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Copper and Iron Homeostasis in Plants: The Challenges of Oxidative Stress

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Abstract

Significance: Photosynthesis, the process that drives life on earth, relies on transition metal (e.g., Fe and Cu) containing proteins that participate in electron transfer in the chloroplast. However, the light reactions also generate high levels of reactive oxygen species (ROS), which makes metal use in plants a challenge. **Recent Advances:** Sophisticated regulatory networks govern Fe and Cu homeostasis in response to metal ion availability according to cellular needs and priorities. Molecular remodeling in response to Fe or Cu limitation leads to its economy to benefit photosynthesis. Fe toxicity is prevented by ferritin, a chloroplastic Fe-storage protein in plants. Recent studies on ferritin function and regulation revealed the interplay between iron homeostasis and the redox balance in the chloroplast. **Critical Issues:** Although the connections between metal excess and ROS in the chloroplast are established at the molecular level, the mechanistic details and physiological significance remain to be defined. The causality /effect relationship between transition metals, redox signals, and responses is difficult to establish. **Future Directions:** Integrated approaches have led to a comprehensive understanding of Cu homeostasis in plants. However, the biological functions of several major families of Cu proteins remain unclear. The cellular priorities for Fe use under deficiency remain largely to be determined. A number of transcription factors that function to regulate Cu and Fe homeostasis under deficiency have been characterized, but we have not identified regulators that mediate responses to excess. Importantly, details of metal sensing mechanisms and cross talk to ROS-sensing mechanisms are so far poorly documented in plants. *Antioxid. Redox Signal.* 19, 919–932.

Introduction

THE METALS IRON (Fe), copper (Cu), manganese (Mn), and zinc (Zn) are employed by organisms to perform a remarkable array of functions that are critical for life. Proteins that carry these metal ions as cofactors mediate diverse biochemical processes, including energy conversion, synthesis, and regulation of nucleic acids, biosynthesis of complex molecules, reactive oxygen species (ROS) detoxification, as well as signaling events that trigger molecular, cellular, and systemic responses.

During the evolution of life, the metal-based biochemical reactions have been selected and conserved because the chemical properties of these metals make them ideal structural and/or active cofactors in enzymes. In particular, the transition metals Fe and Cu take a central place in redox control and electron transport in the cell. Therefore, these transition met-

als are essential for most prokaryotic and eukaryotic cells. However, the same redox properties of Fe and Cu that are utilized by metalloproteins can be harmful to aerobic cells because the uncontrolled production of ROS is deleterious for the cell. Thus, living organisms have developed various and sophisticated cellular and systemic homeostatic processes that allow an efficient use of the metals, despite the unavoidable danger they constitute for the cell.

Among the different living organisms, plants had to face specific challenges related to metal homeostasis. Plants and algae have an additional intracellular organelle where carbon fixation takes place, the chloroplast. Similar to respiration in the mitochondria, the chloroplast relies on a complex protein machinery to mediate electron transport. However, chloroplasts use photosystems to capture sun light in order to drive the electron transport chain and reduce NADP⁺, thereby converting solar energy into chemical energy. The processes

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of photosynthesis are strictly dependent on metals (57, 71). It is now clear that most of the cellular Fe and Cu pools in plants are found in the chloroplast (78). However, photosynthetic electron transport is also a potential ROS generative process. When light is in excess, or when the electron transport capacity in the chloroplast is limited, electrons can directly reduce molecular oxygen (29). Plants are sessile organisms and are therefore extra sensitive to biotic and abiotic stresses. Stress promotes ROS fluctuations in plants, particularly in the chloroplast (9). Therefore, a fine-tuned regulation of homeostatic processes has evolved in plants in order to maintain a tight equilibrium between cellular “labile” pools of metals, ROS detoxification capacity, and photosynthesis efficiency.

In this review we will focus on the interplay between metal use and ROS metabolism in plants, with an emphasis on Cu and Fe in the chloroplast. We will address how plants cope with low Cu availability and the consequences of this adaptation for the photosynthetic apparatus and chloroplast localized redox processes. Another part of this review will emphasize the dark side of Fe, and focuses on ferritin function and regulation. These storage proteins are unique to Fe in metal homeostasis and constitute a molecular tool to identify Fe-specific signaling pathways. In this context, we will discuss the tight interconnection between Fe excess and oxidative stress, both at the physiological and molecular levels in plants.

The Use of Metals in Plant Cell Biology

Metals are important bio-components because they can interact with macromolecules and affect protein structure and activity. Among the transition metals important for biology are Fe, Cu, Mn, Mo, and Ni (53). These metal ions exist in more than one oxidation state when bound to protein active sites. Therefore, they readily accept or donate electrons from their external orbitals to other molecules. Mo and Ni only occur in a limited set of enzymes active in the cytosol and peroxisomes (35). The much more abundant Fe, as well as Cu and Mn ions, serve as critical cofactors for electron transport chain in the mitochondria and in the plastids, the two power generators in plant cells. These three transition metals are also found in active centers of diverse proteins and act as electron acceptors and donors in numerous cellular processes, particularly in the chloroplast in the green lineage (Fig. 1) (56, 94). Among the different transition metals, Fe is the most widely utilized. Fe is used as cofactor in a variety of chemical structures: directly bound to the polypeptide (nonheme iron), incorporated into heme structures or into iron-sulfur (Fe-S) clusters. These various types of Fe-cofactor allow Fe-proteins to cover a very large spectrum of redox potentials (Fig. 2). Therefore, Fe-proteins are ubiquitously utilized in diverse biochemical environments. Transition metals can also have structural functions where the binding of the metal cofactor allows specific protein folding, stability, and functionality. The absence of redox activity for zinc (Zn) explains why Zn is the metal of choice as a safe structural cofactor in many enzymes and transcription factors [reviewed in (17), for plants see (79)].

The use of the different transition metals in modern plants results from a long evolutionary process, involving dramatic changes in cell complexity and environmental fluctuations. Recent advances in large-scale genome and proteome analytic technologies allowed the systematic analysis of the relationship between the temporal emergence of metal-specific ma-

FIG. 1. Major metal-dependent cellular processes in plant chloroplast. The main pathways and proteins that utilize metals in plant chloroplasts are listed. PPO, polyphenol oxidase; TIC55, translocon inner membrane complex 55; NFU, “nitrogen fixing bacteria” (Nif) U-like proteins; SUL, “sulfur limitation” proteins; APR, adenosine 5′-phosphosulfate (APS) reductase; SIR, sulfite reductase; NIR, nitrite reductase; GOGAT, glutamine oxoglutarate aminotransferase (= glutamate synthase); HCAR, 7-hydroxymethyl chlorophyll a reductase; CHL27, chlorophyll deficient 27 (= CRD1: copper response defect 1); LLS1, pheophorbide a oxygenase; SiRB, siroheme ferrochelatase B; FC, ferrochelatase; SOD, superoxide dismutase; APX, ascorbate peroxidase; FER, ferritin.

chineries and metal-binding structures into proteins, their presence in specific kingdoms, and the evolution of the chemistry of the different trace elements in primitive oceans (27). It is now established that the geochemistry of the trace elements has been a driving factor in the evolution of cellular metal ion use. For their redox chemistry, primitive prokaryotic cells mainly used Fe, the most available metal, together with Mn in the anoxic and reducing Archean ocean 4.5 billion years ago. The increase of oxygen levels as a consequence of the invention of photosynthesis roughly 2.4 billion years ago decreased Fe availability in the Proterozoic ocean and some metallo-proteins evolved toward the use of Mn. Over the past 0.8 billion years, the emergence of an aerobic and oxidizing environment led to a dramatic decline of Fe and Mn availabilities (21, 80, 91). Concomitantly, Cu, which was scarcely available to this point, became bio-available and appeared in biological systems (75) together with Zn.

Metal Toxicity in Plants

The chemical properties that make the transition metals key components for beneficial cellular redox reactions can also lead to undesired reactivity that is deleterious for the cell. Partially reduced oxygen species are produced when molecular oxygen accepts electrons from other molecules. Many metal-dependent and metal-independent cellular processes reduce oxygen to superoxide (O_2^-) or hydrogen peroxide (H_2O_2) (8). Although these molecules have by themselves a

FIG. 2. Metals are crucial for the electron transport in the photosynthetic apparatus in plants. The electron transfer from water to the final electron acceptor in the plant photosynthetic complexes is presented (black arrow). The oxygen evolving complex (OEC), photosystem II, the cytochrome b_6/f complex, photosystem I, and ferredoxin (FD) are depicted with respect to their functional arrangement in the electron transport chain and orientation in the chloroplast thylakoids and stroma compartments. Metalloproteins are depicted with black lined symbols. Electron carriers unrelated to metals are presented in gray-lined symbols. Proteins involved in electron transport are depicted according to their standard redox potential (mV). Fe complex, nonheme Fe protein of the photosystem II; Pheo, pheophytin; Q_A , quinone; PQ, plastoquinone; Cyt, cytochrome; PC, plastocyanin; $F_X/F_A/F_B$, electron acceptor X/A/B of photosystem I.

limited cytotoxicity, they are the substrates for the production of the very harmful hydroxyl radical ($\text{OH}\cdot$) which cannot be removed enzymatically and therefore is responsible for most of the oxidative damage in biological systems (34). The production of hydroxyl radicals is catalyzed by Fe ions and, to a lesser extent by Cu ions, in what is known as the Haber-Weiss cycle (Fig. 3):

The first reaction (reaction a) is due to the cycling of the transition metal (M) between its reduced M^n and oxidized M^{n+1} states, which can result in the reduction of molecular oxygen. The single-electron reduction of oxygen is thermodynamically unfavorable because of the electron configuration, but transition metals (M) such as Fe and Cu can harbor unpaired electrons and therefore can be catalysts of oxygen reduction (32, 34). The second reaction (reaction b) consists of the metal-mediated reduction of H_2O_2 leading to the production of the hydroxyl radicals (reaction b). This reaction is known as the Fenton reaction when Fe is the catalyst (33, 34). The net reaction (c) illustrates how the presence of superoxide and H_2O_2 can lead to the production of hydroxyl radicals in the presence of transition metals (Fig. 3). The hydroxyl radicals produced can oxidize biomolecules leading to major cellular alterations and ultimately to cell death. The hydroxyl radical can be responsible for mutations at very high rates. The presence of ROS can also lead to oxidative modifications of proteins and release of metal cofactors. Protein oxidation or

FIG. 3. Photosynthesis and stresses in plant chloroplast promote the Haber-Weiss cycle. Under high light conditions, electrons can escape from the photosynthetic machinery (Fig. 2) and can directly reduce molecular oxygen (O_2) present in the chloroplast. The resulting superoxide ion (O_2^-) reacts with hydrogen peroxide (H_2O_2) in the presence of catalytic transition metals such as Fe and Cu to produce the highly toxic hydroxyl radical ($\text{OH}\cdot$). In plants, stress promotes the accumulation of O_2^- and H_2O_2 in the chloroplast. In that particular context, redox cycling of the metal constitutes a major issue for the plants. (a) and (b) refer to the equations presented in the text.

loss of the cofactor leads to protein misfolding, aggregation, and/or degradation (25, 32). Another deleterious effect of metal reactivity is the peroxidation of lipids (34). Thus, intracellular membrane systems and cell integrity can be altered.

How Do Plants Tackle the Challenge Posed by the Redox-Active Metals?

Plants are sessile organisms and therefore face major fluctuations in environmental conditions such as temperature, moisture (drought), and nutrient availability in addition to biotic stress from pathogens and herbivores. It is interesting to note that ROS are important signaling molecules involved in the plant's response to stress and development, and the chloroplast is emerging as a pivotal organelle for the cellular redox balance. On the other hand, the chemistry of the redox-active metals such as Fe and Cu in the chloroplast has to be tightly tuned to avoid the deleterious effect of the hydroxyl radical production (Fig. 3), as well as the alteration of the ROS-mediated signaling events. Therefore, both biotic and abiotic challenges have driven the evolution of defense mechanisms in plants allowing them to cope with adverse conditions. In addition, photosynthesis itself is a potentially dangerous process that combines molecular oxygen production, electron transport, and the utilization of metal cofactors (Figs. 2 and 3) (29). The presence in chloroplasts of nature's strongest oxidizer (photosystem II [PSII]) and nature's strongest reducer (photosystem I [PSI]) poses a challenge (56). In particular, at high light intensities the saturation of the electron transport capacity of the photosynthetic apparatus leads to the undesired reduction of molecular oxygen in the chloroplast. Such electron congestion in the photosystems can originate not only from excessive light irradiance, but also from diverse environmental and nutritional events that alter the molecular integrity or the proper redox cycling of the photosynthetic apparatus (29). Plants have developed fine-tuned homeostatic mechanisms allowing the delivery of the specific metal to metalloproteins while limiting uncontrolled metal reactivity [reviewed in (64)]. Due to their extremely high reactivity, the concentrations of metals as free ions must be very low inside cells. Metal toxicity avoidance is possible by the chelation of the metal to ligands, metallochaperones, or storage proteins. The control of the subcellular distribution of metals is another strategy that allows plants to cope with metal toxicity. In this context, the accumulation of metals in the vacuole is emerging as an important component of metal homeostasis in plants. For instance, tonoplast-located metal transporters and metal chelating molecules such as nicotianamine play pivotal functions for metal storage and mobilization in the vacuole (36, 42, 43, 45).

To further tackle the toxicity of the redox-active metals, another strategy used by plants is the strict control on the accumulation of superoxide and H_2O_2 (5), the two important players in the undesired Haber-Weiss cycle. Antioxidant enzymes are involved in the breakdown of the reduced forms of oxygen in plant cells: superoxide dismutases (SODs) catalyze the dismutation of $\text{O}_2^{\cdot -}$ into O_2 and H_2O_2 , followed by the catalase and peroxidase mediated decomposition of H_2O_2 to produce H_2O and O_2 [reviewed in (5, 63)]. Strikingly, many of these proteins require Fe or Cu and Zn as cofactors in the chloroplast and the cytosol (Fig. 4), or Mn in the mitochondria.

Comparative genomics and phylogenetic analyses of various classes of metalloproteins suggest that the use of the different metals by living organisms has evolved under the pressure of aerobic conditions on earth (94). Catalase, which is considered as an ancient metalloprotein, is found ubiquitously in all aerobic organisms. Catalase is a hemoprotein and therefore uses Fe as redox-active metal ion. Primitive SOD also used Fe as a cofactor (11, 54). When Fe availability decreased on earth, the use of Fe in SOD enzymes progressively evolved toward the alternative use of Mn. Phylogenetic data support the idea that the MnSODs originate from the FeSOD (4). However, the later emergence of a third category of SOD using both Cu and Zn is a biological novelty since the amino acid sequences of the Cu/ZnSODs are unrelated to the ones of the FeSODs and MnSODs (11, 54). The use of Cu as the redox-active metal in these novel SODs probably emerged in response to the rise in oxygen levels on earth and the consequent decline of bio-available Fe and Mn (75).

Iron and Copper Homeostasis in Plant Chloroplasts

Over the last decade, metal homeostasis research has focused on the identification of molecular factors involved in metal transport, distribution, chelation, and utilization in the model plant *Arabidopsis thaliana*, and more recently in crops and trees. This has been extensively reviewed elsewhere (14, 19, 44, 64, 67). In comparison to Fe, the cellular need for Cu is lower and Cu cofactor use is restricted to a relatively small set of proteins (19). The concentration of each metal in plant leaves illustrates these differential requirements (53). Under optimal growth conditions, Fe accumulates at around $50\text{--}100\ \mu\text{g}\cdot\text{g}^{-1}$ dry weight (DW) when Cu accumulates at $5\text{--}20\ \mu\text{g}\cdot\text{g}^{-1}$ DW in leaves of different plant species. At least half of the Cu is found in the chloroplast in green cells of *A. thaliana* (1, 78, 82). The major copper proteins are plastocyanin and cytochrome-*c* oxidase involved in the electron transport in the chloroplast and the mitochondria, respectively, and the Cu/ZnSODs that are found in the cytosol and the chloroplast in plants (Fig. 5). Other cuproproteins found in plants such as the chloroplastic polyphenol oxidases (PPO) and the extracellular laccases are also redox-active enzymes (Fig. 5) (19).

Fe is more widely utilized in plant redox biology and many of the Fe-dependent proteins still remain to be discovered. With the exception of the Mn-dependent oxygen evolving complex that provides the electrons from the water-splitting complex to PSII and Cu-dependent plastocyanin that carries the electrons from the cytochrome b_6/f complex and PSI, the photosynthetic electron transport machinery relies on Fe-based cofactors (57, 71). Therefore, Fe is indeed qualitatively and quantitatively the most important metal for photosynthesis (Fig. 2). Up to 80% of the cellular Fe is in the chloroplast (35, 78, 84). The PSII complex contains a polypeptide to which Fe is directly coordinated as well as a heme protein subunit. The cytochrome b_6/f complex contains two hemoproteins and a Rieske-type [2Fe-2S] cluster protein. The subunits PsaA, PsaB, and PsaC of the plant PSI are all [4Fe-4S] cluster proteins. The ultimate electron acceptor ferredoxin, which has diverse and crucial redox functions in the chloroplast, is also a [2Fe-2S] cluster protein (10). In total, 22 Fe atoms can be involved in a single electron transfer throughout a photosynthetic chain (71). Apart from this, Fe is ubiquitously used in oxidation-reduction processes (Fig. 1). For instance, the

FIG. 4. The enzymatic reactive oxygen species (ROS)-detoxifying mechanisms in plant chloroplasts are dependent on metal-based reactions. In plant chloroplasts, ROS homeostasis mainly relies on a core machinery in which metalloenzymes are pivotal (*triangle*). Fe- and Cu-dependent enzymes are depicted with black-lined symbols. The superoxide ion produced through the photosynthetic activity is converted into molecular oxygen and H_2O_2 by SODs. SODs found in plant chloroplasts are all dependent on Fe or Cu cofactors. H_2O_2 is then subsequently reduced to water (H_2O). The enzyme APX requires AsA and generates monodehydroascorbate (MDA). The enzyme glutathione peroxidase (GPX) requires GSH and generates glutathione disulphide (GSSG). The Fe-dependent APX is thought to be the major pathway used by plants for H_2O_2 removal. The catabolism of H_2O_2 through these cycles is described as the ascorbate-glutathione cycle. The regeneration of the reducing power (AsA and GSH) is allowed by the cyclic transfer of reducing equivalents (peripheric cycles). Within the chloroplast, MDA can be directly reduced to AsA by the MDA reductase (MDAR). MDA (two molecules) can also be spontaneously converted into AsA and dehydroascorbate (DHA). DHA is then reduced to AsA by the action of the DHA reductase (DHAR), using GSH as the source of reducing equivalents. The GSSG produced by both pathways is further reduced back to GSH using the NADPH as an ultimate electron donor, a reaction catalyzed by the glutathione reductase (GR). Oxidized and reduced forms for each molecule are indicated.

FIG. 5. Cu transport, delivery, utilization, and chelation in plant cell. The different proteins involved in Cu homeostasis are presented with respect to their membrane and organelle location. Rectangles indicate Cu-transporters and arrows Cu transport orientation. White circles represent Cu-chaperones, black circles Cu-utilizing proteins, and gray circles Cu-chelating proteins. COPT, copper transporter; HMA, heavy metal associated transporter; RAN, response to antagonist (= HMA7); PAA, P-type ATPase (PAA1=HMA6, PAA2=HMA8), ATX, antioxidant; CCH, copper chaperone; CCS, copper chaperone for superoxide dismutase; CSD, Cu/ZnSOD; ETR, ethylene receptor; COX, cytochrome *c* oxydase; LAC, laccase; AO, ascorbate oxidase; AAO, amine oxidase; ARPN, plantacyanin.

synthesis of chlorophyll, heme, and siroheme occurs in the chloroplast and is dependent on several Fe-proteins (55, 58, 60, 87). Nitrate and sulfur assimilation also rely on the use of Fe as redox cofactor in essential enzymes such as the nitrate reductase (heme) in the cytosol and nitrite reductase ([4Fe-4S]), glutamate synthetase ([3Fe-4S]), adenosine phosphosulfate reductase ([2Fe-2S]), and sulfite reductase ([4Fe-4S]) in the chloroplast (10).

With such a critical demand for Fe and Cu in the oxygen-producing chloroplast, photosynthetic organisms have evolved regulatory mechanisms that serve to constantly adjust the organism's physiology in order to optimize metal use. In addition, while metal toxicity is a constant challenge at the cellular and molecular levels, plants have to face low metal bioavailability in the rhizosphere (38). Plants therefore have systems that allow the increase of metal bioavailability

through soil acidification, reduction of the metal, or excretion of phytosiderophores [reviewed in (69)]. A common feature of plant responses to metal limitation is the upregulation of high-affinity transporters in concert with subsequent distribution mechanisms. Under metal excess, plants limit their metal uptake, induce the synthesis of metal-chelating molecules and proteins, and activate ROS detoxifying mechanisms (67).

How Plants Deal with Cu Scarcity: A Prioritization of Cu Toward Plastocyanin and a Metal-Switch to Maintain SOD Activity in the Chloroplast

The progressive discovery of almost all Cu-proteins in plants is important for our understanding of Cu homeostasis (Fig. 5) (19). In addition, we identified what probably is the majority of all Cu transporters and Cu chaperones. Further understanding of Cu homeostasis has been made possible by integration of genetic and biochemical data obtained for individual components into larger models *via* systems approaches, thus linking regulatory mechanisms and physiological responses. The identification and analyses of regulatory transcription factors from algae and plants indicate that copper homeostasis relies on highly conserved mechanisms and that the intracellular Cu levels are the main regulators of its distribution and use [reviewed in (19, 56)].

Plastocyanin, a copper-containing protein in the lumen of the thylakoids, is essential for photosynthesis in plants (92). Any environmental or genetic limitation of the delivery of Cu to the chloroplast decreases the plastocyanin content likely because the protein is less stable in the absence of Cu (1, 78). As a consequence, the electron transport rate throughout the photosynthetic apparatus is decreased under Cu deficiency. Under these conditions, ROS accumulation may occur in the

FIG. 6. Plant response to Cu deficiency: the "economy" model. In plants, Cu limitation triggers a large molecular remodeling that allows Cu to be preferentially allocated to PC in the chloroplast lumen. Central to this response is the conserved transcription factor SPL7 (for squamosa promoter binding protein-like 7) which is active under Cu limitation and which regulates almost all Cu responses in *Arabidopsis* (lower panel). SPL7 is required for the up-regulation of the expression of the plasma membrane COPT1 Cu transporter involved in the primary Cu uptake from the rhizosphere. SPL7 induces also the plasma membrane COPT6 Cu transporter in the shoot, likely involved in the Cu loading into the photosynthetic cells. Concomitantly, SPL7 induces the expression of the so-called Cu microRNAs, which in turn down-regulate transcripts encoding for most of the Cu proteins in various subcellular compartments (see text for details). This regulatory mechanism allows the prioritization of Cu use toward PC, which is not a target of the Cu microRNAs. Cu transport into the chloroplast lumen is also facilitated by the stabilization of the P-type ATPase PAA2 at the thylakoids under low Cu condition. This regulation is not dependent on SPL7. Therefore, this additional layer of regulation allows the chloroplast to control its own Cu allocation, independently to the other subcellular compartments. One major consequence of this Cu-microRNA-dependent economy model is the depletion of Cu/ZnSOD activity in both the chloroplast and the cytosol. Interestingly, an FeSOD is up-regulated by SPL7 and accumulates in the chloroplast to compensate for the lack of the Cu-requiring functional counterpart. FSD, FeSOD.

light because of photo-reduction of oxygen at PSII in the absence of downstream electron acceptors. In addition, photosynthetic electron transport is required for the maintenance of high NADPH/NADP⁺ and GSH/GSSG ratios and such a reduced state of the stroma is important for ROS scavenging systems such as glutathione dependent peroxidases. In the green alga *Chlamydomonas*, a heme-containing cytochrome *c₆* is expressed under Cu deficiency and serves to functionally replace plastocyanin while the latter is actively degraded (56). However, there is no evidence for compensation by a cytochrome *c₆*-homolog in higher plants (59, 78). Instead, plants prioritize Cu distribution toward plastocyanin (Fig. 6) (73, 82).

In plants, Cu homeostasis is regulated by the SPL7 (squamosa promoter binding protein-like) transcription factor that is active under Cu starvation (Fig. 6) (12, 93). In *Arabidopsis* important targets of SPL7 are the *COPT1*, *COPT5*, and *COPT6* genes. *COPT1* encodes for the high-affinity Cu transporter of the roots that is responsible for the primary uptake of Cu (76). *COPT6* is expressed in shoots and is located at the plasma membrane (40). Both are upregulated in Cu-deficient plants in order to increase the absorption capacity at a systemic level (*COPT1*) and more specifically in photosynthetic cells (*COPT6*) (40, 76). *COPT5* is also induced by Cu deficiency and is responsible for Cu efflux from the vacuole, suggesting its function in Cu remobilization (43). At the top of the Irving-Williams series, Cu binds very tightly to its targets (50). Therefore, any competing Cu sinks (*i.e.*, Cu-utilizing proteins) must be eliminated when Cu becomes limiting in order to allow the preferential Cu delivery to plastocyanin. In plants, this mechanism of "copper economy" involves the post-transcriptional regulation of dispensable Cu-enzymes by various microRNAs, which are themselves under the control of SPL7 (2, 93). Interestingly, transcripts encoding the essential Cu-proteins such as plastocyanin are not targeted for degradation by the microRNAs (2), suggesting that these proteins are priority targets for scarcely available Cu.

To date, five microRNA families have been shown to down-regulate dispensable Cu-proteins in plants: *miR397*, *miR398*, *miR408*, *miR857*, and *miR1444* (2, 52, 73). These microRNAs, which are up-regulated by Cu limitation, are known as the copper microRNAs [Cu-microRNAs; see (19)]. *MiR398* targets transcripts of the Cu/ZnSOD proteins as well as the transcript of copper chaperone for superoxide dismutase, the chaperone responsible for Cu delivery to Cu/ZnSOD [reviewed in (19)]. *MiR398* also targets one of two isoforms of COX5b, a subunit of cytochrome *c* oxidase in the mitochondria, but the relevance of this mild regulation by *miR398* is not understood (93). Three other families of miRNAs, *miR397*, *miR408*, and *miR857*, target transcripts of extracellular Cu-proteins such as laccases and a secreted plastocyanin-like protein, plantacyanin (2). The *miR1444* family targets the chloroplastic PPO (51, 52, 73). These Cu-microRNAs accumulate at extremely low Cu concentrations and disappear at higher Cu concentrations. This observation suggests that the Cu economy strategy is employed when the use of the few available Cu atoms has to be optimized (19). Both laccase and PPO are present in many copies in plants. Either 4 or 2 Cu atoms per monomer, respectively, are necessary for their activities. Cu/ZnSOD functions as a dimer and requires 1 Cu atom per monomeric subunit. The relative abundance of each Cu-protein in a plant cell remains to be determined, which limits any firm conclusion about the

magnitude of Cu-microRNA-mediated Cu pool savings. However, given the fact that Cu/ZnSOD and PPO are abundant proteins in the chloroplast (73, 95) at least under Cu sufficiency, it is likely that the absence of competition for Cu in this organelle, and potentially also at the cellular and systemic levels, gives an advantage to maturation of plastocyanin when Cu is deficient (Fig. 6). In this context, a recent study in poplar revealed that plastocyanin is the first protein to be recovered when Cu is re-supplied to previously Cu-deficient plants (73). Strikingly, the photosynthetic efficiency was quickly recovered after Cu-resupply, while the restoration of other Cu-dependent enzymatic activities, such as the Cu/ZnSOD or the PPO activities, was delayed for several days (73). These data lend strong support to the idea that Cu delivery to plastocyanin is indeed a priority and that the Cu-microRNAs serve to allow Cu economy, the optimal use of Cu when it is scarce.

While this economy strategy may be efficient for continued biomass production through photosynthesis, the depletion of the Cu/ZnSODs would at the same time have the potential to impact the cellular redox balance. Cu/ZnSODs are by far the most abundant both in the cytosol and the chloroplast under Cu-replete growth conditions. Other SOD enzymes in plants are the mitochondrial MnSOD and the plastidial FeSOD [reviewed in (68)]. In the chloroplast of mature plants, Cu/ZnSOD was the only SOD active at detectable level in native gel-assays in Cu-sufficient conditions (24). Interestingly, FeSOD accumulates in the chloroplast under Cu deficiency conditions (Fig. 6). The transcription of the corresponding gene is upregulated by SPL7 and therefore its expression is tightly coordinated with the loss of the Cu/ZnSODs (19). Likewise the metal-based switch between the plastocyanin and cytochrome *c₆* in *Chlamydomonas* (56), the synthesis of the FeSOD likely serves to compensate for the loss of the Cu/ZnSOD; however, there is so far little insight into the physiological relevance of the Cu-regulated SOD enzyme switch, because both Cu/ZnSOD and FeSOD seem redundant under various laboratory growth conditions [reviewed in (68)].

In addition to the preferential allocation of Cu to plastocyanin facilitated by the removal of competitive sinks, copper translocation into the thylakoids is also activated in response to Cu deficiency (Fig. 6) (82). Plastocyanin is a nuclear-encoded protein that needs to be translocated into the thylakoid lumen, prior to acquisition of its metal cofactor for maturation. Similar to the peptide, Cu has to be transported across two membrane systems in the chloroplast: the inner envelope of the chloroplast and the thylakoids (19). Two P-type ATPases (PAA) act as Cu transporters. PAA1/HMA6 and PAA2/HMA8 (HMA for Heavy-Metal Associated protein) are responsible for Cu transport through the inner envelope of the chloroplast and the thylakoids, respectively (1, 78). Upon Cu deficiency, the PAA2 transporter accumulates in the thylakoids. PAA2 half-life is increased by 2-fold when plants are grown on very low Cu concentrations similar to those that activate the SPL7 pathway (82). However, this PAA2 stabilization is not dependent on SPL7 since the regulation is still observed in the SPL7-deleted *Arabidopsis*. Instead, low Cu levels in the chloroplast could be the signal for PAA2 stabilization (82). The modulation of PAA2 accumulation, simultaneously with the SPL7-mediated responses, likely controls the flow of available Cu ions in the chloroplast stroma to the thylakoid lumen.

The available data support a model where regulatory mechanisms have evolved to largely respond to Cu limitation.

All the known Cu-specific regulatory mechanisms are activated at very low Cu concentrations ($<1 \mu\text{M}$ CuSO_4 in agar media or $<50 \text{ nM}$ CuSO_4 in hydroponics), which is far below the reported toxicity levels (2, 19). It is tempting to consider that Cu deficiency is more prevalent than Cu toxicity in nature. Most of the Cu-proteins are abundant under nonlimiting Cu conditions, but their activities are seemingly dispensable. These Cu-proteins may themselves act as Cu buffers when Cu is present at levels above the optimum. Interestingly, with respect to electron transport function plastocyanin accumulates at supra-optimal levels in plants grown under Cu-repleted conditions (3, 73). This suggests that plastocyanin might buffer excess Cu when Cu levels in the thylakoid lumen exceed the needs for electron transfer. Abundance of plastocyanin transcripts is not affected by high Cu concentration in the media (2). Therefore, a significant fraction of plastocyanin may be in the apo form and could readily bind excess Cu within the thylakoid lumen. Interestingly, the stabilization of PAA2 observed in response to low copper levels is abolished in *Arabidopsis* by plastocyanin depletion (82). This indicates that the presence of apo- and holo-plastocyanin, as an electron carrier or as a chelating protein, may be a prerequisite for loading of Cu into the thylakoid lumen in order to avoid nonspecific reactions of Cu.

How Plants Deal with Fe Toxicity: Ferritin's Function and Regulation and Interplay with ROS

The Fe proteome (ironome) is much larger than the Cu proteome. The identification of the complete ironome in plants is essential in order to get a much better view of Fe homeostasis. Another challenge for studies of Fe homeostasis is the lack of conservation of regulatory mechanisms in different kingdoms. In yeast and mammals, Fe homeostasis signaling involves Fe-S clusters because disruption of Fe-S cluster assembly systems disrupts the regulation of cellular Fe uptake and storage. In yeast, the master transcriptional regulator called activator of ferrous transport (AFT) shuttles from the cytosol to the nucleus, where it activates Fe-responsive genes. AFT activation is negatively regulated by Fe-S cluster-containing glutaredoxin complexes in the cytosol. In this fashion iron uptake in yeast is regulated in response to cytosolic Fe-S cluster availability, which in turn relies on the mitochondrial Fe-S assembly system (49). In animals, the iron responsive element-iron regulatory protein (IRE-IRP) system regulates at the post-transcriptional level Fe acquisition and storage. Mammalian IRP is a cytosolic form of aconitase, a 4Fe-4S cluster-binding protein (37). These IRE-IRP systems are not conserved in plants and the involvement of Fe-S cluster proteins in Fe status sensing is not yet demonstrated. Therefore, molecular studies in plants have first focused on the identification and biochemical characterization of individual components of Fe homeostasis, and the analysis of their regulation. Because of its major consequences for the world's feedstock quality and quantity, plant responses to Fe deficiency have received most attention. Major progress has been made in the identification of the high affinity transport systems responsible for Fe uptake from the soil as well as in the identification of the signaling pathways responsible for their induction upon Fe deficiency [for a recent review, see (44)]. Long distance signaling events are involved in this control. Among the potential signal molecules involved in Fe

homeostasis, the phytohormone ethylene as well as the free radical nitric oxide (NO) and derived nitroso- and glutathione complexes are receiving particular attention (30, 70). Ethylene and nitric oxide have the potential to signal over longer distances and might therefore help to communicate the need for iron in distant plant parts.

Fe is abundant in plant cells and is widely implicated in redox machineries. However, Fe is a relatively "labile" co-factor when compared to Zn and Cu and free iron is highly reactive and toxic. Thus, iron homeostasis has to be tightly controlled. Among the transition metals, Fe is the only one that required the evolution of a dedicated storage protein called ferritin. Ferritin is almost ubiquitously found in all kingdoms of life with the exception of some fungi, including yeast. Yeast tackles Fe toxicity using the vacuole as a storage compartment (48). In plants also, Fe storage in the vacuole is emerging as an important component of Fe homeostasis as shown by the involvement of the tonoplast-located VIT1, NRAMP3, and NRAMP4 transporters in Fe storage and mobilization during the development of *Arabidopsis* (42, 45). However, ferritin is found from bacteria to humans and higher plants and has important physiological functions (16, 85). The recent studies on ferritin function and regulation in plants have revealed a tight interplay between these proteins, the efficient use of Fe, and the protection against Fe-mediated oxidative stress (Figs. 7 and 8) (6, 72, 74).

FIG. 7. Ferritin is important for the redox balance in the chloroplast and allows plants to benefit from increased Fe bioavailability. Fe is considered as a limiting factor in most soils for plant growth. The supply of a bioavailable source of Fe to the soil increases the growth of *Arabidopsis* plants. The absence of ferritin does not lead to any macroscopic phenotype when grown in soil containing low Fe (– Fe), yet the lack of Fe chelation leads to an increase of ROS levels. The mutant can circumvent oxidative damage by increasing ROS detoxifying enzyme activities. By contrast, under high Fe condition (+ Fe), while the wild-type takes advantage of the available Fe, the ferritin mutant undergoes severe oxidative stress. The ROS detoxifying mechanisms are overwhelmed and are not capable anymore of preventing ROS toxicity. Consequently, pleiotropic defects both in vegetative and reproductive organs are observed in the mutant, including a reduced carbon assimilation that ultimately results in a decreased biomass. Thus, the beneficial effect of the high Fe availability fully relies on the presence of ferritin in the chloroplast.

FIG. 8. A model for ferritin regulation: the interconnections between Fe and ROS signals. Ferritin is regulated at the transcriptional level by iron (Fe) through a *cis*-element iron dependent regulatory sequence (IDRS), which is necessary for the repression of the plant ferritin gene under low Fe condition. This pathway involves the accumulation of nitric oxide (NO) in the chloroplast. The ferritin gene expression is also controlled by H_2O_2 at the transcriptional level, but this regulation is independent on the IDRS. This suggests that two different pathways lead to the accumulation of ferritin transcripts in response to Fe and H_2O_2 . Ferritin expression is also controlled at the post-transcriptional level by Fe and H_2O_2 . Fe and H_2O_2 promote the ferritin mRNA decay few hours after the accumulation of the transcripts. This additional layer of regulation likely constitutes a feedback control allowing the plants to prevent overproduction of the ferritin transcript. Ultimately, the storage of Fe in the newly synthesized ferritin decreases the free Fe present in the chloroplast, leading to the arrest of the transcription and the re-stabilization of the transcript. This multilayered regulatory network allows a tight control of ferritin synthesis, with respect with the presence of Fe and the production of ROS.

Ferritins are macromolecular complexes made of 24 subunits that form a cage-like structure in which up to 4500 Fe atoms can be stored in a nonreactive form. The Fe storage mechanism is based on two intrinsic catalytic activities of the peptide, (i) the ferroxidase activity that first oxidizes Fe^{2+} in Fe^{3+} , and then (ii) the nucleation of the Fe^{3+} in a mineral core in which Fe is associated with phosphate (90). In mammals, ferritins are located in the cytosol and constitute a pool of Fe required for early development (86). They are also expressed in the mitochondria of specific cell types where they protect against oxidative stress (47). Recent studies in both algae and plants have revealed that, in these species, ferritin does not constitute a major iron pool, but is solely involved in the protection against Fe-mediated oxidative stress (20, 72). Ferritins are thought to localize mainly to the chloroplast in plants, but low levels are detected in the mitochondria as well (83). Ferritins accumulate when plants are grown on high Fe concentrations [reviewed in (15, 16)]. Their involvement in Fe chelation to prevent its reactivity with oxygen has been proposed (13). Indeed, recent characterization of *Arabidopsis* possessing single, double, triple, and quadruple disruptions of ferritin genes showed that the levels of ROS and the activity of the ROS-detoxifying enzymes are elevated in the absence of ferritin (Fig. 7) (72). Notably, among the increased enzyme

activities are catalase and ascorbate peroxidase (APX), both Fe-dependent activities. Strikingly, while control plants grew faster when the soil was supplemented with Fe, suggesting that Fe is limiting, the growth of the ferritin-less mutant was reduced (Fig. 7) (72). This result illustrates that ferritins are required to take advantage of Fe bioavailability, while avoiding its toxicity. The absence of ferritin had a strong impact on the carbon fixation rate, but had no effect on the photosynthetic electron transport itself (72). Therefore, the observed reduction of biomass in the ferritin-less mutant likely results from the effects of the Fe-mediated oxidative stress on the subsequent carbon fixation reactions, and not from a direct oxidation of the photosystem components. Other Fe toxicity-related phenotypes are observed in the seed and the flower of the ferritin mutants (72), suggesting that ferritins not only are important for photosynthesis in green tissues but also for versatile functions in different cell types in plants.

When ferritins are experimentally overexpressed in plant cells, two effects are observed. First is the induction of the Fe acquisition system because the chelation of Fe at higher than desired levels likely alters the proper delivery of Fe to the metalloproteins (89). Second is the activation of the ROS-detoxifying mechanism. Illegitimate Fe accumulation resulting from the overexpression of ferritin in the chloroplast leads to an increase of various peroxidase, catalase, and glutathione reductase activities (13). These plants show phenotypic signs of Fe excess and chloroplast structure alterations (13). Therefore, both the absence and the overaccumulation of ferritins impair the redox balance (13, 89). Because the proper synthesis of ferritin is a key feature of the control of Fe homeostasis, the mechanism controlling ferritin expression has been extensively studied. In animals, ferritin synthesis, together with the Fe acquisition systems, is regulated at the post-transcriptional level in response to the cellular Fe levels and oxidative stress. This regulation involves the IRP/IRE system (37). In plants, this regulatory mechanism is not present (7) and ferritin accumulation in response to Fe is primarily controlled at the transcriptional level through a *cis*-element, called iron-dependent regulatory sequence (IDRS) present in the promoter region of some ferritin genes (Fig. 8). This sequence is necessary for the repression of the ferritin synthesis under low Fe conditions (65). Thus, ferritin accumulation in response to Fe addition to the media results from the de-repression of the transcription of the ferritin gene. Interestingly, an accumulation of $NO\bullet$ in the chloroplast is detected already a few minutes after Fe treatment and this $NO\bullet$ burst is necessary for the de-repression of the ferritin synthesis (6). The $NO\bullet$ -mediated de-repression is dose dependent and active even when $NO\bullet$ is artificially produced in plant cell in absence of Fe, suggesting that $NO\bullet$ acts as a downstream signaling molecule in the pathway (Fig. 8) (6, 61). Oxidative signals are definitively in the signal transduction pathway since extracellular treatments with antioxidant such as N-acetylcysteine and glutathione abolish ferritin accumulation (31, 77). While the source of the $NO\bullet$ production in the chloroplast is still under investigation, it seems dependent on the glutathione levels (88).

H_2O_2 also triggers the increase of ferritin transcripts in plants (66, 77). The pathway by which H_2O_2 activates ferritin transcription is independent of the IDRS-mediated Fe-induced pathway described above (6). Co-treatment with Fe

and H_2O_2 revealed an additive accumulation of ferritin transcripts, relative to the accumulation observed for individual treatments (6). Therefore, two synergistic pathways involving specific oxidative signals lead to ferritin transcript accumulation (Fig. 8). However, H_2O_2 only resulted in a mild ferritin accumulation at the protein level, while Fe triggered a strong ferritin accumulation. Fe incorporation into ferritin is also an important determinant of its stability (46). Therefore, the lack of Fe may prevent ferritin protein accumulation in response of H_2O_2 . In the absence of free iron ions, H_2O_2 is not nearly as dangerous for the cell when compared to the presence of free Fe that can promote the Haber-Weiss cycle (5). Therefore, it is tempting to hypothesize that the H_2O_2 -induced transcription of ferritin genes serves as a mechanism to prepare cells so that they have increased Fe sequestering capacity in an oxidizing environment.

Fe regulates ferritins along with other ROS-detoxifying systems. In some plants, a Cu/ZnSOD transcript accumulation in response to Fe has been reported (81). This also occurs in response to high light, other metals, and various oxidative stresses [reviewed in (68)]. However, due to the very large effect of Cu on their expression, each of these conditions previously shown to affect Cu/ZnSOD expression should be revised under controlled Cu nutrition conditions. An unambiguous Fe-responsive protein is the cytosolic enzyme ascorbate peroxidase APX1 (28). Similar to SOD, APX1 is also regulated by high light (41). The effect of Fe on APX1 promoter activity has been clearly documented at the molecular level (28). Interestingly, this APX member is located in the cytosol and the expression of the chloroplastic counterparts is not affected by Fe (28). It is now accepted that ROS detoxification relies on the concerted action of different cellular compartments especially in the case of H_2O_2 which is known to diffuse through the biological membranes (34). Interestingly, the expression of APX in response to Fe and high light is also dependent on the glutathione level (28, 41). Ferritins are also transcriptionally regulated by the circadian clock, and this regulation is important for Fe homeostasis in *Arabidopsis* (26). Given the dramatic effect of the daily light oscillations on chloroplast metabolism and on the abundance of the Fe-proteins in the plastid, it is likely that the clock-dependent ferritin regulation serves to anticipate the potential Fe reactivity during transitions from dark to light.

Ferritins are also regulated together with other Fe-responsive proteins at the post-transcriptional level (Fig. 8) (74). Surprisingly, this regulatory mechanism decreases the mRNA stability of some early Fe-induced genes. This apparent paradox was explained by kinetic studies: this additional level of regulation by mRNA turnover, together with the activation of transcription, allows some proteins to accumulate only transiently in response to Fe or H_2O_2 . Both Fe and H_2O_2 signaling involve an oxidative step, since treatments with antioxidants abolished both the Fe- and the H_2O_2 -mediated ferritin mRNA destabilization (74). Thus, the existence of two independent pathways is not yet established. Interestingly, a transcriptomic study showed that, next to ferritin, most of the other genes affected by this regulation are transcription factors. Among these Fe-induced transcription factors, some are important for protection against oxidative stress in plants (22, 23). Transient expression of these transcription factors is pivotal for their proper function in gene regulation. In the case of ferritin, this regulation likely constitutes a feedback mech-

anism preventing the deleterious effects of the excessive accumulation of the protein in plant cell. At the molecular level, this mRNA degradation process relies on the ribo-nucleolytic activity of the previously identified mRNA destabilization (DST) machinery (62). Indeed, two mutants affected in the mRNA degradation machinery (39) showed an over-accumulation of ferritin mRNA and other target transcripts and as a consequence, the plant response to oxidant and pro-oxidant treatment such as Fe and high light was impaired, and photosynthesis and plant growth was decreased (74).

Therefore, ferritins are at the heart of the Fe-oxygen chemistry in the chloroplast. They act as fine regulators of the free Fe in the chloroplast, providing the most advantageous balance between Fe use and sequestration. Both the protein and its mode of regulation are intimately interconnected with oxygen-derived molecules (Figs. 7 and 8). In this context, ferritins definitively function as homeostatic proteins. Loss of ferritin regulation or function results in the activation of oxidative stress responses. The next challenge is to understand the significance of these oxidative stress responses. What is really metal toxicity: what is specific to a metal and what is due to a general oxidative stress response? The interplay between metals and ROS make it challenging to tease apart cause and effect. *In vitro* studies are also hard to interpret because of the difficulty to reproduce the cellular redox environment. A major barrier to tackle is the identification of the molecular actors responsible for ferritin responses, this will require the identification of transcription factors, for instance through yeast one-hybrid experiments, as well as identification of the sensors that signal ferritin transcript turn-over, which might require a genetic approach. Physiological and pharmacological experiments (6, 61, 72) as well as the recent progress in cellular imaging of ROS using specific probes (6, 72) can provide information concerning the nature of the ROS involved. However, genetic approaches are now required to further investigate the primary signals responsible for the sensing of Fe. The identification of the source of $NO\bullet$ production in response to Fe in the chloroplast, as well as the identification of the protein involved in Fe-induced decay of ferritin mRNA, would be an excellent entry point. This would lead to further researches toward the discovery of the Fe sensor and signaling pathway(s). The integration of these Fe-signals in the oxidative stress response network should ultimately allow a better understanding on the interplay between Fe and oxygen.

Conclusion and Outlook

Photosynthetic organisms frequently grow faster when Fe is supplied to their growth site, indicating that this micronutrient is limiting in their native habitat (18, 72). Cu deficiency is rare in nature, but in agricultural systems Cu supplementation can improve crop yields (19). An explanation for the beneficial effect of Fe feeding is that the high requirement for Fe is thwarted by its poor bioavailability in most of the arable soils. Classic symptoms of Fe and Cu deficiencies include reduction in vegetative biomass, chlorosis, decreased photosynthetic activity, defects in plant morphology and seed production, or in the case of Cu deficiency lodging of cereals. Deficient plants are also, directly or indirectly, more susceptible to various stresses (drought, diseases, pathogens). Metal toxicity is not as prevalent in nature.

However, plants that are grown in flooded fields such as rice, which represents an important part of the staple food worldwide, are particularly prone to accumulate Fe in excess, as well as toxic metals such as cadmium.

The importance of Fe in plant biology makes it a challenging ion to study. Constant progress is made with inputs from molecular, biochemical and phenotypic analyses yet we still have a very elusive picture of Fe homeostasis. A particularly understudied field is Fe utilization by plant cells. In a context of Fe-limiting conditions, prioritization of the use of the metal has to be orchestrated. A minimal activity of essential metabolisms such as respiration and photosynthesis is required for plant survival. The depletion of the Fe-dependent ROS detoxifying mechanisms, simultaneously with the progressive imbalance of the metabolism that leads to the production of ROS, must be limited to avoid cell death. Therefore, the integrated approaches that led to a nearly complete understanding of Cu homeostasis in plants, if applied to Fe, should provide new insights into Fe utilization by plants.

An important remaining challenge for Cu homeostasis is the discovery of the biological function of several Cu-utilizing proteins. Among them, SOD should deserve most of the attention. Despite the numerous functions attributed to this protein, we have to admit that the phenotypic and molecular characterization of mutants virtually depleted of SOD activity in the chloroplast and the cytosol belies all biology textbooks because such mutants do not show dramatic phenotypes even under stress conditions. These SOD have been conserved in plants during evolution and the switch between the Fe and Cu dependent SOD in plants gives rise to a strict requirement of SOD activity maintenance in the chloroplast. The extracellular laccases form an important Cu-protein family with poorly understood biological function. Some laccase family members (3 out of 17 in *Arabidopsis*) have been implicated in cell wall formation and remodeling, but the majority of the laccases have not been characterized functionally. Homeostatic processes allow immobile plants to respond and acclimate to environmental stresses. Molecular remodeling triggers physiological adaptation in order to cope with challenging conditions. While molecular studies can elucidate the regulatory mechanisms that control these changes in response to metal availability, pivotal will be the identification of metal sensing mechanisms that are so far poorly defined in plants.

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Abbreviations Used

AAO = amine oxidase
AFT = activator of ferrous transport
AO = ascorbate oxidase
APR = adenosine 5'-phosphosulfate (APS) reductase
APX = ascorbate peroxidase
ARPN = plastocyanin
AsA = ascorbate (reduced form)
ATX = antioxidant
CCH = copper chaperone
CCS = copper chaperone for superoxide dismutase
CHL27 = chlorophyll deficient 27 (=CRD1: copper response defect 1)
COPT = copper transporter
COX = cytochrome *c* oxidase
CSD = Cu/ZnSOD
Cyt = cytochrome

DHA = dehydroascorbate
DHAR = DHA reductase
DST = destabilization element
DW = dry weight
ETR = ethylene receptor
FC = ferrochelatase
FD = ferredoxin
FER = ferritin
 $F_X/F_A/F_B$ = electron acceptor X/A/B of photosystem I
GOGAT = glutamine oxoglutarate aminotransferase (=glutamate synthase)
GPX = glutathione peroxidase
GR = glutathione reductase
GSH = glutathione (reduced form)
GSSG = glutathione disulphide
HCAR = 7-hydroxymethyl chlorophyll a reductase
HMA = heavy metal associated transporter
IDRS = iron dependent regulatory sequence
IRE = iron responsive element
IRP = iron regulatory protein
LAC = laccase
LLS1 = pheophorbide a oxygenase
M = metal
MDA = monodehydroascorbate
MDAR = MDA reductase
NFU = "nitrogen fixing bacteria" (Nif) U-like proteins
NiR = nitrite reductase
NO = nitric oxide
NRAMP = natural resistance-associated macrophage protein
PAA = P-type ATPase
PC = plastocyanin
Pheo = pheophytin
PPO = polyphenol oxidase
PQ = plastoquinone
PSI = photosystem I
PSII = photosystem II
Q = quinone
RAN = response to antagonist (=HMA7)
ROS = reactive oxygen species
SIR = sulfite reductase
SiRB = sirohydrochlorin ferrochelatase B
SOD = superoxide dismutase
SPL7 = squamosa promoter binding protein-like 7
SUF = sulfur limitation proteins
TIC55 = translocon inner membrane complex 55
VIT1 = vacuolar iron transporter 1