytosol (Bréhélin *et al.*, 2004; Geigenberger *et al.*, 2017), although TRX *h2* and TRX *h9* has been demonstrated to be located in mitochondria and associated to the membrane, respectively (Meng *et al.*, 2010). TRX *h3* is the highest expressed among TRX *h* genes (Reichheld *et al.*, 2002) and has long been recognized as a cytosolic isoform (Gelhaye *et al.*, 2004b; Park *et al.*, 2009; Ito *et al.*, 2011), although this protein has also been identified in two different chloroplast proteomic studies (Peltier *et al.*, 2006; Zybailov *et al.*, 2008). The Arabidopsis interactome revealed that TRX *h3* interacts with 50 proteins that can be clustered in seven different groups according to their function in plant cells. Intriguingly, five of these groups are chloroplastic enzymes related to photosynthesis, C and N metabolism, redox enzymes, and carbonic anhydrases (Figure 7). TRX *h3* supposedly

interacts with the chloroplastic redox-related enzymes GST F8, DHAR3, Prx IIE, MSR A4, Fdx 1 and Fdx 2 (Figure 7). Furthermore, different photosynthetic proteins related to the CBC (e.g. SBPase, TK, R5Pepi, PRK, FBAldolase, RubisCO activase, CP12) and the oxygen evolution complex (OEC) seem to interact with TRX *h3* (Figure 7). However, it is important to highlight that there is no evidence confirming that TRX *h3* is a chloroplastic-located TRX. By contrast, TRX *h3*-GFP assay indicates that this protein is in fact located in the cytosol (Park *et al.*, 2009). Thus, despite the high number of interactions with chloroplastic proteins, we argue that this might be false-positive results or that these proteins interact with TRX *h3* during their translocation to the chloroplast. The other two groups that interacted with TRX *h3* in our analysis include cytosolic enzymes such as APX1, MDH, GAPDH and FBPase and nine other proteins with unknown function or unknown subcellular location. Interestingly, cytosolic MDH, ICDH, FBPase and GAPDH all interacted with TRX *h3*. This suggests that TRX *h3* may serve as a key regulator of C metabolism in the cytosol.

The Arabidopsis interactome also showed that TRX *h3* interacts with three β carbonic anhydrases (βCA1, βCA2, and βCA4). These enzymes are key regulators of stomatal movements in response to CO_2 (Engineer *et al.*, 2016). βCA1 and βCA4 are highly expressed in guard cells and the *ca1 ca4* double mutant has impaired stomatal responses to CO_2 (Hu *et al.*, 2015). Redox regulation of carbonic anhydrases has already been demonstrated in the marine diatom *Phaeodactylum tricornutum* (Kikutani *et al*., 2012). These facts suggest that βCAs and consequently stomatal responses to CO_2 can be redox regulated by TRX *h3*. However, confirmation that TRX *h3* regulates these enzymes *in vivo* remains to be experimentally assessed. The characterization of plants lacking TRX *h3* may bring important information concerning the general function of this protein in the regulation of stomatal movement and C metabolism (Table 2).

D. Lethality and centrality in chloroplastic redox network

Systemic analyses are important to identify essential nodes of biological networks, in which its removal from the network leads to disturbed or abolished physiological responses which can lead to the death of the organism (Jeong *et al.*, 2001; Li *et al.*, 2006). Hubs have been described as essential nodes of protein-protein interaction networks, in which the mutation in genes coding these proteins are lethal or have severe consequences for the organism (Jeong *et al.*, 2001; Albert, 2005; Yu *et al.*, 2008). By contrast, depletion of

lightly connected nodes in scale-free networks has minor impact throughout the network and in consequence to the organism (Barabási and Oltvai, 2004). In this context, the most connected nodes of the co-expression network are NTRC and PRXQ with 27 links each followed by GR2 and the two 2-Cys PRX A and B with 25 links each (Figure 4). In the case of protein-protein chloroplastic interaction network, NTRC and TRX *y1* were shown to be the main hubs, presenting 10 and 12 interactions, respectively, including the interaction between themselves (Figure 5). According to the centrality and lethality theory of scale-free networks (Jeong *et al.*, 2001; Barabási and Oltvai, 2004), hubs are very important components that confer robustness to biological networks (Albert *et al.*, 2001; Albert, 2005). Therefore, the expectation is that mutation in hubs may substantially alter the topology of the network which may have severe consequences for the organism. Indeed, plants lacking NTRC or GR2 demonstrate a drastic reduction in growth and/or high susceptibility to stress conditions (Thormählen *et al.*, 2015; Ding *et al.*, 2016b), most probably due to the perturbation of the chloroplastic redox network caused by those mutations. However, plants lacking PRX Q and TRX *y1* did not show any distinguishable phenotype compared to the WT (Petersson *et al.*, 2006). In the case of *ntrc* mutant, reduced growth in comparison to WT was observed, probably due to the overaccumulation of oxidized forms of 2-Cys PRXs (Pérez-Ruiz *et al.*, 2017), highlighting the interconnection of these genes that are co-expressed (Figure 4) and also interact to each other at protein level (Figure 5).

Taken together, these observations suggest that these hub-like proteins may have central roles in the chloroplastic redox network. In fact, NTRC is crucial for the activation of enzymes of CBC, antioxidative defence system, synthesis of starch, ATP and chlorophyll. Furthermore, NTRC are known to regulate chloroplast gene expression. Although less studied, TRX *y1* has been also implicated in the activation of the antioxidative defence system and chlorophyll and starch synthesis and degradation (Geigenberger *et al.*, 2017). However, despite the centrality of NTRC in the chloroplastic redox network, by contrast to animal cells (Conrad *et al.*, 2004), single mutation in any NTR protein is not lethal in plants. This suggests that the lethality and centrality theory of scale-free networks may have a further level of complexity in plant networks. Probably, the higher phenotypic plasticity of plants, which have been accomplished by, for example, an increased number of gene duplications, ultimately leads to the formation of different compensatory mechanisms that overcome the

mutation in central nodes and avoid the propagation of the negative effects of the mutations throughout the whole network. This idea is based on the fact that double mutation in both components of the chloroplastic TRX reductase systems, namely NTRC and FTR, is lethal in Arabidopsis (Yoshida and Hisabori, 2016), indicating that the absence of lethality in the *ntrc* mutant is probably due to a compensatory role performed by FTR. Similar to this phenomenon, several other components of the redox network can compensate for each other and thus explain the absence of apparent phenotype, justifying the need to characterize plants lacking different components of the redox system simultaneously. Further experiments are needed to confirm that the theory of lethality and centrality differs in plant from animal networks as well as to test the hypothesis that the higher phenotypic plasticity of plants is related to a lesser degree of lethality in the hubs of their networks.

Concluding remarks and future perspectives

Plant redox networks possess a higher degree of complexity when compared to animal and microorganismal redox networks. This is evidenced by the higher number of isoforms of each component of the redox network and the complementarity observed between them. Whilst mutation in key components of the redox system is lethal or, more commonly, has severe consequences for animal cells, several single Arabidopsis or rice mutants do not show any distinguishable phenotype than non-transformed plants. In evolutionary terms, this may be an adaptive feature acquired by plants to grow and survive under constant adverse conditions which is a common situation of plant life due to their sessile nature. The higher number of isoforms of redox components may be therefore a mechanism that cooperatively adjust plant cell metabolism to avoid oxidative stress under a constant input of energy through the process of photosynthesis. Thus, both the redox regulation of metabolism and the regulation of the redox metabolic network itself have been singled out as important mechanisms for plant growth and plant stress tolerance. However, despite the fact that the understanding of the redox regulation of metabolism under stress conditions has received great attention, little is known concerning how the components of redox metabolism are regulated and interact with each other. We contend that this is mainly based on our failure to consider this regulation from a systemic perspective. As such adopting systems biology approaches may help to fill some of these gaps. In parallel, the redundancy and the compensatory role

among the components of the redox network may eventually be completely unravelled by using multi-transgene approaches. Such strategies will likely assume a paramount importance in improving plant metabolic engineering for stress tolerance.

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References

Abadie, C., Lothier, J., Boex-Fontvieille, E., Carroll, A., and Tcherkez, G. 2017. Direct assessment of the metabolic origin of carbon atoms in glutamate from illuminated leaves using13C-NMR. *New Phytol.* **216**: 1079–1089.

Albert, R. 2005. Scale-free networks in cell biology. *J. Cell Sci.* **118**: 4947–4957.

Albert, R., Jeong, H., and Barabasi, A.L. 2001. Error and attack tolerance of complex networks. *Nature*. **409**: 542–542.

Angeli, A. De, Zhang, J., Meyer, S., and Martinoia, E. 2013. AtALMT9 is a malate-activated vacuolar chloride channel required for stomatal opening in Arabidopsis. *Nat. Commun.* **4**: 1804.

Arabidopsis Genome Initiative 2000. Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. *Nature* **408**: 796–815.

Arabidopsis Interactome Mapping Consortium 2011. Evidence for Network Evolution in an Arabidopsis Interactome. Map *Science*. **333**: 601–607.

Araújo, W.L., Nunes-Nesi, A., and Fernie, A.R. 2011a. Fumarate: Multiple functions of a simple metabolite. *Phytochemistry*. **72**: 838–843.

Araújo, W.L., Nunes-Nesi, A., Osorio, S., Usadel, B., Fuentes, D., Nagy, R., Balbo, I., Lehmann, M., Studart-Witkowski, C., Tohge, T., et al. 2011b. Antisense Inhibition of the Iron-Sulphur Subunit of Succinate Dehydrogenase Enhances Photosynthesis and Growth in Tomato via an Organic Acid–Mediated Effect on Stomatal Aperture. *Plant Cell* **23**: 600–627.

Asada, K. 1999. The water-water cycle in chloroplasts: Scavenging of Active Oxygens and Dissipation of Excess Photons. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **50**: 601–639.

Baba, K., Nakano, T., Yamagishi, K., and Yoshida, S. 2001. Involvement of a nuclear-encoded basic helix-

loop-helix protein in transcription of the light-responsive promoter of psbD. *Plant Physiol.* **125**: 595–603.

Barabási, A., and Oltvai, Z.N. 2004. Network biology: understanding the cell's functional organization. *Nat. Rev. Genet.* **5**: 101–113.

Bashandy, T., Taconnat, L., Renou, J.P., Meyer, Y., and Reichheld, J.P. 2009. Accumulation of flavonoids in an ntra ntrb mutant leads to tolerance to UV-C. *Mol. Plant* **2**: 249–258.

Bauwe, H., Hagemann, M., and Fernie, A.R. 2010. Photorespiration: players, partners and origin. *Trends Plant Sci.* **15**: 330–336.

Belin, C., Bashandy, T., Cela, J., Delorme-Hinoux, V., Riondet, C., and Reichheld, J.P. 2015. A comprehensive study of thiol reduction gene expression under stress conditions in Arabidopsis thaliana. *Plant, Cell Environ.* **38**: 299–314.

Benitez-Alfonso, Y., Cilia, M., Roman, A.S., Thomas, C., Maule, A., Hearn, S., and Jackson, D. 2009. Control of Arabidopsis meristem development by thioredoxin-dependent regulation of intercellular transport. *Proc. Natl. Acad. Sci.* **106**: 3615–3620.

Bertalanffy, L. von 1968. General system theory New York: George Braziller.

Biteau, B., Labarre, J., and Toledano, M.B. 2003. ATP-dependent reduction of cysteine–sulphinic acid by S. cerevisiae sulphiredoxin. *Nature*. **425**: 980–984.

Blanco, N.E., Guinea-Diaz, M., Whelan, J., and Strand, A. 2014. Interaction between plastid and mitochondrial retrograde signalling pathways during changes to plastid redox status. *Philos. Trans. R. Soc. B Biol. Sci.* **369**: 20130231–20130231.

Bréhélin, C., Laloi, C., Setterdahl, A.T., Knaff, D.B., and Meyer, Y. 2004. Cytosolic, mitochondrial thioredoxins and thioredoxin reductases in Arabidopsis thaliana. *Photosynth. Res.* **79**: 295–304.

Broin, M., and Rey, P. 2003. Potato plants lacking the CDSP32 plastidic thioredoxin exhibit overoxidation of the BAS1 2-cysteine peroxiredoxin and increased lipid Peroxidation in thylakoids under photooxidative stress. *Plant Physiol.* **132**: 1335–1343.

Buchanan, B.B. 2016a. The carbon (formerly dark) reactions of photosynthesis. *Photosynth. Res.* **128**: 215–217.

Buchanan, B.B. 2016b. The Path to Thioredoxin and Redox Regulation in Chloroplasts. *Annu. Rev. Plant Biol.* **67**: 1–24.

Buchanan, B.B., Holmgren, A., Jacquot, J.P., and Scheibe, R. 2012. Fifty years in the thioredoxin field and a bountiful harvest. *Biochim. Biophys. Acta - Gen. Subj.* **1820**: 1822–1829.

Burritt, P.D.D.J. 2017. Glutathione in Plant Growth, Development, and Stress Tolerance Cham: Springer International Publishing. 128-139.

Calderón, A., Sánchez-Guerrero, A., Ortiz-Espín, A., Martínez-Alcalá, I., Camejo, D., Jiménez, A., and Sevilla, F. 2018. Lack of mitochondrial thioredoxin o 1 is compensated by antioxidant components under salinity in Arabidopsis thaliana plants. *Physiol. Plant.* **7418**: 1–24.

Carrillo, L.R., Froehlich, J.E., Cruz, J.A., Savage, L.J., and Kramer, D.M. 2016. Multi-level regulation of the

chloroplast ATP synthase: the chloroplast NADPH thioredoxin reductase C (NTRC) is required for redox modulation specifically under low irradiance. *Plant J.* **87**: 654–663.

Caverzan, A., Bonifacio, A., Carvalho, F.E.L., Andrade, C.M.B., Passaia, G., Schünemann, M., Maraschin, F. dos S., Martins, M.O., Teixeira, F.K., Rauber, R., et al. 2014. The knockdown of chloroplastic ascorbate peroxidases reveals its regulatory role in the photosynthesis and protection under photo-oxidative stress in rice. *Plant Sci.* **214**: 74–87.

Cerveau, D., Kraut, A., Stotz, H.U., Mueller, M.J., Couté, Y., and Rey, P. 2016. Characterization of the Arabidopsis thaliana 2-Cys peroxiredoxin interactome. *Plant Sci.* **252**: 30–41.

Cha, S.K., Navrot, N., Didierjean, C., Rouhier, N., Hirasawa, M., Knaff, D.B., Wingsle, G., Samian, R., Jacquot, J.P., Corbier, C., et al. 2008. An atypical catalytic mechanism involving three cysteines of thioredoxin. *J. Biol. Chem.* **283**: 23062–23072.

Cheung, C.Y.M., Poolman, M.G., Fell, D.A., Ratcliffe, R.G., and Sweetlove, L.J. 2014. A Diel Flux Balance Model Captures Interactions between Light and Dark Metabolism during Day-Night Cycles in C3 and Crassulacean Acid Metabolism Leaves. *Plant Physiol.* **165**: 917–929.

Chew, O., Whelan, J., and Millar, A.H. 2003. Molecular Definition of the Ascorbate-Glutathione Cycle in Arabidopsis Mitochondria Reveals Dual Targeting of Antioxidant Defenses in Plants. *J. Biol. Chem.* **278**: 46869–46877.

Chronopoulou, E., Madesis, P., Tsaftaris, A., and Labrou, N.E. 2014. Cloning and characterization of a biotic-stress-inducible glutathione transferase from phaseolus vulgaris. *Appl. Biochem. Biotechnol.* **172**: 595–609.

Collin, V., Issakidis-Bourguet, E., Marchand, C., Hirasawa, M., Lancelin, J.M., Knaff, D.B., and Miginiac-Maslow, M. 2003. The Arabidopsis plastidial thioredoxins. New functions and new insights into specificity. *J. Biol. Chem.* **278**: 23747–23752.

Comelli, R.N., and Gonzalez, D.H. 2007. Conserved homeodomain cysteines confer redox sensitivity and influence the DNA binding properties of plant class III HD-Zip proteins. *Arch. Biochem. Biophys.* **467**: 41–47.

Conrad, M., Jakupoglu, C., Moreno, S., Lippl, S., Banjac, A., Schneider, M., Beck, H., Hatzopoulos, A., Just, U., Sinowatz, F., et al. 2004. Essential role for mitochondrial thioredoxin reductase in hematopoiesis, heart development, and heart function. *Mol.* **24**: 9414.

Courteille, A., Vesa, S., Sanz-Barrio, R., Cazale, A.-C., Becuwe-Linka, N., Farran, I., Havaux, M., Rey, P., and Rumeau, D. 2013. Thioredoxin m4 Controls Photosynthetic Alternative Electron Pathways in Arabidopsis. *Plant Physiol.* **161**: 508–520.

Dahal, K., and Vanlerberghe, G.C. 2017. Alternative oxidase respiration maintains both mitochondrial and chloroplast function during drought. *New Phytol.* **213**: 560–571.

Daloso, D.M., Müller, K., Obata, T., Florian, A., Tohge, T., Bottcher, A., Riondet, C., Bariat, L., Carrari, F., Nunes-Nesi, A., et al. 2015. Thioredoxin, a master regulator of the tricarboxylic acid cycle in plant mitochondria. *Proc. Natl. Acad. Sci.* **112**: E1392–E1400.

Dangoor, I., Peled-Zehavi, H., Levitan, A., Pasand, O., and Danon, A. 2009. A Small Family of Chloroplast Atypical Thioredoxins. *Plant Physiol.* **149**: 1240–1250.

Danna, C.H. 2003. Thylakoid-Bound Ascorbate Peroxidase Mutant Exhibits Impaired Electron Transport and Photosynthetic Activity. *Plant Physiol.* **132**: 2116–2125.

Davletova, S. 2005. Cytosolic Ascorbate Peroxidase 1 is a Central Component of the Reactive Oxygen Gene Network of Arabidopsis. *Plant Cell Online*. **17**: 268–281.

Del-Saz, N.F., Florez-Sarasa, I., Clemente-Moreno, M.J., Mhadhbi, H., Flexas, J., Fernie, A.R., and Ribas-Carbó, M. 2016. Salinity tolerance is related to cyanide-resistant alternative respiration in Medicago truncatula under sudden severe stress. *Plant Cell Environ.* **39**: 2361–2369.

Del-Saz, N.F., Ribas-Carbo, M., McDonald, A.E., Lambers, H., Fernie, A.R., and Florez-Sarasa, I. 2018. An In Vivo Perspective of the Role(s) of the Alternative Oxidase Pathway. *Trends Plant Sci.* **23**: 206–219.

Del-saz, N.F., Ribas-carbo, M., Mcdonald, A.E., Lambers, H., Fernie, R., and Florez-sarasa, I. 2018. An In Vivo Perspective of the Role (s) of the Alternative Oxidase Pathway. **23**: 1–14.

Delorme-Hinoux, V., Bangash, S.A.K., Meyer, A.J., and Reichheld, J.P. 2016. Nuclear thiol redox systems in plants. *Plant Sci.* **243**: 84–95.

Dietz, K.-J. 2011. Peroxiredoxins in Plants and Cyanobacteria. *Antioxid. Redox Signal.* **15**: 1129–1159.

Dietz, K.J. 2015. Efficient high light acclimation involves rapid processes at multiple mechanistic levels. *J. Exp. Bot.* **66**: 2401–2414.

Dietz, K.J., Jacob, S., Oelze, M.L., Laxa, M., Tognetti, V., Miranda, S.M.N. De, Baier, M., and Finkemeier, I. 2006. The function of peroxiredoxins in plant organelle redox metabolism. *J. Exp. Bot.* **57**: 1697–1709.

Ding, S., Jiang, R., Lu, Q., Wen, X., and Lu, C. 2016a. Glutathione reductase 2 maintains the function of photosystem II in Arabidopsis under excess light. *Biochim. Biophys. Acta Bioenerg.* **1857**: 665–677.

Ding, S., Wang, L., Yang, Z., Lu, Q., Wen, X., and Lu, C. 2016b. Decreased glutathione reductase2 leads to early leaf senescence in Arabidopsis. *J. Integr. Plant Biol.* **58**: 29–47.

Dixon, D.P., and Edwards, R. 2010. Glutathione Transferases. *Arab. B.* **8**: e0131.

Dixon, D.P., Hawkins, T., Hussey, P.J., and Edwards, R. 2009. Enzyme activities and subcellular localization of members of the arabidopsis glutathione transferase superfamily. *J. Exp. Bot.* **60**: 1207–1218.

Eisenhut, M., Bräutigam, A., Timm, S., Florian, A., Tohge, T., Fernie, A.R., Bauwe, H., and Weber, A.P.M. 2017. Photorespiration Is Crucial for Dynamic Response of Photosynthetic Metabolism and Stomatal Movement to Altered CO2 Availability. *Mol. Plant* **10**: 47–61.

Engineer, C.B., Hashimoto-Sugimoto, M., Negi, J., Israelsson-Nordström, M., Azoulay-Shemer, T., Rappel, W.J., Iba, K., and Schroeder, J.I. 2016. CO_2 sensing and CO_2 regulation of stomatal conductance: advances and open questions. *Trends Plant Sci.* **21**: 16–30.

Exposito-Rodriguez, M., Laissue, P.P., Yvon-Durocher, G., Smirnoff, N., and Mullineaux, P.M. 2017. Photosynthesis-dependent H_2O_2 transfer from chloroplasts to nuclei provides a high-light signalling mechanism.

Nat. Commun. **8**: 49.

Farnese, F.S., Menezes-Silva, P.E., Gusman, G.S., and Oliveira, J.A. 2016. When Bad Guys Become Good Ones: The Key Role of Reactive Oxygen Species and Nitric Oxide in the Plant Responses to Abiotic Stress. *Front. Plant Sci.* **7**: 1–15.

Fernie, A.R. 2012. Grand Challenges in Plant Systems Biology: Closing the Circle(s). *Front. Plant Sci.* **3**: 1–4.

Fernie, A.R., and Martinoia, E. 2009. Malate. Jack of all trades or master of a few? *Phytochemistry*. **70**: 828–832.

Florez-Sarasa, I., Noguchi, K., Araújo, W.L., Garcia-Nogales, A., Fernie, A.R., Flexas, J., and Ribas-Carbo, M. 2016a. Impaired Cyclic Electron Flow around Photosystem I Disturbs High-Light Respiratory Metabolism. *Plant Physiol.* **172**: 2176–2189.

Florez-Sarasa, I., Ribas-Carbo, M., Del-Saz, N.F., Schwahn, K., Nikoloski, Z., Fernie, A.R., and Flexas, J. 2016. Unravelling the *in vivo* regulation and metabolic role of the alternative oxidase pathway in C_3 species under photoinhibitory conditions. *New Phytol.* **212**: 66–79.

Flügel, F., Timm, S., Arrivault, S., Florian, A., Stitt, M., Fernie, A.R., and Bauwe, H. 2017. The Photorespiratory Metabolite 2-Phosphoglycolate Regulates Photosynthesis and Starch Accumulation in Arabidopsis. *Plant Cell* **29**: 2537–2551.

Fomenko, D.E., Koc, A., Agisheva, N., Jacobsen, M., Kaya, A., Malinouski, M., Rutherford, J.C., Siu, K.-L., Jin, D.-Y., Winge, D.R., et al. 2011. Thiol peroxidases mediate specific genome-wide regulation of gene expression in response to hydrogen peroxide. *Proc. Natl. Acad. Sci.* **108**: 2729–2734.

Foyer, C.H., and Halliwell, B. 1976. Planta. **5**.

Foyer, C.H., Neukermans, J., Queval, G., Noctor, G., and Harbinson, J. 2012. Photosynthetic control of electron transport and the regulation of gene expression. *J. Exp. Bot.* **63**: 1637–1661.

Foyer, C.H., and Noctor, G. 2003. Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiol. Plant.* **119**: 355–364.

Foyer, C.H., and Noctor, G. 2011. Ascorbate and Glutathione: The Heart of the Redox Hub. *Plant Physiol.* **155**: 2–18.

Foyer, C.H., and Noctor, G. 2013. Redox Signaling in Plants. *Antioxid. Redox Signal.* **18**: 2087–2090.

Friboulet, A., and Thomas, D. 2005. Systems Biology an interdisciplinary approach. *Biosens. Bioelectron.* **20**: 2404–2407.

Friso, G., and Wijk, K.J. van 2015. Update: Post-translational protein modifications in plant metabolism. *Plant Physiol.* **169**: pp.01378.2015.

Geigenberger, P., and Fernie, A.R. 2014. Metabolic Control of Redox and Redox Control of Metabolism in Plants. *Antioxid. Redox Signal.* **21**: 1389–1421.

Geigenberger, P., Thormählen, I., Daloso, D.M., and Fernie, A.R. 2017. The Unprecedented Versatility of the Plant Thioredoxin System. *Trends Plant Sci.* **22**: 249–262.

Gelhaye, E., Rouhier, N., Gerard, J., Jolivet, Y., Gualberto, J., Navrot, N., Ohlsson, P.-I., Wingsle, G., Hirasawa, M., Knaff, D.B., et al. 2004a. A specific form of thioredoxin h occurs in plant mitochondria and regulates the alternative oxidase. *Proc. Natl. Acad. Sci.* **101**: 14545–14550.

Gelhaye, E., Rouhier, N., and Jacquot, J.P. 2004b. The thioredoxin h system of higher plants. *Plant Physiol. Biochem.* **42**: 265–271.

Giacomelli, L., Masi, A., Ripoll, D.R., Lee, M.J., and Wijk, K.J. Van 2007. Arabidopsis thaliana deficient in two chloroplast ascorbate peroxidases shows accelerated light-induced necrosis when levels of cellular ascorbate are low. *Plant Mol. Biol.* **65**: 627–644.

Hägglund, P., Finnie, C., Yano, H., Shahpiri, A., Buchanan, B.B., Henriksen, A., and Svensson, B. 2016. Seed thioredoxin h. *Biochim.* Biophy: *Proteins Proteomics.* **1864**: 974–982.

Hara, S., Motohashi, K., Arisaka, F., Romano, P.G.N., Hosoya-Matsuda, N., Kikuchi, N., Fusada, N., and Hisabori, T. 2006. Thioredoxin-h1 reduces and reactivates the oxidized cytosolic malate dehydrogenase dimer in higher plants. *J. Biol. Chem.* **281**: 32065–32071.

Harshavardhan, V.T., Wu, T., and Hong, C. 2017. Glutathione Reductase and Abiotic Stress Tolerance in Plants. 265–286.

Herbette, S., Lenne, C., Leblanc, N., Julien, J.L., Drevet, J.R., and Roeckel-Drevet, P. 2002. Two GPX-like proteins from Lycopersicon esculentum and Helianthus annuus are antioxidant enzymes with phospholipid hydroperoxide glutathione peroxidase and thioredoxin peroxidase activities. *Eur. J. Biochem.* **269**: 2414–2420.

Herbette, S., Roeckel-Drevet, P., and Drevet, J.R. 2007. Seleno-independent glutathione peroxidases: More than simple antioxidant scavengers. *FEBS J.* **274**: 2163–2180.

Heyno, E., Innocenti, G., Lemaire, S.D., Issakidis-Bourguet, E., and Krieger-Liszkay, A. 2014. Putative role of the malate valve enzyme NADP-malate dehydrogenase in H2O2 signalling in Arabidopsis. *Philos. Trans. R. Soc. B Biol. Sci.* **369**: 20130228–20130228.

Hills, A., Chen, Z.-H., Amtmann, A., Blatt, M.R., and Lew, V.L. 2012. OnGuard, a computational platform for quantitative kinetic modeling of guard cell physiology. *Plant Physiol.* **159**: 1026–42.

Hiltscher, H., Rudnik, R., Shaikhali, J., Heiber, I., Mellenthin, M., Meirelles Duarte, I., Schuster, G., Kahmann, U., and Baier, M. 2014. The radical induced cell death protein 1 (RCD1) supports transcriptional activation of genes for chloroplast antioxidant enzymes. *Front. Plant Sci.* **5**: 1–14.

Hu, H., Rappel, W.-J., Occhipinti, R., Ries, A., Böhmer, M., You, L., Xiao, C., Engineer, C.B., Boron, W.F., and Schroeder, J.I. 2015. Distinct Cellular Locations of Carbonic Anhydrases Mediate Carbon Dioxide Control of Stomatal Movements. *Plant Physiol.* **169**: 1168–1178.

Huang, J., Niazi, A.K., Young, D., Rosado, L.A., Vertommen, D., Bodra, N., Abdelgawwad, M.R., Vignols, F., Wei, B., Wahni, K., et al. 2018. Self-protection of cytosolic malate dehydrogenase against oxidative stress in Arabidopsis. *J. Exp. Bot.* **69**: 3491–3505.

Hyun, A.W., Jeong, W., Chang, T.S., Kwang, J.P., Sung, J.P., Jeong, S.Y., and Sue, G.R. 2005. Reduction of

cysteine sulfinic acid by sulfiredoxin is specific to 2-Cys peroxiredoxins. *J. Biol. Chem.* **280**: 3125–3128.

Iqbal, A., Yabuta, Y., Takeda, T., Nakano, Y., and Shigeoka, S. 2006. Hydroperoxide reduction by thioredoxin-specific glutathione peroxidase isoenzymes of Arabidopsis thaliana. *FEBS J.* **273**: 5589–5597.

Ito, J., Batth, T.S., Petzold, C.J., Redding-Johanson, A.M., Mukhopadhyay, A., Verboom, R., Meyer, E.H., Millar, A.H., and Heazlewood, J.L. 2011. Analysis of the Arabidopsis cytosolic proteome highlights subcellular partitioning of central plant metabolism. *J. Proteome Res.* **10**: 1571–1582.

Jeong, H., Mason, S.P., Barabasi, A.-L., and Oltvai, Z.N. 2001. Lethality and centrality in protein networks. **411**: 41–42.

Jeong, W., Sung, J.P., Chang, T.S., Lee, D.Y., and Sue, G.R. 2006. Molecular mechanism of the reduction of cysteine sulfinic acid of peroxiredoxin to cysteine by mammalian sulfiredoxin. *J. Biol. Chem.* **281**: 14400–14407.

Jung, B.G., Lee, K.O., Lee, S.S., Chi, Y.H., Jang, H.H., Kang, S.S., Lee, K., Lim, D., Yoon, S.C., Yun, D.J., et al. 2002. A Chinese cabbage cDNA with high sequence identity to phospholipid hydroperoxide glutathione peroxidases encodes a novel isoform of thioredoxin-dependent peroxidase. *J. Biol. Chem.* **277**: 12572–12578.

Kangasjärvi, S., Lepistö, A., Hännikäinen, K., Piippo, M., Luomala, E.-M., Aro, E.-M., and Rintamäki, E. 2008. Diverse roles for chloroplast stromal and thylakoid-bound ascorbate peroxidases in plant stress responses. *Biochem. J.* **412**: 275–285.

Kikutani, S., Tanaka, R., Yamazaki, Y., Hara, S., Hisabori, T., Kroth, P.G., and Matsuda, Y. 2012. Redox regulation of carbonic anhydrases via thioredoxin in chloroplast of the marine diatom Phaeodactylum tricornutum. *J. Biol. Chem.* **287**: 20689–20700.

Kim, C.G., Park, K.W., Lee, B., Kim, D.I., Park, J.Y., Kim, H.J., Park, J.E., An, J.H., Cho, K.H., Jeong, S.C., et al. 2009. Gene flow from genetically modified to conventional chili pepper (Capsicum annuum L.). *Plant Sci.* **176**: 406–412.

König, K., Vaseghi, M.J., Dreyer, A., and Dietz, K.J. 2018. The significance of glutathione and ascorbate in modulating the retrograde high light response in Arabidopsis thaliana leaves. *Physiol. Plant.* **162**: 262–273.

Krueger, S., Steinhauser, D., Lisec, J., and Giavalisco, P. 2014. Analysis of Subcellular Metabolite Distributions Within Arabidopsis thaliana Leaf Tissue: A Primer for Subcellular Metabolomics In: Sanchez-Serrano, J., and J. Salinas, Eds., Arabidopsis Protocols. Methods in Molecular Biology. Totowa, NJ: Humana Press, pp. 575–596.

Labrou, N.E., Papageorgiou, A.C., Pavli, O., and Flemetakis, E. 2015. Plant GSTome: Structure and functional role in xenome network and plant stress response. *Curr. Opin. Biotechnol.* **32**: 186–194.

Lallement, P.A., Brouwer, B., Keech, O., Hecker, A., and Rouhier, N. 2014. The still mysterious roles of cysteine-containing glutathione transferases in plants. *Front. Pharmacol.* **5**: 1–22.

Laugier, E., Tarrago, L., Courteille, A., Innocenti, G., Eymery, F., Rumeau, D., Issakidis-Bourguet, E., and Rey, P. 2013. Involvement of thioredoxin y2 in the preservation of leaf methionine sulfoxide reductase capacity and growth under high light. *Plant, Cell Environ.* **36**: 670–682.

Lemaire, S.D., Michelet, L., Zaffagnini, M., Massot, V., and Issakidis-Bourguet, E. 2007. Thioredoxins in chloroplasts. *Curr. Genet.* **51**: 343–365.

Lepistö, A., Pakula, E., Toivola, J., Krieger-Liszkay, A., Vignols, F., and Rintamäki, E. 2013. Deletion of chloroplast NADPH-dependent thioredoxin reductase results in inability to regulate starch synthesis and causes stunted growth under short-day photoperiods. *J. Exp. Bot.* **64**: 3843–3854.

Li, S., Assmann, S.M., and Albert, R. 2006. Predicting essential components of signal transduction networks: A dynamic model of guard cell abscisic acid signaling. *PLoS Biol.* **4**: 1732–1748.

Liebthal, M., Maynard, D., and Dietz, K.-J. 2017. Peroxiredoxins and Redox Signaling in Plants. *Antioxid. Redox Signal.* **28**: 609-623.

Lima-Melo, Y., Carvalho, F.E.L., Martins, M.O., Passaia, G., Sousa, R.H.V., Neto, M.C.L., Margis-Pinheiro, M., and Silveira, J.A.G. 2016. Mitochondrial GPX1 silencing triggers differential photosynthesis impairment in response to salinity in rice plants. *J. Integr. Plant Biol.* **58**: 737–748.

Margis, R., Dunand, C., Teixeira, F.K., and Margis-Pinheiro, M. 2008. Glutathione peroxidase family - An evolutionary overview. *FEBS J.* **275**: 3959–3970.

Marty, L., Siala, W., Schwarzländer, M., Fricker, M.D., Wirtz, M., Sweetlove, L.J., Meyer, Y., Meyer, A.J., Reichheld, J.-P., and Hell, R. 2009. The NADPH-dependent thioredoxin system constitutes a functional backup for cytosolic glutathione reductase in Arabidopsis. *Proc. Natl. Acad. Sci. U. S. A.* **106**: 9109–9114.

Maruta, T., Sawa, Y., Shigeoka, S., and Ishikawa, T. 2016. Diversity and evolution of ascorbate peroxidase functions in chloroplasts: More than just a classical antioxidant enzyme? *Plant Cell Physiol.* **57**: 1377–1386.

Maruta, T., Tanouchi, A., Tamoi, M., Yabuta, Y., Yoshimura, K., Ishikawa, T., and Shigeoka, S. 2010. Arabidopsis chloroplastic ascorbate peroxidase isoenzymes play a dual role in photoprotection and gene regulation under photooxidative stress. *Plant Cell Physiol.* **51**: 190–200.

Medeiros, D.B., Barros, K., Barros, J.A., Omena-Garcia, R.P., Arrivault, S., Vincis Pereira Sanglard, L., Detmann, K.C., Silva, W.B., Daloso, D.M., DaMatta, F., et al. 2017. Impaired malate and fumarate accumulation due the mutation of tonoplast dicarboxylate transporter has little effects on stomatal behaviour. *Plant Physiol.* **175**: 1-34.

Medeiros, D.B., Martins, S.C.V., Cavalcanti, J.H.F., Daloso, D.M., Martinoia, E., Nunes-Nesi, A., DaMatta, F.M., Fernie, A.R., and Araújo, W.L. 2016. Enhanced Photosynthesis and Growth in *atquac1* Knockout Mutants Are Due to Altered Organic Acid Accumulation and an Increase in Both Stomatal and Mesophyll Conductance. *Plant Physiol.* **170**: 86–101.

Meng, L., Wong, J.H., Feldman, L.J., Lemaux, P.G., and Buchanan, B.B. 2010. A membrane-associated thioredoxin required for plant growth moves from cell to cell, suggestive of a role in intercellular communication. *Proc. Natl. Acad. Sci. U. S. A.* **107**: 3900–3905.

Meyer, Y., Belin, C., Delorme-Hinoux, V., Reichheld, J.-P., and Riondet, C. 2012. Thioredoxin and Glutaredoxin Systems in Plants: Molecular Mechanisms, Crosstalks, and Functional Significance. *Antioxid.*

Redox Signal. **17**: 1124–1160.

Meyer, Y., Buchanan, B.B., Vignols, F., and Reichheld, J.-P. 2009. Thioredoxins and Glutaredoxins: Unifying Elements in Redox Biology. *Annu. Rev. Genet.* **43**: 335–367.

Merlot, S., Mustilli, A.-C., Genty, B., North, H., Lefebvre, V., Sotta, B., Vavasseur, A., and Giraudat, J. 2002. Use of infrared thermal imaging to isolate Arabidopsis mutants defective in stomatal regulation. *Plant J.* **30**: 601–9.

Mhamdi, A., Hager, J., Chaouch, S., Queval, G., Han, Y., Taconnat, L., Saindrenan, P., Gouia, H., Issakidis-Bourguet, E., Renou, J.-P., et al. 2010. Arabidopsis Gluthatione Reductase 1 Plays a Crucial Role in Leaf Responses to Intracellular Hydrogen Peroxide and in Ensuring Appropriate Gene Expression through Both Salicylic Acid and Jasmonic Acid Signaling Pathways. *Plant Physiol.* **153**: 1144–1160.

Miao, Y., Lv, D., Wang, P., Wang, X.-C., Chen, J., Miao, C., and Song, C.-P. 2006. An Arabidopsis Glutathione Peroxidase Functions as Both a Redox Transducer and a Scavenger in Abscisic Acid and Drought Stress Responses. *Plant Cell Online*. **18**: 2749–2766.

Michalska, J., Zauber, H., Buchanan, B.B., Cejudo, F.J., and Geigenberger, P. 2009. NTRC links built-in thioredoxin to light and sucrose in regulating starch synthesis in chloroplasts and amyloplasts. *Proc. Natl. Acad. Sci. U. S. A.* **106**: 9908–9913.

Michelet, L., Zaffagnini, M., Morisse, S., Sparla, F., Pérez-Pérez, M.E., Francia, F., Danon, A., Marchand, C.H., Fermani, S., Trost, P., et al. 2013. Redox regulation of the Calvin–Benson cycle: something old, something new. *Front. Plant Sci.* **4**: 1–21.

Mignolet-Spruyt, L., Xu, E., Idänheimo, N., Hoeberichts, F.A., Mühlenbock, P., Brosche, M., Breusegem, F. Van, and Kangasjärvi, J. 2016. Spreading the news: Subcellular and organellar reactive oxygen species production and signalling. *J. Exp. Bot.* **67**: 3831–3844.

Miller, G., Suzuki, N., Rizhsky, L., Hegie, A., Koussevitzky, S., and Mittler, R. 2007. Double Mutants Deficient in Cytosolic and Thylakoid Ascorbate Peroxidase Reveal a Complex Mode of Interaction between Reactive Oxygen Species, Plant Development, and Response to Abiotic Stresses. *Plant Physiol.* **144**: 1777–1785.

Mittler, R. 2017. ROS Are Good. *Trends Plant Sci.* **22**: 11–19.

Møller, I.M. 2001. Plant mitochondria and oxidative stress : Electron Transport, NADPH Turnover, and Metabolism of Reactive Oxygen Species. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **52**: 561–591.

Møller, I.M., Jensen, P.E., and Hansson, A. 2007. Oxidative Modifications to Cellular Components in Plants. *Annu. Rev. Plant Biol.* **58**: 459–481.

Møller, I.M., and Rasmusson, A.G. 1998. The role of NADP in the mitochondrial matrix. *Trends Plant Sci.* **3**: 21–27.

Montrichard, F., Alkhalfioui, F., Yano, H., Vensel, W.H., Hurkman, W.J., and Buchanan, B.B. 2009. Thioredoxin targets in plants: The first 30 years. *J. Proteomics*. **72**: 452–474.

Munne-Bosch, S., Queval, G., and Foyer, C.H. 2013. The Impact of Global Change Factors on Redox Signaling Underpinning Stress Tolerance. *Plant Physiol.* **161**: 5–19.

Murata, N., and Nishiyama, Y. 2018. ATP is a driving force in the repair of photosystem II during photoinhibition. *Plant Cell Environ.* **41**: 285–299.

Naranjo, B., Mignée, C., Krieger-Liszkay, A., Hornero-Méndez, D., Gallardo-Guerrero, L., Cejudo, F.J., and Lindahl, M. 2016. The chloroplast NADPH thioredoxin reductase C, NTRC, controls non-photochemical quenching of light energy and photosynthetic electron transport in Arabidopsis. *Plant Cell Environ.* **39**: 804–822.

Navrot, N., Collin, V., Gualberto, J., Gelhaye, E., Hirasawa, M., Rey, P., Knaff, D.B., Issakidis, E., Jacquot, J.-P., and Rouhier, N. 2006. Plant Glutathione Peroxidases Are Functional Peroxiredoxins Distributed in Several Subcellular Compartments and Regulated during Biotic and Abiotic Stresses. *Plant Physiol.* **142**: 1364–1379.

Nianiou-Obeidat, I., Madesis, P., Kissoudis, C., Voulgari, G., Chronopoulou, E., Tsaftaris, A., and Labrou, N.E. 2017. Plant glutathione transferase-mediated stress tolerance: functions and biotechnological applications. *Plant Cell Rep.* **36**: 791–805.

Nietzel, T., Mostertz, J., Hochgräfe, F., and Schwarzländer, M. 2017. Redox regulation of mitochondrial proteins and proteomes by cysteine thiol switches. *Mitochondrion* **33**: 72–83.

Nikkanen, L., Toivola, J., and Rintamäki, E. 2016. Crosstalk between chloroplast thioredoxin systems in regulation of photosynthesis. *Plant Cell Environ.* **39**: 1691–1705.

Nikoloski, Z., Perez-Storey, R., and Sweetlove, L.J. 2015. Inference and Prediction of Metabolic Network Fluxes. *Plant Physiol* **169**: 1443–1455.

Noctor, G., and Foyer, C.H. 2016. Intracellular Redox Compartmentation and ROS-Related Communication in Regulation and Signaling. *Plant Physiol.* **171**: 1581–1592.

Noctor, G., Mhamdi, A., Chaouch, S., Han, Y., Neukermans, J., Marquez-Garcia, B., Queval, G., and Foyer, C.H. 2012. Glutathione in plants: An integrated overview. *Plant, Cell Environ.* **35**: 454–484.

Noctor, G., Reichheld, J.-P., and Foyer, C.H. 2018. ROS-related redox regulation and signaling in plants. *Semin. Cell Dev. Biol.* **80**: 3–12.

Nonn, L., Williams, R.R., Erickson, R.P., and Powis, G. 2003. The absence of mitochondrial thioredoxin 2 causes massive apoptosis, exencephaly, and early embryonic lethality in homozygous mice. *Mol. Cell. Biol.* **23**: 916–22

Obata, T., Florian, A., Timm, S., Bauwe, H., and Fernie, A.R. 2016. On the metabolic interactions of (photo)respiration. *J. Exp. Bot.* **67**: 3003–3014.

Okegawa, Y., and Motohashi, K. 2015. Chloroplastic thioredoxin m functions as a major regulator of Calvin cycle enzymes during photosynthesis in vivo. *Plant J.* **84**: 900–913.

Park, S.K., Jung, Y.J., Lee, J.R., Lee, Y.M., Jang, H.H., Lee, S.S., Park, J.H., Kim, S.Y., Moon, J.C., Lee, S.Y., et al. 2009. Heat-Shock and Redox-Dependent Functional Switching of an h-Type Arabidopsis Thioredoxin

from a Disulfide Reductase to a Molecular Chaperone. *Plant Physiol.* **150**: 552–561.

Passaia, G., and Margis-Pinheiro, M. 2015. Glutathione peroxidases as redox sensor proteins in plant cells. *Plant Sci.* **234**: 22–26.

Passaia, G., Spagnolo Fonini, L., Caverzan, A., Jardim-Messeder, D., Christoff, A.P., Gaeta, M.L., Araujo Mariath, J.E. de, Margis, R., and Margis-Pinheiro, M. 2013. The mitochondrial glutathione peroxidase GPX3 is essential for H_2O_2 homeostasis and root and shoot development in rice. *Plant Sci.* **208**: 93–101.

Peltier, J.-B., Cai, Y., Sun, Q., Zabrouskov, V., Giacomelli, L., Rudella, A., Ytterberg, A.J., Rutschow, H., and Wijk, K.J. van 2006. The Oligomeric Stromal Proteome of *Arabidopsis thaliana* Chloroplasts. *Mol. Cell. Proteomics*. **5**: 114–133.

Perez-Ruiz, J.M., Spínola, M.C., Kirchsteiger, K., Moreno, J., Sahrawy, M., and Cejudo, F.J. 2006. Rice NTRC is a High-Efficiency Redox System for Chloroplast Protection against Oxidative Damage. *Plant Cell Online*. **18**: 2356–2368.

Pérez-Ruiz, J.M., Guinea, M., Puerto-Galán, L., and Cejudo, F.J. 2014. NADPH thioredoxin reductase C is involved in redox regulation of the Mg-chelatase I subunit in Arabidopsis thaliana chloroplasts. *Mol. Plant*. **7**: 1252–1255.

Pérez-Ruiz, J.M., Naranjo, B., Ojeda, V., Guinea, M., and Cejudo, F.J. 2017. NTRC-dependent redox balance of 2-Cys peroxiredoxins is needed for optimal function of the photosynthetic apparatus. *Proc. Natl. Acad. Sci.* **114**: 12069–12074.

Pesaresi, P., Schneider, A., Kleine, T., and Leister, D. 2007. Interorganellar communication. *Curr. Opin. Plant Biol.* **10**: 600–606.

Petersson, U.A., Kieselbach, T., García-Cerdán, J.G., and Schröder, W.P. 2006. The Prx Q protein of Arabidopsis thaliana is a member of the luminal chloroplast proteome. *FEBS Lett.* **580**: 6055–6061.

Pires, M. V., Pereira Júnior, A.A., Medeiros, D.B., Daloso, D.M., Pham, P.A., Barros, K.A., Engqvist, M.K.M., Florian, A., Krahnert, I., Maurino, V.G., et al. 2016. The influence of alternative pathways of respiration that utilize branched-chain amino acids following water shortage in Arabidopsis. *Plant Cell Environ.* **39**: 1304–1319.

Polle, A. 2001. Dissecting the Superoxide Dismutase-Ascorbate-Glutathione-Pathway in Chloroplasts by Metabolic Modeling. Computer Simulations as a Step towards Flux Analysis. *Plant Physiol.* **126**: 445–462.

Powis, G., and Montfort, W.R. 2001.Properties and Biological Activities of Thioredoxins. *Annu. Rev. Biophys. Biomol. Struct.* **30**: 261-295.

Pracharoenwattana, I., Zhou, W., Keech, O., Francisco, P.B., Udomchalothorn, T., Tschoep, H., Stitt, M., Gibon, Y., and Smith, S.M. 2010. Arabidopsis has a cytosolic fumarase required for the massive allocation of photosynthate into fumaric acid and for rapid plant growth on high nitrogen. *Plant J.* **62**: 785–795.

Puerto-Galán, L., Pérez-Ruiz, J.M., Guinea, M., and Cejudo, F.J. 2015. The contribution of NADPH thioredoxin reductase C (NTRC) and sulfiredoxin to 2-Cys peroxiredoxin overoxidation in Arabidopsis thaliana

chloroplasts. *J. Exp. Bot.* **66**: 2957–2966.

Rasmusson, A.G., Soole, K.L., and Elthon, T.E. 2004. Alternative NAD(P)H Dehydrogenases of Plant Mitochondria. *Annu. Rev. Plant Biol.* **55**: 23–39.

Reichheld, J.-P., Khafif, M., Riondet, C., Droux, M., Bonnard, G., and Meyer, Y. 2007. Inactivation of thioredoxin reductases reveals a complex interplay between thioredoxin and glutathione pathways in Arabidopsis development. *Plant Cell* **19**: 1851–1865.

Reichheld, J.P., Mestres-Ortega, D., Laloi, C., and Meyer, Y. 2002. The multigenic family of thioredoxin h in Arabidopsis thaliana: Specific expression and stress response In: Plant Physiology and Biochemistry. pp. 685–690.

Reichheld, J.-P., Khafif, M., Riondet, C., Droux, M., Bonnard, G., and Meyer, Y. 2007. Inactivation of thioredoxin reductases reveals a complex interplay between thioredoxin and glutathione pathways in Arabidopsis development. *Plant Cell*. **19**: 1851–1865.

Reichheld, J.P., Riondet, C., Delorme, V., Vignols, F., and Meyer, Y. 2010. Thioredoxins and glutaredoxins in development. *Plant Sci.* **178**: 420–423.

Richter, A.S., Peter, E., Rothbart, M., Schlicke, H., Toivola, J., Rintamaki, E., and Grimm, B. 2013. Posttranslational Influence of NADPH-Dependent Thioredoxin Reductase C on Enzymes in Tetrapyrrole Synthesis. *Plant Physiol.* **162**: 63–73.

Río, L.A. Del, Corpas, F.J., Sandalio, L.M., Palma, J.M., and Barroso, J.B. 2003. Plant peroxisomes, reactive oxygen metabolism and nitric oxide. *IUBMB Life*. **55**: 71–81.

Robaina-Estévez, S., Daloso, D.M., Zhang, Y., Fernie, A.R., and Nikoloski, Z. 2017. Resolving the central metabolism of Arabidopsis guard cells. *Sci. Rep.* **7**: 1-13.

Rodriguez Milla, M.A., Maurer, A., Huete, A.R., and Gustafson, J.P. 2003. Glutathione peroxidase genes in Arabidopsis are ubiquitous and regulated by abiotic stresses through diverse signaling pathways. *Plant J.* **36**: 602–615.

Rouhier, N., Santos, C.V. Dos, Tarrago, L., and Rey, P. 2006. Plant methionine sulfoxide reductase A and B multigenic families. *Photosynth. Res.* **89**: 247–262.

Rudnik, R., Bulcha, J.T., Reifschneider, E., Ellersiek, U., and Baier, M. 2017. Specificity versus redundancy in the RAP2.4 transcription factor family of Arabidopsis thaliana: Transcriptional regulation of genes for chloroplast peroxidases. *BMC Plant Biol.* **17**: 1–17.

Sanz-Barrio, R., Corral-Martinez, P., Ancin, M., Segui-Simarro, J.M., and Farran, I. 2013. Overexpression of plastidial thioredoxin f leads to enhanced starch accumulation in tobacco leaves. *Plant Biotechnol. J.* **11**: 618–627.

Schenk, H., Klein, M., Erdbrügger, W., Dröge, W., and Schulze-Osthoff, K. 1994. Distinct effects of thioredoxin and antioxidants on the activation of transcription factors NF-kB and AP-1. *Proc. Nat. Acad. Sci. USA* **91**: 1672–1676.

Schmidtmann, E., König, A.-C., Orwat, A., Leister, D., Hartl, M., and Finkemeier, I. 2014. Redox regulation of Arabidopsis mitochondrial citrate synthase. *Mol. Plant*. **7**: 156–69.

Selles, B., Hugo, M., Trujillo, M., Srivastava, V., Wingsle, G., Jacquot, J.-P., Radi, R., and Rouhier, N. 2012. Hydroperoxide and peroxynitrite reductase activity of poplar thioredoxin-dependent glutathione peroxidase 5: kinetics, catalytic mechanism and oxidative inactivation. *Biochem. J.* **442**: 369–380.

Sperry, J.S. Evolution of Water Transport and Xylem Structure. 2013. Evolution of Functional Traits in Plants. **164**: S115-S127

Serrato, A.J., Pérez-Ruiz, J.M., Spínola, M.C., and Cejudo, F.J. 2004. A novel NADPH thioredoxin reductase, localised in the chloroplast, which deficiency causes hypersensitivity to abiotic stress in Arabidopsis thaliana. *J. Biol. Chem.* **279**: 43821–43827.

Sevilla, F., Camejo, D., Ortiz-Espín, A., Calderón, A., Lázaro, J.J., and Jiménez, A. 2015. The thioredoxin/peroxiredoxin/sulfiredoxin system: Current overview on its redox function in plants and regulation by reactive oxygen and nitrogen species. *J. Exp. Bot.* **66**: 2945–2955.

Sewelam, N., Jaspert, N., Kelen, K. Van Der, Tognetti, V.B., Schmitz, J., Frerigmann, H., Stahl, E., Zeier, J., Breusegem, F. Van, and Maurino, V.G. 2014. Spatial H2O2 signaling specificity: H_2O_2 from chloroplasts and peroxisomes modulates the plant transcriptome differentially. *Mol. Plant*. **7**: 1191–1210.

Shannon, P., Markiel, A., Owen Ozier, 2, Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., and Ideker, T. 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* 2498–2504.

Shahpiri, A., Svensson, B., and Finnie, C. 2009. From proteomics to structural studies of cytosolic/mitochondrial-type thioredoxin systems in barley seeds. *Mol. Plant*. **2**: 378–389.

Shaikhali, J., Heiber, I., Seidel, T., Ströher, E., Hiltscher, H., Birkmann, S., Dietz, K.J., and Baier, M. 2008. The redox-sensitive transcription factor Rap2.4a controls nuclear expression of 2-Cys peroxiredoxin A and other chloroplast antioxidant enzymes. *BMC Plant Biol.* **8**: 1–14.

Shaikhali, J., and Wingsle, G. 2017. Redox-regulated transcription in plants: Emerging concepts. *AIMS Mol. Sci.* **4**: 301–338.

Sheth, B.P., and Thaker, V.S. 2014. Plant systems biology: Insights, advances and challenges. *Planta*. **240**: 33–54.

Sousa, R.H.V., Carvalho, F.E.L., Ribeiro, C.W., Passaia, G., Cunha, J.R., Lima-Melo, Y., Margis-Pinheiro, M., and Silveira, J.A.G. 2015. Peroxisomal APX knockdown triggers antioxidant mechanisms favourable for coping with high photorespiratory H_2O_2 induced by CAT deficiency in rice. *Plant Cell Environ.* **38**: 499–513.

Souza, G.M., Prado, C.H.B.A., Ribeiro, R. V., Barbosa, J.P.R.A.D., Gonçalves, A.N., and Habermann, G. 2016. Toward a systemic plant physiology. *Theor. Exp. Plant Physiol*. **28**:341-346.

Su, T., Shao, Q., Wang, P., Ma, C., Peroxisome, Á.P.Á., and Pexophagy, Á. 2016. Redox State as a Central Regulator of Plant-Cell Stress Responses.

Sun, Y., and Oberley, L.W. 1996. Redox regulation of transcriptional activators. *Free Radic Biol Med*. **21**: 335–348.

Sweetlove, L.J., Lytovchenko, A., Morgan, M., Nunes-Nesi, A., Taylor, N.L., Baxter, C.J., Eickmeier, I., and Fernie, A.R. 2006. Mitochondrial uncoupling protein is required for efficient photosynthesis. *Proc Natl Acad Sci U S A.* **103**: 19587–19592.

Szklarczyk, D., Morris, J.H., Cook, H., Kuhn, M., Wyder, S., Simonovic, M., Santos, A., Doncheva, N.T., Roth, A., Bork, P., et al. 2017. The STRING database in 2017: Quality-controlled protein-protein association networks, made broadly accessible. *Nucleic Acids Res.* **45**: D362–D368.

Tan, S.X., Greetham, D., Raeth, S., Grant, C.M., Dawes, I.W., and Perrone, G.G. 2010. The thioredoxin-thioredoxin reductase system can function in vivo as an alternative system to reduce oxidized glutathione in Saccharomyces cerevisiae. *J. Biol. Chem.* **285**: 6118–6126.

Tcherkez, G., Boex-Fontvieille, E., Mahé, A., and Hodges, M. 2012. Respiratory carbon fluxes in leaves. *Curr. Opin. Plant Biol.* **15**: 308–314.

Teixeira, F.K., Menezes-Benavente, L., Galvão, V.C., Margis, R., and Margis-Pinheiro, M. 2006. Rice ascorbate peroxidase gene family encodes functionally diverse isoforms localized in different subcellular compartments. *Planta* **224**: 300–314.

Thormählen, I., Meitzel, T., Groysman, J., Öchsner, A.B., Roepenack-Lahaye, E. von, Naranjo, B., Cejudo, F.J., and Geigenberger, P. 2015. Thioredoxin f1 and NADPH-dependent thioredoxin reductase C have overlapping functions in regulating photosynthetic metabolism and plant growth in response to varying light conditions. *Plant Physiol.* **169**: 1766–1786.

Thormählen, I., Ruber, J., Roepenack-Lahaye, E. Von, Ehrlich, S.M., Massot, V., Hümmer, C., Tezycka, J., Issakidis-Bourguet, E., and Geigenberger, P. 2013. Inactivation of thioredoxin f1 leads to decreased light activation of ADP-glucose pyrophosphorylase and altered diurnal starch turnover in leaves of Arabidopsis plants. *Plant, Cell Environ.* **36**: 16–29.

Thormählen, I., Zupok, A., Rescher, J., Leger, J., Weissenberger, S., Groysman, J., Orwat, A., Chatel-Innocenti, G., Issakidis-Bourguet, E., Armbruster, U., et al. 2017. Thioredoxins Play a Crucial Role in Dynamic Acclimation of Photosynthesis in Fluctuating Light. *Mol. Plant*. **10**: 168–182.

Timm, S., Florian, A., Arrivault, S., Stitt, M., Fernie, A.R., and Bauwe, H. 2012a. Glycine decarboxylase controls photosynthesis and plant growth. *FEBS Lett.* **586**: 3692–3697.

Timm, S., Florian, A., Arrivault, S., Stitt, M., Fernie, A.R., and Bauwe, H. 2012b. Glycine decarboxylase controls photosynthesis and plant growth. *FEBS Lett.* **586**: 3692–3697.

Timm, S., Florian, A., Fernie, A.R., and Bauwe, H. 2016. The regulatory interplay between photorespiration and photosynthesis. *J. Exp. Bot.* **67**: 2923–2929.

Timm, S., Florian, A., Wittmiss, M., Jahnke, K., Hagemann, M., Fernie, A.R., and Bauwe, H. 2013. Serine Acts as a Metabolic Signal for the Transcriptional Control of Photorespiration-Related Genes in Arabidopsis. *Plant*

Physiol. **162**: 379–389.

Timm, S., Giese, J., Engel, N., Wittmiß, M., Florian, A., Fernie, A.R., and Bauwe, H. 2018. T-protein is present in large excess over the other proteins of the glycine cleavage system in leaves of Arabidopsis. *Planta*. **247**: 41–51.

Timm, S., Wittmiß, M., Gamlien, S., Ewald, R., Florian, A., Frank, M., Wirtz, M., Hell, R., Fernie, A.R., and Bauwe, H. 2015. Mitochondrial Dihydrolipoyl Dehydrogenase Activity Shapes Photosynthesis and Photorespiration of *Arabidopsis thaliana*. *Plant Cell* **27**: 1968–1984.

Tomaz, T., Bagard, M., Pracharoenwattana, I., Linden, P., Lee, C.P., Carroll, A.J., Stroher, E., Smith, S.M., Gardestrom, P., and Millar, A.H. 2010. Mitochondrial Malate Dehydrogenase Lowers Leaf Respiration and Alters Photorespiration and Plant Growth in Arabidopsis. *Plant Physiol.* **154**: 1143–1157.

Toubiana, D., Fernie, A.R., Nikoloski, Z., and Fait, A. 2013. Network analysis: tackling complex data to study plant metabolism. *Trends Biotechnol.* **31**: 29–36.

Tovar-Méndez, A., Miernyk, J.A., and Randall, D.D. 2003. Regulation of pyruvate dehydrogenase complex activity in plant cells. *Eur. J. Biochem.* **270**: 1043–1049.

Traverso, J.A., Micalella, C., Martinez, A., Brown, S.C., Satiat-Jeunemaître, B., Meinnel, T., and Giglione, C. 2013. Roles of N-Terminal Fatty Acid Acylations in Membrane Compartment Partitioning: Arabidopsis h-Type Thioredoxins as a Case Study. *Plant Cell*. **25**: 1056–1077.

Tzafrir, I., Pena-muralla, R., Dickerman, A., Berg, M., Rogers, R., Hutchens, S., Sweeney, T.C., Mcelver, J., Aux, G., Patton, D., et al. 2004. Identification of Genes Required for Embryo Development in Arabidopsis 1 [w]. *Plant Physiol.* **135**: 1206–1220.

Uhmeyer, A., Cecchin, M., Ballottari, M., and Wobbe, L. 2017. Impaired Mitochondrial Transcription Termination Disrupts the Stromal Redox Poise in *Chlamydomonas*. *Plant Physiol.* **174**: 1399–1419.

Ursini, F., Maiorino, M., and Gregolin, C. 1985. The selenoenzyme phospholipid hydroperoxide glutathione peroxidase. *Biochim. Biophys. Acta - Gen. Subj.* **839**: 62–70.

Viola, I.L., Guttlein, L.N., and Gonzalez, D.H. 2013. Redox Modulation of Plant Developmental Regulators from the Class I TCP Transcription Factor Family. *Plant Physiol.* **162**: 1434–1447.

Vishwakarma, A., Bashyam, L., Senthilkumaran, B., Scheibe, R., and Padmasree, K. 2014. Physiological role of AOX1a in photosynthesis and maintenance of cellular redox homeostasis under high light in Arabidopsis thaliana. *Plant Physiol. Biochem.* **81**: 44–53.

Wang, J., and Vanlerberghe, G.C. 2013. A lack of mitochondrial alternative oxidase compromises capacity to recover from severe drought stress. *Physiol. Plant.* **149**: 461–473.

Wang, P., Liu, J., Liu, B., Feng, D., Da, Q., Wang, P., Shu, S., Su, J., Zhang, Y., Wang, J., et al. 2013. Evidence for a Role of Chloroplastic m-Type Thioredoxins in the Biogenesis of Photosystem II in Arabidopsis. *Plant Physiol.* **163**: 1710–1728.

Wang, X., Fang, G., Yang, J., and Li, Y. 2017. A Thioredoxin-Dependent Glutathione Peroxidase (OsGPX5) Is

Required for Rice Normal Development and Salt Stress Tolerance. *Plant Mol. Biol. Report.* **35**: 333–342.

Welchen, E., and Gonzalez, D.H. 2016. Cytochrome c, a hub linking energy, redox, stress and signaling pathways in mitochondria and other cell compartments. *Physiol. Plant.* **157**: 310–321.

Williams, T.C.R., Poolman, M.G., Howden, A.J.M., Schwarzlander, M., Fell, D.A., Ratcliffe, R.G., and Sweetlove, L.J. 2010. A Genome-Scale Metabolic Model Accurately Predicts Fluxes in Central Carbon Metabolism under Stress Conditions. *Plant Physiol.* **154**: 311–323.

Wingler, A., Lea, P.J., Quick, W.P., and Leegood, R.C. 2000. Photorespiration: metabolic pathways and their role in stress protection. *Philos. Trans. R. Soc. B Biol. Sci.* **355**: 1517–1529.

Wolosiuk, R.A., Buchanan, B.B., and Crawford, N.A. 1977. Regulation of NADP-malate dehydrogenase by the light-actuated ferredoxin/thioredoxin system of chloroplasts. *FEBS Lett.* **81**: 253–258.

Wu, J., Sun, Y., Zhao, Y., Zhang, J., Luo, L., Li, M., Wang, J., Yu, H., Liu, G., Yang, L., et al. 2015. Deficient plastidic fatty acid synthesis triggers cell death by modulating mitochondrial reactive oxygen species. *Cell Res.* **25**: 621–633.

Yoshida, K., Hara, S., and Hisabori, T. 2015. Thioredoxin selectivity for thiol-based redox regulation of target Proteins in Chloroplasts. *J. Biol. Chem.* **290**: 14278–14288.

Yoshida, K., and Hisabori, T. 2014. Mitochondrial isocitrate dehydrogenase is inactivated upon oxidation and reactivated by thioredoxin-dependent reduction in Arabidopsis. *Front. Environ. Sci.* **2**: 1–7.

Yoshida, K., and Hisabori, T. 2016a. Adenine nucleotide-dependent and redox-independent control of mitochondrial malate dehydrogenase activity in Arabidopsis thaliana. *Biochim. Biophys. Acta*. **1857**: 810–818.

Yoshida, K., and Hisabori, T. 2016b. Two distinct redox cascades cooperatively regulate chloroplast functions and sustain plant viability. *Proc. Natl. Acad. Sci.* **113**: E3967–E3976.

Yu, H., Braun, P., Yildirim, M.A., Lemmens, I., Venkatesan, K., Sahalie, J., Hirozane-Kishikawa, T., Gebreab, F., Li, N., Simonis, N., et al. 2008. High-Quality Binary Protein Interaction Map of the Yeast Interactome Network. *Science (80-.).* **322**: 104–110.

Yu, X., Pasternak, T., Eiblmeier, M., Ditengou, F., Kochersperger, P., Sun, J., Wang, H., Rennenberg, H., Teale, W., Paponov, I., et al. 2013. Plastid-Localized Glutathione Reductase2-Regulated Glutathione Redox Status Is Essential for Arabidopsis Root Apical Meristem Maintenance. *Plant Cell*. **25**: 4451–4468.

Zhang, Y., and Fernie, A.R. 2018. On the role of the tricarboxylic acid cycle in plant productivity. *J. Integr. Plant Biol.*

Zubimendi, J.P., Martinatto, A., Valacco, M.P., Moreno, S., Andreo, C.S., Drincovich, M.F., and Tronconi, M.A. 2018. The complex allosteric and redox regulation of the fumarate hydratase and malate dehydratase reactions of Arabidopsis thaliana Fumarase 1 and 2 gives clues for understanding the massive accumulation of fumarate. *FEBS J.* **285**: 2205–2224.

Zybailov, B., Rutschow, H., Friso, G., Rudella, A., Emanuelsson, O., Sun, Q., and Wijk, K.J. van 2008. Sorting signals, N-terminal modifications and abundance of the chloroplast proteome. *PLoS One* **3**: 1–19.

Table 1 List of redox proteins found in the chloroplasts. The identification, abbreviation and location of each protein of the chloroplastic redox network are described using Arabidopsis as model plant.

Arabidopsis Gene ID	Abbreviation	Protein Name	Protein location
AT1G03680	Trx *m1*	Thioredoxin m1 isoform	Chloroplast
AT4G03520	Trx *m2*	Thioredoxin m2 isoform	Chloroplast
AT2G15570	Trx *m3*	Thioredoxin m3 isoform	Chloroplast
AT3G15360	Trx *m4*	Thioredoxin m4 isoform	Chloroplast
AT3G02730	Trx *f1*	Thioredoxin f1 isoform	Chloroplast
AT5G16400	Trx *f2*	Thioredoxin f2 isoform	Chloroplast
AT1G76760	Trx *y1*	Thioredoxin y1 isoform	Chloroplast
AT1G43560	Trx *y2*	Thioredoxin y2 isoform	Chloroplast
AT1G50320	Trx *x*	Thioredoxin type x	Chloroplast
AT3G06730	Trx *z*	Thioredoxin type z	Chloroplast
AT4G09010	APX 4	Ascorbate Peroxidase isoform 4	Lumen
AT4G08390	sAPX	Stromal Ascorbate Peroxidase	Stroma
AT1G77490	tAPX	Thylakoid Ascorbate Peroxidase	Thylakoid
At3g54660	GR2	Glutathione Reductase isoform 2	Chloroplast/Mitochondrion
AT2G25080	GPX 1	Glutathione Peroxidase 1	Chloroplast
AT4G31870	GPX 7	Glutathione Peroxidase 7	Chloroplast
AT2G20270	GRXS12	Glutaredoxin S12	Chloroplast
AT1G31170	SRX	Sulfiredoxin	Chloroplast
AT3G11630	2-Cys Prx A	2-Cys Peroxiredoxin A	Chloroplast
AT5G06290	2-Cys Prx B	2-Cys Peroxiredoxin B	Chloroplast/Mitochondrion
AT3G26060	PrxQ	Peroxiredoxin Q	Chloroplast
AT3G52960	PrxIIE	Peroxiredoxin-II-E,	Chloroplast
AT3G54900	GRXS14	Glutaredoxin S14	Chloroplast
AT2G38270	GRXS16	Glutaredoxin S16	Chloroplast
AT2G41680	NTRC	NADPH Thioredoxin Reductase	Chloroplast
AT5G23440	FTR A1	Ferredoxin/Thioredoxin Reductase Subunit A1	Chloroplast
AT5G08410	FTR A2	Ferredoxin/Thioredoxin Reductase Subunit A2	Chloroplast
AT2G04700	FTR B	Ferredoxin/Thioredoxin Reductase Subunit B	Chloroplast

Table 2 List of mutants that may be characterized in the near future in order to fulfill the gap of our knowledge regarding the function of specific genes and the complementarity among the components of the redox network.

Mutants	Compartments involved	Possible outputs
Thioredoxins		
trxh2	Cytosol, mitochondria, endoplasmatic membrane	The *trxh2* is an uncharacterized mutant. Functional genomic approaches should be applied to investigate the function of TRX *h2*, which is located at cytosol, mitochondria and endoplasmatic membrane (Meng *et al.*, 2010; Traverso *et al.*, 2013).
trxh3	Cytosol	Three carbonic anhydrases (CA) βCA1, βCA2 and βCA4 interacted with TRX *h3*. Could this TRX regulate these CAs and thus the stomatal response to CO_2? The characterization of CO_2 stomatal responses of *trxh3* may provide important insights into this question.
trxh9	Plasma membrane, endoplasmatic membrane	The mutation in TRX *h9* is lethal for Arabidopsis (Meng *et al.*, 2010; Traverso *et al.*, 2013), although the reasons for this essentiality remains unclear. Further characterization of this mutant may bring important information whether other redox systems are also involved in this phenotype.
trxh1 trxh2 trxh3 trxh4 trxh5	Cytosol	It has been shown that both FUM and cMDH are positively regulated by TRX *h2* (Hara *et al.*, 2006; Daloso *et al.*, 2015; Huang *et al.*, 2018) . Notably, recent evidence indicates that cMDH is regulated by five different TRX *h* (Huang *et al.*, 2018).The characterization of *trxh* multiple mutants may show whether FUM and cMDH can be activated by other redox components and what it is the impact of TRX *h* mutations for plant growth, especially under stress conditions.
trxo1trxo2trxh2	Mitochondria	These three TRXs are described as being mitochondrially located, although information's concerning TRX *o2* location is still missing. Assuming that TRX *o2* it is in fact found in the mitochondria, this triple mutant will lacks all mitochondrial TRX activity. Mutation in mitochondrial TRX is lethal for mammalian cells (Nonn *et al.*, 2003). The questions to be addressed by the characterization of this triple mutant are: Is the plant mitochondrial TRX system essential for plants? Are the components of plant mitochondrial TRX system redundant? Could mitochondrial GRXs compensate the absence of the entire mitochondrial TRX system?
trxy1 ntrc	Chloroplast	These enzymes were characterized as hub-like nodes in the chloroplastic redox network. What is the effect of this double mutation for the entire chloroplastic redox network? The characterization of this double mutant may help to answer this important question.
Thioredoxin reductases		
ntra ntrb ntrc	Chloroplast, cytosol and mitochondria	NTR absence is lethal for mammalian cells (Conrad *et al.*, 2004). Surprisingly, neither NTRC nor NTRA-NTRB double mutation are lethal for plants. The characterization of the triple *ntra ntrb ntrc* mutant will answer whether plant NTRs are essential for plants. If not, which redox components can compensate the absence of these enzymes?

Glutathione metabolism		
gr1 gr2	Chloroplast, cytosol and mitochondria.	GR1 and GR2 encode glutathione reductases located at the cytosol and at both chloroplast and mitochondria, respectively. Mutation in genes of other reductases such as NTRC and NTRA:NTRB has severe consequences for plant growth. The characterization of *gr1 gr2* double mutant may demonstrate the importance of this reductase system for plants.
ghr1 ghr4	Chloroplast	It was suggested that GHRs are central to the regulation of the quinone redox state(Lallement *et al.*, 2014). Photochemistry measurements in single or double *ghr1* and *ghr4* mutants may prove if this glutathione transferase can regulate plastoquinone redox state. No characterization of these mutants was performed so far.
Peroxidases		
gpx5	Plasma membrane	While the other seven GPX isoforms are relatively well characterized in Arabidopsis, not much is known about AtGPX5.
Multiple mutants		
ntrc gr2; *ftra ftrb gr2*		The complementarity among NTRC and FTR has been recently demonstrated (Yoshida and Hisabori, 2016). However, whether GR2 can also compensate the absence of the other chloroplastic reductases remains to be determined.
gpx3 rcd1	Cytosol and nucleus	The radical induced cell death protein 1 (RCD1) supports regulation of genes encoding chloroplast antioxidant enzymes and glutathione biosynthesis (Hiltscher *et al.*, 2014). AtGPX3 interacts with RCD1 (Miao *et al.*, 2006). Studies with *gpx3 rcd1* double mutant would indicate which redox pathways are directly involved with the interaction between these proteins.
gpx3 abi1; *gpx3 abi2*	Cytosol, nucleus and plasma membrane	Abscisic acid insensitive 1 (ABI1) and 2 (ABI2) are involved with the abscisic acid signaling pathway (Merlot *et al.*, 2002). AtGPX3 interacts with ABI1 and stronger with ABI2, leading to stomatal closure via the activation Ca^{2+} channels at the plasma membrane (Miao *et al.*, 2006). There is no information about *gpx3 abi1* double mutant, while *gpx3 abi2* double mutant seems to be insensitive to ABA (Miao *et al.*, 2006). Experiments with *gpx3 abi1* and *gpx3 abi2* double mutants would improve the understanding about ABA signaling mediated by regulation of the redox state.

TABLE S1 List of proteins that interact with TRX *h3* according to the Arabidopsis interactome study (Arabidopsis Interactome Mapping Consortium, 2011).

Arabidopsis Gene ID	Abbreviation	Protein Name	Protein location
AT3G23940	DHAD	DIHYDROXYACID DEHYDRATASE	Chloroplast
AT5G26000	β-Glucosidade	BETA GLUCOSIDASE	Chloroplast
AT5G35630	GS2	GLUTAMINE SYNTHETASE 2	Chloroplast
AT3G60750	TK	TRANSKETOLASE	Chloroplast
AT3G04790	-	EMBRYO DEFECTIVE	Chloroplast
AT5G14740	βCA 2	BETA CARBONIC ANHYDRASE 2	Chloroplast
AT1G65930	cIDH	CYTOSOLIC NADP+-DEPENDENT ISOCITRATE DEHYDROGENASE	Cytosolic
AT1G17290	AlaAT	ALANINE AMINOTRANSFERASE	Chloroplast
AT1G32060	PRK	PHOSPHORIBULOKINASE	Chloroplast
AT5G53490	TL17	THYLAKOID LUMENAL 17.4 KDA PROTEIN	Chloroplast
AT5G58330	NADP-MDH	NADP-DEPENDENT MALATE DEHYDROGENASE	Chloroplast
AT2G35370	GDC-H	GLYCINE DECARBOXYLASE COMPLEX H	Chloroplast
AT3G52930	FBA-8	FRUCTOSE-BISPHOSPHATE ALDOLASE 8	Chloroplast
AT2G21170	TPI	PLASTID ISOFORM TRIOSE PHOSPHATE ISOMERASE	Chloroplast
AT2G39730	Rcbs-activase	RCA, RUBISCO ACTIVASE	Chloroplast
AT5G61410	R5Pepi	D-RIBULOSE-5-PHOSPHATE-3-EPIMERASE	Chloroplast
AT2G43560	-	FKBP-PEPTIDYL-PROLYL CIS-TRANS ISOMERASE	Chloroplast
AT3G62410	CP12-1	CP12 DOMAIN-CONTAINING PROTEIN 1	Chloroplast
AT3G50820	OEC33	OEC33 COMPLEX OXYGEN	Chloroplast
AT1G21750	ATPDI5	PROTEIN DISULFIDE ISOMERASE 5, ATPDIL1-1	Chloroplast
AT3G62030	CYP20-3	Cyclopinhil 20-3	Chloroplast

AT2G15620	NR1	Ferrodoxin Nitri reductase	Chloroplast
AT2G47400	CP12-1	CP12 DOMAIN-CONTAINING PROTEIN 1	Chloroplast
AT5G66530	-	Galactose mutarose superfamily protein	Chloroplast
AT5G66570	OEC	OEE33, OXYGEN EVOLVING COMPLEX 33 KILODALTON PROTEIN	Chloroplast
AT4G25130	MSRA4	METHIONINE SULFOXIDE REDUCTASE A4	Chloroplast
AT4G26530	FBA5	FRUCTOSE-BISPHOSPHATE ALDOLASE 5	Chloroplast
AT1G04410	cMDH	CYTOSOLIC-NAD-DEPENDENT MALATE DEHYDROGENASE 1, Cyt-NADMDH	Cytosol
AT4G19700	ATILP, BOI	BOTRYTIS SUSCEPTIBLE1 INTERACTOR	Cytosol
AT1G43670	cFBPase	FRUCTOSE INSENSITIVE 1, FRUCTOSE-1,6-BISPHOSPHATASE	Cytosol
AT2G24270	GAPDH	ALDH11A3 GLYCERALDEHYDE 3 PHOSPHATE DEHYDROGENASE	Cytosol
AT3G18490	ASPG1	ASPARTIC PROTEASE IN GUARD CELL 1	Endoplasm reticulum
AT5G60360	SAG2	ALEURAIN-LIKE PROTEASE, ALP, (Senescence gene)	Extracellular space
AT2G01950	BRL2	BRI1-LIKE 2, VASCULAR HIGHWAY 1, VH1	Integral component of membranes
AT1G48030	MTLPD1	LIPOAMIDE DEHYDROGENASE 1	Mitochondrion
AT1G23310	AOAT1	ALANINE-2-OXOGLUTARATE AMINOTRANSFERASE	Peroxisome
AT4G39330	CAD9	CINNAMYL ALCOHOL DEHYDROGENASE 9	Apoplast
AT2G42580	TTL3	TTL3	nucleus
AT1G75040	PR5	PATHOGENESIS-RELATED GENE 5, PR-5	Cell wall

Figure 1 Schematic representation of the chloroplastic redox network. Abiotic and biotic stress conditions can lead to an overproduction of harmful reactive oxygen species (ROS) such as singlet oxygen (O_2^-) and hydrogen peroxide (H_2O_2)**.** Superoxide dismutase (SOD) is responsible to convert 2 O_2^- in H_2O_2, which can be metabolized by different redox systems including peroxiredoxins (Prx) and peroxidases dependent on ascorbate (APX) and glutathione (GPX). GPX activity depends on reduced glutathione (GSH), which can be provided by glutathione reductase (GR) that uses chloroplastic electron transport chain (ETC)-derived NADPH to convert oxidized glutathione (GSSG) into GSH. Similarly, APX is also capable of converting H_2O_2 in H_2O, but using reducing power from the ascorbate cycle instead, which includes the conversion of ascorbate into monodehydroascorbate (MDHA) by the enzyme monodehydroascorbate reductase (MDHAR). The degradation of H_2O_2 by 2-Cys PRX seems to involve different states of oxidation/reduction of these proteins given that their reduced form (2-Cys PRX_{red}) can be oxidized (2-Cys PRX_{ox}) or overoxidized (2-Cys PRX_{oox}) by H_2O_2, and have their reduction state rescued by the activity of sulfiredoxin (SRX), and/or NADPH-dependent thioredoxin reductase C (NTRC) and thioredoxins (TRXs). In parallel, the chloroplastic ETC-generated NADPH can be used to sustain the NTRC-TRX system, which is able to reduce different proteins of the redox network (note that S-S disulfide bound in the enzymes already showed to be TRX-mediated redox regulated). The chloroplast ETC is activated by light and leads to the reduction of ferredoxin (Fd) via ferredoxin $NADPH^+$ reductase (FNR). Reduced Fd is then used by ferredoxin thioredoxin reductase (FTR) that, together with NTRC, can reduce TRXs. GPX, malate dehydrogenase (MDH) and methionine sulfoxide reductase (MSR), whose function is to reduce methionine sulfoxide (MetSo) into methionine (Met), are examples of TRX-mediated redox regulated enzymes.

Figure 2 Plant thioredoxin (TRX)-mediated redox regulation cascade. The TRX system is composed by reductases, TRXs and their target proteins (right panel). The left panel demonstrate the relative number of proteins of each of these components of the TRX system. The reductases NADPH-dependent TRX reductase (NTR), ferredoxin reductase (FTR), and glutathione reductase (GR) are responsible to reduce the different

isoforms of TRXs, which are divided in TRXs *f*, *h*, *m*, *o*, *x*, *y*, and *z*. Reduced TRXs can then (de)activate a wide range of target proteins.

Figure 3 Role(s) of the circulating malate for the redox regulation of plant cells under light (left) and dark (right) conditions. Malate metabolism is closely related to the generation of NAD(P)(H) in the chloroplast, cytosol and mitochondria by the activity of key enzymes such as malate (MDH), isocitrate (ICDH), and succinate (SDH) dehydrogenases and associated enzymes such as fumarase (FUM1, mitochondrial; FUM2, cytosolic), citrate synthase (CS), and phospho*enol*pyruvate carboxylase (PEPc). Light energy activates the chloroplastic electron transport chain that ultimately results in the production of NADPH, which can be used by the Calvin-Benson (CB) cycle and/or by the malate valve that involves an exchange of malate (Mal)/oxaloacetate (OAA) and the activity of MDH in the plastids (pMDH) and in the cytosol (cMDH). Another pathway that provides cytosolic OAA involves the activity of PEPc that utilizes PEP from glycolysis. Cytosolic OAA can be used for the synthesis of Mal and Fum in the cytosol or for Glu/Gln synthesis. Evidence suggests that the synthesis of Glu depends on C coming from PEPc fixation and also from previous stored C, which may be night-stored citrate, OAA, Mal and Fum. Both pMDH and cMDH are key enzymes to control the balance of $NADPH/NADP^+$ and $NADH/NAD^+$ in the light given that the fluxes through the TCA cycle are inhibited under this condition, likely due that pyruvate dehydrogenase (PDH) is inhibited and both FUM1 and SDH are deactivated by TRX *o1*. In the mitochondria, the NAD(P)H seems to be generated mainly by the activity of both ICDH and NAD kinase (NADk). It has been proposed that mitochondrial MDH preferentially acts in the direction of Mal synthesis, which can then be exported to the cytosol for the synthesis of Fum or being translocate to the vacuole. The synthesis of Fum is stimulated by the accumulation of Gln, Asn, and OAA by FUM2 activation. By contrast to the light condition, all the mitochondrial TCA cycle enzymes seem to be activated whilst the chloroplast metabolism and the malate valve are deactivated in the dark. Under this condition, the metabolism seems likely to be involved in ATP production within the mitochondria and to store organic acids in the vacuole to be used in the following light period. Abbreviations: AcCoA, acetyl CoA; Asn, asparagine; CB, Calvin-Benson; Cit, citrate; Fd, ferredoxin; Fum, Fumarate; Gln, glutamine; Glu, glutamate;

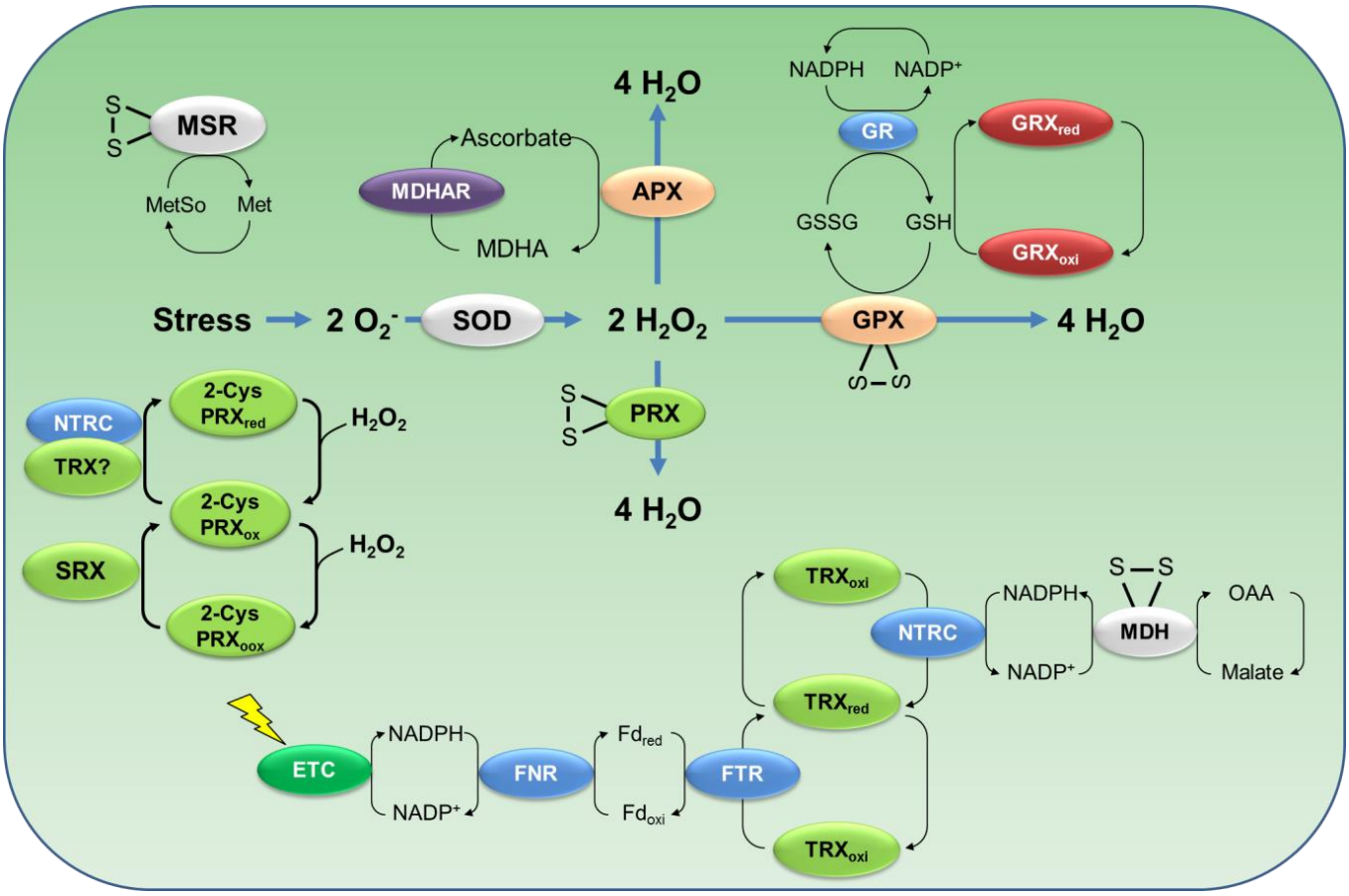
Mal, malate; OAA, oxaloacetate. PEP, phospho*enol*pyruvate; Pyr, pyruvate; Succ, succinate; TCA, tricarboxylic acid; TRX, thioredoxin.

Figure 4 Gene co-expression network between the main components of the chloroplastic redox network. The nodes (genes) are connected to each other when their genes are co-expressed. Gene co-expression network was carried out using String database platform (Szklarczyk *et al.*, 2017). The co-expression gene network was generated using Cytoscape® v.3.6.1 (Shannon *et al.*, 2003). The proteins used to construct this co-expression network were: GR2; FTRA1-2; FTRB; NTRC; TRXs *m1-4*, *f1-2*, *y1-2*, *x*, and *z*; SRX; tAPX, sAPX; GPX1 and 7; GRXS12, S14, and S16; 2-Cys PRX A-B, PRXQ, and PRXII-E. Detailed information regarding the proteins of this network is described in the Table 1.

Figure 5 Protein-protein interaction network between the main components of the chloroplastic redox network. The nodes (enzymes) are connected by a link when their proteins interact to each other according to the Arabidopsis interactome (Arabidopsis Interactome Mapping Consortium, 2011). The color of the nodes represents different groups of proteins: light blue, transcription factors; dark blue, reductases; light brown, peroxidases; green fluorescent, redoxins; light green, other proteins. One-way arrows represent regulations between a regulatory protein (tail of the arrow) and a specific regulated protein (head of the arrow). Red edges represent protein-protein interaction or regulation already observed by *in vitro* or *in vivo* studies. Abbreviations and identification of the proteins of this network are described in the table 1.

Figure 6 Protein-protein interaction network between NTRC and TRX *y1* and different transcription factors (TFs). The nodes (enzymes) are connected by a link when their proteins interact to each other according to the Arabidopsis interactome (Arabidopsis Interactome Mapping Consortium, 2011). Blue nodes are TFs that interact directly or indirectly with NTRC and/or TRX *y1*. White nodes are different proteins that interact with the TFs.

Figure 7 TRX *h3* protein-protein interaction network. The nodes (enzymes) are connected by a link when their proteins interact to each other according to the Arabidopsis interactome (Arabidopsis Interactome Mapping Consortium, 2011). Green and yellow nodes denote chloroplastic and cytosolic enzymes, respectively. Proteins identified with asterisks (*) are located to both chloroplast and cytosol. White nodes are unknown proteins that interact with TRX *h3*. Abbreviations and the identification of the proteins of this network are described in the supplemental table S1.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Stress
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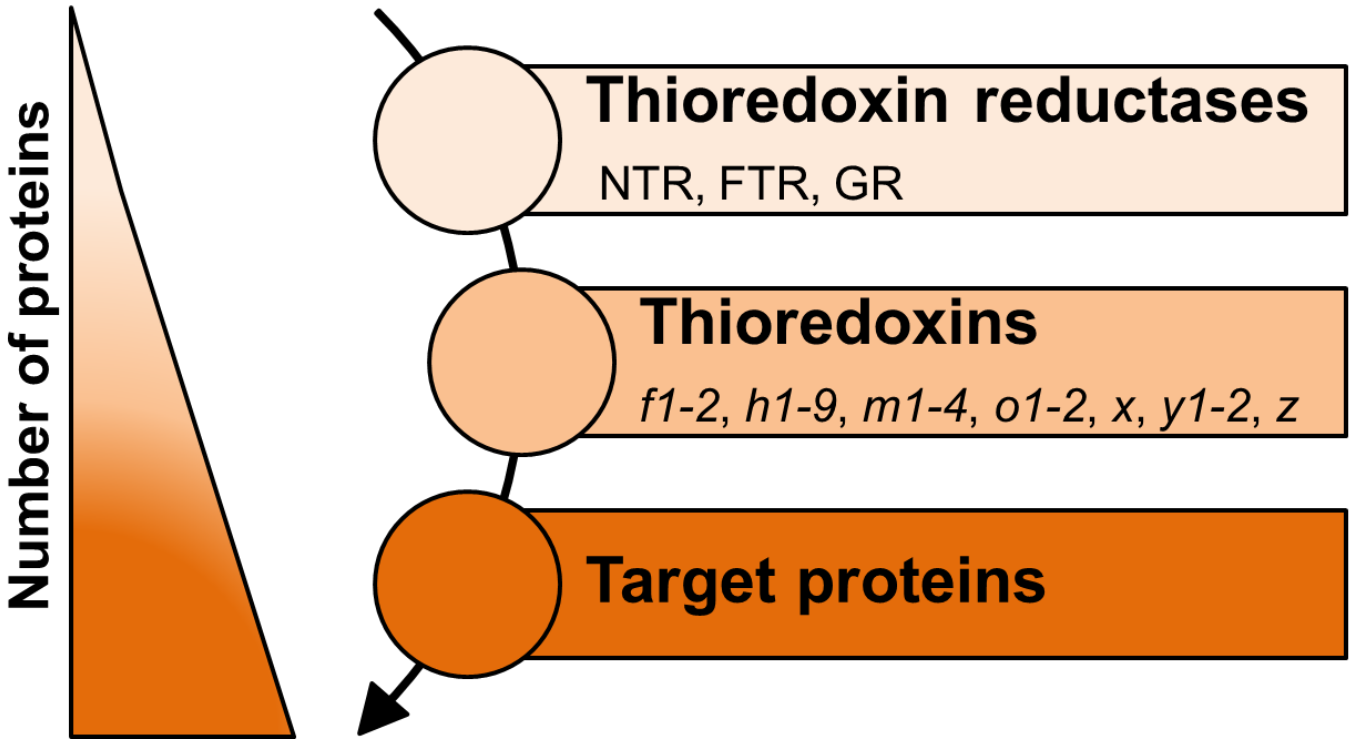
Figure 1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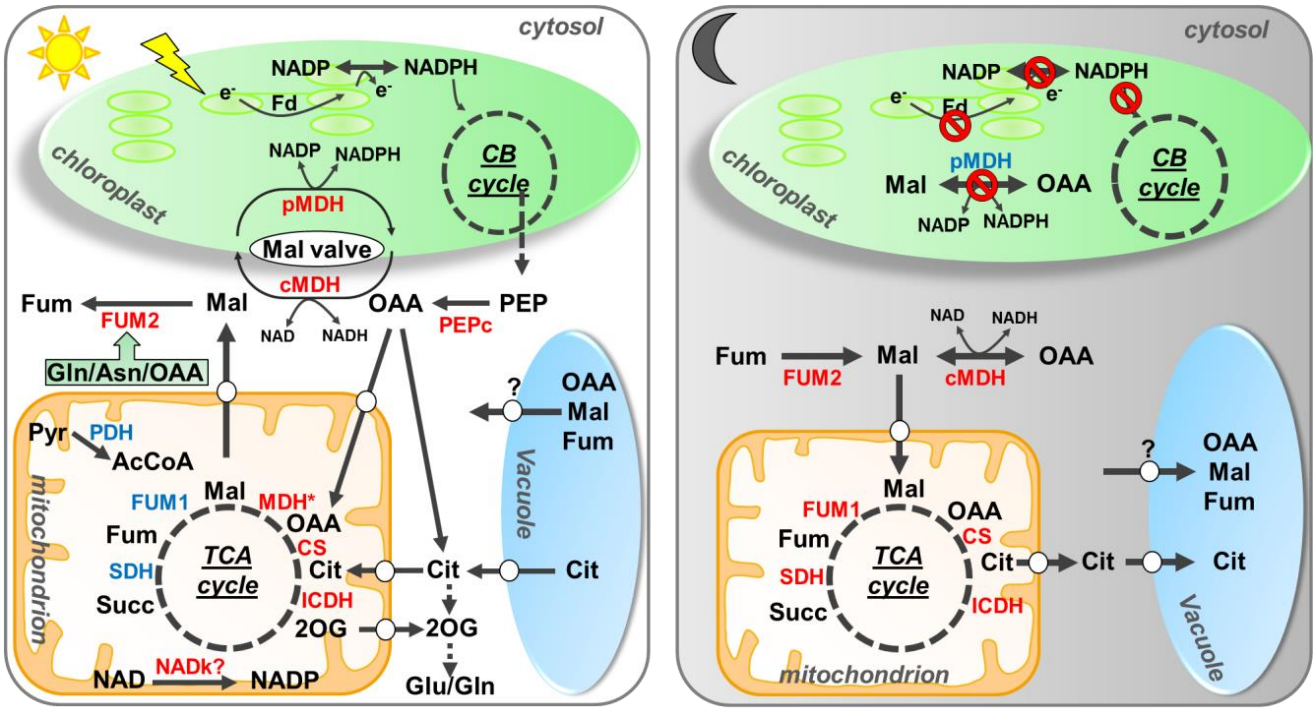
Thioredoxins
f1-2, h1-9, m1-4, o1-2, x, y1-2, z
Target proteins

Figure 2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CB cycle
pMDH
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cMDH
NAD
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Mal
OAA
PEP
PEPc
Gln/Asn/OAA
Pyr
PDH
AcCoA
mitochondrion
FUM1
MDH*
TCA cycle
CS
SDH
Succ
Cit
ICDH
2OG
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Vacuole
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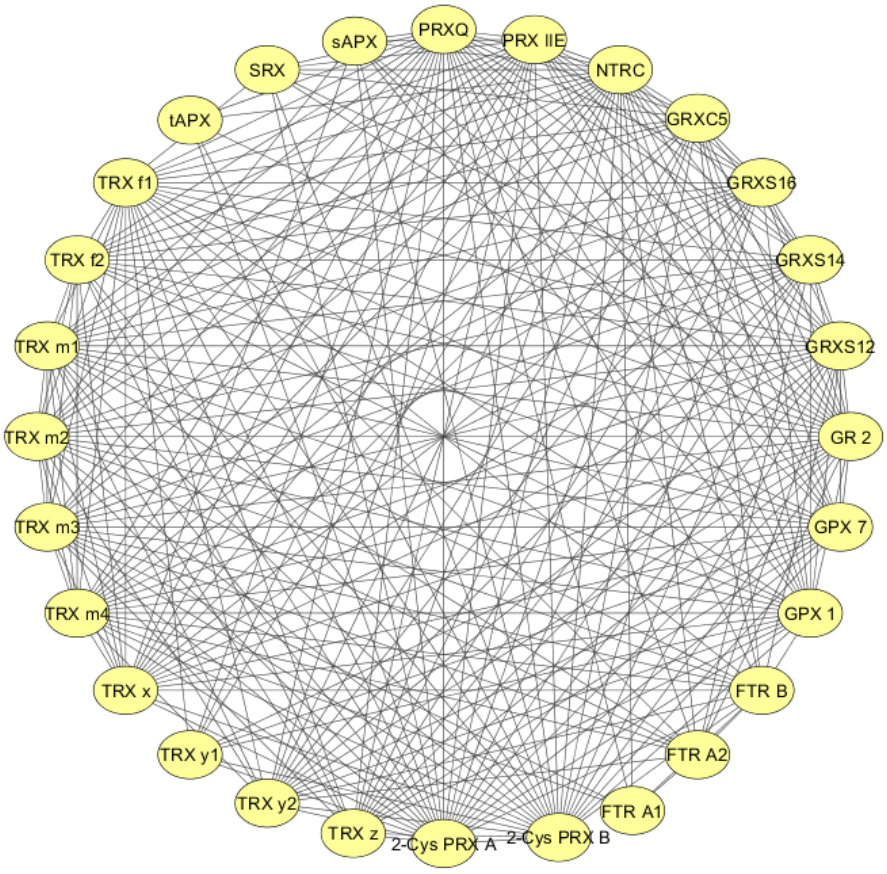
NAD
NADH

Figure 3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GRXS12
TRX m2
GR 2
TRX m3
GPX 7
TRX m4
GPX 1
TRX x
FTR B
TRX y1
FTR A2
TRX y2
FTR A1
TRX z
2-Cys PRX A
2-Cys PRX B

Figure 4

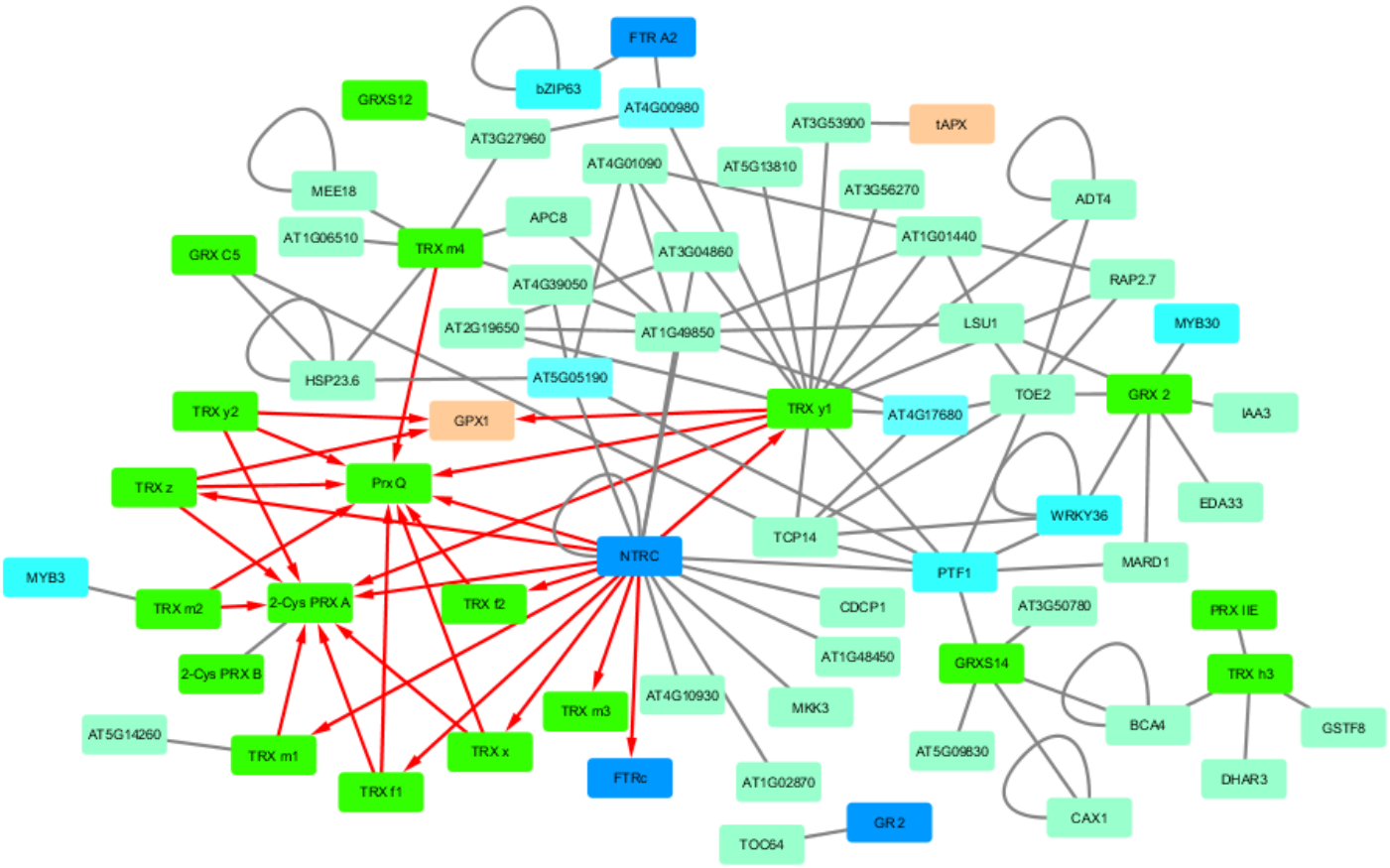
FTR A2
bZIP63
GRXS12
AT4G00980
AT3G27960
AT3G53900
tAPX
AT4G01090
AT5G13810
AT3G56270
ADT4
MEE18
APC8
AT1G06510
TRX m4
AT1G01440
GRX C5
AT3G04860
RAP2.7
AT4G39050
AT2G19650
AT1G49850
LSU1
MYB30
HSP23.6
AT5G05190
TOE2
GRX 2
IAA3
TRX y2
GPX1
TRX y1
AT4G17680
TRX z
Prx Q
EDA33
WRKY36
TCP14
NTRC
MARD1
MYB3
PTF1
TRX m2
2-Cys PRX A
TRX f2
CDCP1
AT3G50780
PRX IIE
AT1G48450
GRXS14
2-Cys PRX B
TRX h3
AT4G10930
TRX m3
MKK3
BCA4
AT5G14260
GSTF8
AT5G09830
TRX m1
TRX x
FTRc
DHAR3
TRX f1
AT1G02870
CAX1
GR 2
TOC64

Figure 5

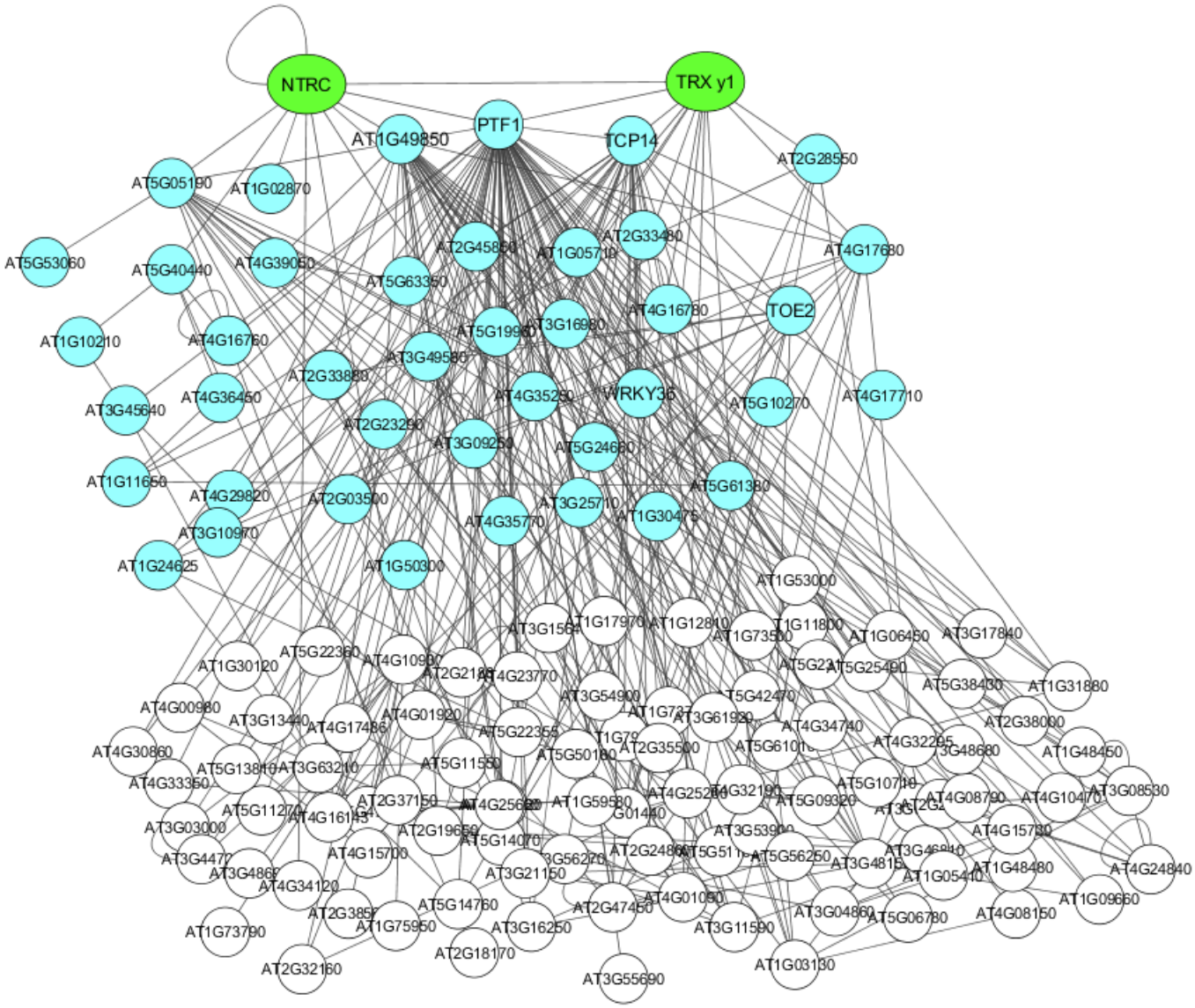
NTRC
TRX y1
PTF1
AT1G49850
TCP14
AT2G28550
AT5G05190
AT1G02870
AT5G53060
AT5G40440
AT4G39050
AT2G45850
AT1G05710
AT2G33480
AT4G17680
AT5G63350
AT4G16780
TOE2
AT1G10210
AT4G16760
AT5G19960
AT3G16980
AT3G49580
AT2G33880
AT3G45640
AT4G36450
AT4G35250
WRKY35
AT5G10270
AT4G17710
AT2G23290
AT3G09250
AT5G24660
AT1G11650
AT4G29820
AT2G03500
AT3G25710
AT5G61380
AT1G30475
AT4G35770
AT3G10970
AT1G24625
AT1G50300
AT1G53000
AT1G17970
AT1G12810
AT1G11800
AT1G06450
AT3G17840
AT5G22360
AT1G30120
AT4G10990
AT4G23770
AT5G25490
AT5G38430
AT1G31880
AT4G00980
AT3G13440
AT4G17486
AT4G01920
AT3G54900
AT3G61920
AT4G34740
AT2G38000
AT4G30860
AT5G22365
AT5G50180
AT2G35500
AT4G32295
AT1G48450
AT5G13810
AT3G63210
AT5G11550
AT4G33350
AT5G10710
AT4G08790
AT4G10470
AT3G08530
AT5G11270
AT2G37150
AT1G59580
AT4G25280
AT4G32190
AT5G09320
AT3G03000
AT4G16143
AT2G19650
AT5G14070
AT3G53900
AT4G15730
AT3G44770
AT4G15700
AT3G56270
AT2G24860
AT5G51110
AT5G56250
AT3G48410
AT1G48480
AT4G24840
AT3G48680
AT4G34120
AT3G21150
AT1G05410
AT1G09660
AT5G14760
AT2G47450
AT4G01090
AT3G04860
AT5G06780
AT4G08150
AT2G38570
AT1G75950
AT3G16250
AT3G11590
AT1G73790
AT2G18170
AT1G03130
AT2G32160
AT3G55690

Figure 6

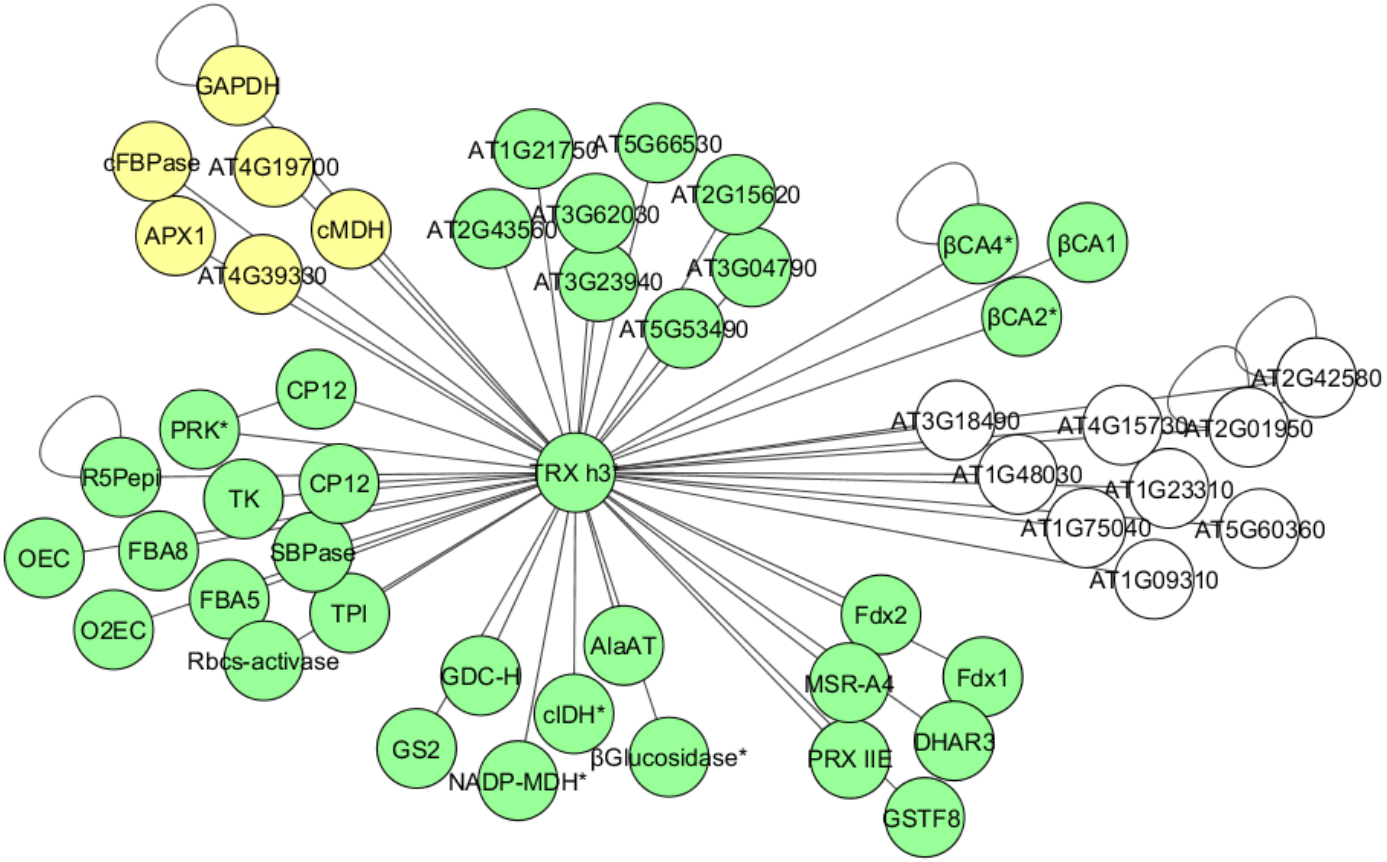
GAPDH
cFBPase
AT4G19700
APX1
cMDH
AT4G39330
AT1G21750
AT5G66530
AT2G43560
AT3G62030
AT2G15620
AT3G23940
AT3G04790
AT5G53490
βCA4*
βCA1
βCA2*
CP12
PRK*
R5Pepi
TK
CP12
OEC
FBA8
SBPase
FBA5
TPI
O2EC
Rbcs-activase
TRX h3
AT2G42580
AT3G18490
AT4G15730
AT2G01950
AT1G48030
AT1G23310
AT1G75040
AT5G60360
AT1G09310
GDC-H
AlaAT
cIDH*
GS2
NADP-MDH*
βGlucosidase*
Fdx2
MSR-A4
Fdx1
DHAR3
PRX IIE
GSTF8

Figure 7