Plant Science 181 (2011) 582–592

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Nitric oxide, nitrosyl iron complexes, ferritin and frataxin: A well equipped team to preserve plant iron homeostasis

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ARTICLE INFO

Article history:
Received 28 January 2011
Received in revised form 12 April 2011
Accepted 13 April 2011
Available online 21 April 2011

Keywords:
Ferritin
Frataxin
Iron nutrition and metabolism
Iron homeostasis
Nitric oxide
Nitrosyl iron complexes

ABSTRACT

Iron is a key element in plant nutrition. Iron deficiency as well as iron overload results in serious metabolic disorders that affect photosynthesis, respiration and general plant fitness with direct consequences on crop production.

More than 25% of the cultivable land possesses low iron availability due to high pH (calcareous soils). Plant biologists are challenged by this concern and aimed to find new avenues to ameliorate plant responses and keep iron homeostasis under control even at wide range of iron availability in various soils. For this purpose, detailed knowledge of iron uptake, transport, storage and interactions with cellular compounds will help to construct a more complete picture of its role as essential nutrient. In this review, we summarize and describe the recent findings involving four central players involved in keeping cellular iron homeostasis in plants: nitric oxide, ferritin, frataxin and nitrosyl iron complexes. We attempt to highlight the interactions among these actors in different scenarios occurring under iron deficiency or iron overload, and discuss their counteracting and/or coordinating actions leading to the control of iron homeostasis.

1. Nitric oxide is involved in plant responses triggered by iron deficiency

Interveinal chlorosis is the most typical symptom in plants growing under iron deficient conditions and it is caused by a reduction of the pigments and proteins in the photosynthetic apparatus with consequent decrease in the photosynthetic rate. Also, the chlorosis caused by reduced solubility of iron in calcareous soils is a major agricultural concern, since it is responsible for diminished crop yields worldwide. Thereby, agronomical and biotechnological approaches aimed to improve plant iron uptake constitute a challenging issue in plant science.

Nitric oxide (NO) is a lipophylic diatomic gas under atmospheric conditions generated by bacteria, plants and animals. NO has a small radius and its neutral charge allows rapid membrane diffusion. NO stability and decay depend on its concentration, the redox status of the system and the concentration of its target molecules. NO entered the chemical hall of fame in 1992, when Science Journal named NO as the molecule of the year, followed by Nobel Price award to R.F. Furchgott, L.J. Ignarro, and F. Murad for their research on NO as signal in the cardiovascular system. In plants, one of the first roles attributed to NO was in plant–pathogen interactions [1,2]. After those works, different research groups demonstrated that NO is involved as a signal in several growth and developmental processes, as well as in the plant response against biotic and abiotic stresses [3,4]. Nonetheless, several crucial questions about NO biosynthesis in plants are still open [5] which are, however, beyond the scope of the present work.

Graziano et al. [6] showed for the first time that treatment with the NO donor sodium nitroprusside (SNP) prevented the typical interveinal chlorosis in maize plants grown under iron deficiency. The same beneficial effect was observed when iron deficient maize plants were treated with another NO donor, S-nitroso-N-acetylpenicillamine (SNAP) or with a gaseous NO-enriched air. Interestingly, the reversion of the interveinal chlorosis correlates with significant increases of the chlorophyll content and growing rate of the NO-treated iron deficient maize plants [6]. NO treatment was able to rescue normal growth of maize plants supplied with very low iron concentration in the nutrient solution (10 μM Fe-EDTA), which would otherwise not survive [6].

It is known since years that iron deficiency profoundly affects the formation, in the chloroplasts, of the light-harvesting pigments and electron transport chain. Also, electron microscope studies have shown that iron deficiency markedly reduces the number of grana and stromal lamellae in chloroplasts [7]. On the reverse, grana stacking and ultrastructure of mesophyll chloro-

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doi:10.1016/j.plantsci.2011.04.006

plasts from iron deficient maize plants appeared similar to that of control plants when treated with NO donor [6]. Also, the transcript abundance of the *RBCL* (RuBisCO large subunit) and *PSBA* (D1 protein) chloroplast-encoded genes in NO-treated iron deficient maize plants attained the level observed in plants growing under iron sufficiency [6].

Treatment with the NO donor SNP was even able to revert intervenal chlorosis in two maize mutants *yellow stripe1* (*ys1*) and *yellow stripe3* (*ys3*) which are defective in iron acquisition mechanisms thus displaying interveinal chlorosis under iron sufficient conditions [6]. *Yellow stripe1-like 15* (*YSL*15) genes in rice are supposed to play a crucial role in iron homeostasis during the first stages of growth [8]. OsYSL15 has a role in uptake and distribution of iron in rice plants [9]; *oysl15* rice KO mutants show, under iron deficiency, a more pronounced chlorotic phenotype than control plants but such chlorosis could be prevented upon SNP treatment [9]. A key observation for understanding the role of NO in the plant responses triggered by iron deficiency is that the NO donor SNP improves internal iron availability and it increases the Labile Iron Pool (LIP) in sorghum embryonic axes [10] without increasing total iron content [6,10]. It is known from literature that increased expression of iron-uptake related genes is not sufficient to confer a noticeable enhancement of iron content when plants are grown under conditions of iron deprivation, NO might thus resolve iron chlorosis by either (i) increasing the availability of endogenous iron or by (ii) facilitating the delivery of iron for chlorophyll synthesis through the formation of mononitrosyl iron complexes (MNICs) and dinitrosyl iron complexes (DNICs). The formation and role of nitrosyl iron complexes in plant iron metabolism will be deeply discussed later.

2. Nitric oxide as a signal molecule mediating iron deficiency responses

As stated above, the effects of the exogenously applied NO all suggested that endogenous NO should play indeed a major role in the plant responses to counteract iron deficiency. NO is rapidly produced in the root epidermis of tomato plants growing in iron deficient conditions, indicating that endogenous NO might be involved in responses to iron deficiency. Experimental evidence demonstrated that NO increase is required for the expression of genes associated to iron uptake in roots, the basic helix-loop-helix transcription factor *LeFER*, the ferric-chelate reductase, *LeFRO1*, and the Fe(II) transporter, *LeIRT1* [11]. FER is believed to be a transcription factor that positively regulates *LeFRO1* and *LeIRT1* gene expression, both proteins FRO and IRT1 are postulated to function together for iron uptake from the soil [12]. Additional evidence also showed that NO treatment resulted ineffective in inducing iron deficiency responses in the tomato *fer* mutant, indicating that FER is necessary to mediate the effect of NO [11].

Recently, it was shown that Cd^{2+} induces NO production both in roots and leaves of Arabidopsis, and that NO contributes, in turn, to the Cd^{2+}-dependent root growth inhibition [13]. In that work, a correlation was found between the ability of NO to promote Cd^{2+} accumulation and *IRT1* up-regulation in roots, suggesting that NO contributes to Cd^{2+} toxicity. It has been also shown that zinc treatment triggered an increase of NO production within several hours in roots of *Solanum nigrum* seedlings [14]. This increase in NO concentration would be involved in the induction of programmed cell death (PCD) in root tips exposed to excess of zinc. PCD would be a response to long-term zinc toxicity by modulating root system architecture and subsequent adaptation to zinc stress [14]. The authors propose a model in which NO contributes to rapid ROS accumulation and subsequent PCD in root tips in response to heavy metal-induced stresses and in which NO could be an important regulator of Zn-modulated root system architecture.

More evidences supporting a role for NO regulating iron deficiency responses were provided by Jin et al. [15]. The authors showed that elevated atmospheric carbon dioxide (CO_2) concentrations can enhance plant growth and change their nutrient demands when plants grown in iron deprivation. In addition, they prove that NO levels in the roots increase in iron limited plants and these levels were higher in response to elevated CO_2. These results suggest that the adaptive responses induced by elevated CO_2 and iron deficiency may be downstream regulated by NO.

NO coordinates many different signalling pathways in a concerted way with hormones [3]. Very recent reports highlighted the close relationships between hormone and NO actions in plant responses to iron deficiency.

Microarray analysis allowed the identification of several genes up-regulated by iron deficiency whose induction was ethylene-dependent; such genes were named iron deficiency-induced ethylene-dependent (IDED). The identified IDED genes have key roles in iron acquisition and homeostasis. Interestingly, GSNO (nitrosogluthatione, a natural compound that serves as NO precursor and storage in plants) up regulates all the identified iron-related IDED genes, indicating that the iron-related genes induced by ethylene are also responsive to NO [16].

The interaction between auxin and NO signalling was investigated in Arabidopsis plants growing under iron deficient conditions [17]. In the auxin insensitive mutant *aux1-7*, NO levels in roots were only slightly induced by iron deficiency, whereas exogenous NAA (a permeable auxin analog) application failed to induce additional NO accumulation and ferric chelate reductase activity in roots of *noa1* and *nia1nia2*, two mutants with reduced NO synthesis. Moreover neither iron deficiency nor NAA application stimulated the gene expression of *FRO2* and *FIT* in *noa1* or *nia1 nia2* mutants. These results would suggest that during iron deficiency, auxin acts upstream of NO in regulating the gene expression of *FRO2* and *FIT* in roots [17].

Overall, the results suggest that NO could be working as a general signal implicated in the regulation and coordination of the signalling network leading to physiological, biochemical and morphological adaptive changes to iron deficient conditions [18]. However, the identity of the molecular NO targets and precise mechanisms underlying the NO action during regulation of plant iron deficiency responses are still unknown. The different redox species of NO (nitrosonium cation, NO^+; nitroxyl anion, NO^- and NO radical, NO) exhibit different chemical properties and interconversion of these different species can occur, according to the redox state of their environment. Proteins can be modified by NO through reactions with different amino acids or prosthetic groups. The main NO-associated protein modifications in the biological context are the covalent modifications of cysteine (*S*-nitrosylation), tyrosine (tyrosine 3-nitration) residues and NO binding to transition metals (metal nitrosylation) [3,19]. These post-translational modifications are probably at the core of the molecular NO-dependent mechanisms leading to enhance plant responses to cope against iron deficiency.

3. Is nitric oxide contributing to compensate redox modifications generated by imbalance of iron levels?

Interestingly, the NO-mediated reversion of chlorosis in maize and rice plants was not correlated with an increase in iron concentration in the whole plants and, consequently, it was suggested that NO effect could be attained by improving the internal iron availability inside the plants [6,9,20].

In biological systems iron exists as ferrous(II), ferric(III), and ferryl(IV) states. It is required for a variety of iron-containing proteins, and utilized as catalyst at the active site of enzymes involved in oxygen metabolism, as well as electron transport. A percentage of cellular iron is sequestered in a poorly or non reactive form in storage proteins. Indeed, when iron is present in the form of low molecular compounds, iron has the potential to act as a prooxidant for the formation of reactive oxygen species or act as a redox signal molecule [21]. However, it has also been reported that nicotianamine and phytochelatin prevent Fenton reaction by sequestering iron [22].

The reactions of NO with iron include the direct reaction of NO with metal centers, the interaction with oxocomplexes and the reaction with high valent metal complexes [23]. The reaction of NO with transition metals will be further detailed in the section 'Formation of nitrosyl iron compounds'. Even though NO binds iron-proteins, it is relatively inert to direct one electron reduction processes that can occur under physiological conditions [24], leading to the formation of NO^- (nitroxyl anion). On the other hand, the oxidised form NO^+ (nitrosonium cation) is a key species in the process of nitrosation in which the NO^+ group is transferred from a carrier compound to a nucleophilic center. One important biological carrier of NO^+ is S-nitrosoglutathione (GSNO). In addition, nitrosyl complexes, for instance SNP, may be effective nitrosating agents at physiological pH, particularly towards sulfur centers. Similar nitrosyl complexes may be formed intracellularly by reaction of NO with iron-centers [25].

Once the iron has been taken by roots from the environment, it is distributed to different organs and tissues, and then the intracellular iron is compartmentalized. Iron must cross several cellular membranes to reach its final destination. The uptake and transport of iron in plants require particular processes such as its chelation or reduction because ferric iron has a very low solubility [26]. An important iron proportion remains insoluble in the apoplast. The iron reduction to ferrous state is a prerequisite for iron transport across the plasma membrane in root cells and the enzyme involved is a FRO whose activity has been postulated to be pH-regulated [27]. However, it has been also suggested that ROS or high intensity of blue light may also contribute to the obligatory reduction of ferric iron prior to uptake [28]. The complex redox chemistry of NO, which is dynamically associated to changes in the redox state, is hypothesized to provide a general mechanism contributing to keep cell redox homeostasis [3,29]. In this context, NO could be an important regulator of ferrous iron availability in tissues [20].

It has been demonstrated that diverse environmental stresses and iron starvation led to dramatic down regulation in the expression of the chloroplast iron–sulfur protein Ferredoxin (Fd) [30–32]. Fds are ubiquitous [2Fe–2S]-proteins involved in many different electron transfer pathways in plants, animals and microorganisms [33]. In a novel biotechnological approach for improving plant fitness under adverse environmental conditions, the physiological role of Fd was replaced by expressing an isofunctional protein, a cyanobacterial flavodoxin (Fld) in chloroplasts [31,32]. Fld is absent in plants but it showed to be able to transfer electrons to Fd-target proteins. Transgenic tobacco and Arabidopsis lines expressing Fld in chloroplast but not in cytosol showed a wide-range tolerance to biotic and abiotic stresses including iron deficient conditions [31,32,34]. Transgenic lines expressing Fld in chloroplast are significantly more tolerant to growth in iron limited media avoiding chlorophyll breakdown and chlorosis. Interestingly, these transgenic plants behaviour is reminiscent of those iron deficient plants treated with NO donors and it could be indicating the similarities of mechanisms and targets underlying Fld and NO actions. Several questions emerge from these observations: (i) has NO a possible role in improving activities of the redox-sensitive Calvin–Benson cycle enzymes? Some key enzymes involved in photosynthesis, including the Calvin–Benson cycle, have been indeed identified as targets for S-nitrosylation in Arabidopsis [35]. (ii) Are the transgenic plants expressing Fld producing more NO? (iii) Since Fd donates electrons for nitrite reductase, is there a possible link between Fe–Fd and nitrite reductase-dependent NO production?

Several reports demonstrated the NO-mediated effect preventing photo damage and photo inhibition in ROS-exacerbated situations. The bipyridinium herbicide diquat is responsible for the photooxidative damage caused by a break in the electron transport chain in chloroplasts and an increased production of superoxide ($O_2^{-\bullet}$) and hydrogen peroxide (H_2O_2). Under diquat treatment, potato plants sprayed with the NO donor SNP showed a restored photosynthetic electron transport rate. In addition, the level of D1 protein was drastically reduced in membrane fractions of intact chloroplasts incubated with diquat and rapidly restored to control levels in presence of NO [36]. Similar antioxidant effect of NO was reported by Sun et al. [37] in plants growing under iron deficiency. It was shown that H_2O_2 and $O_2^{-\bullet}$ generation was increased in iron deficient maize leaves but SNP treatment significantly reduced the ROS level [37]. All these observations point to a possible role of NO in contributing to adjust the cell redox balance in plant growing under iron deficient conditions.

4. Nitric oxide is involved in keeping iron excess under control

Ferritins are iron storage proteins involved in iron homeostasis of almost all living organisms, from bacteria to mammalian cells, with the exception of yeast [38–41].

In eukaryotic cells, ferritins are formed by 24 subunits assembled in a cage like structure in which iron can be stored in safe and bioavailable form, thanks to their ability to react with Fe(II) and to induce its oxidation by ferroxidase activity. Ferritins can release iron upon cellular demand [39]. The reaction of Fe(II) oxidation is followed by nucleation and growth of the mineral iron core. Plant ferritin core has high phosphate content and is amorphous, differently from the human and horse ferritin consisting of a crystal of ferrihydrite [41]. Another major difference between mammalian and plant ferritin is that localization of the former is mainly cytoplasmic [39] whereas the latter is mainly chloroplastic. Furthermore, ferritin can be also localized in mitochondria of both cell types [42–46].

Arabidopsis thaliana possesses 4 ferritin isoforms, *AtFer1-4* whose expression is environmentally and developmentally regulated [40,45–52]. Beside iron excess, different oxidative conditions (ozone, photoinhibition, wounding) can induce plant ferritin expression due to the necessity to keep Fenton reaction under control when ROS production increases [48,49]. Indeed, it has been recently demonstrated that the major role of Arabidopsis ferritin is not to function as iron reservoir but to limit oxidative damage by protecting against free iron toxicity [51].

The interplay between ferritin metabolism, ROS and NO production was known to occur in mammalian cells since years [53]. In plants, evidence of the involvement of NO in the regulation of ferritin was first demonstrated by Murgia et al. [54]. In that work, it was demonstrated that the NO donor SNP mimicked iron excess in the induction of *AtFer1* expression. The NO-mediated induction of ferritin is dose dependent and active even when iron is sequestered by different iron-chelating agents, suggesting that NO acts downstream of iron in the induction of *AtFer1* expression [54]. Such evidence was reinforced by the observation that *AtFer1* expression is inhibited by the NO scavenger cPTIO, even under conditions of iron excess [54]. Nitric oxide acts on *AtFer1* promoter through the IDRS sequence (Iron Dependent Regulatory Sequence), a 15 bp stretch responsible for repression of *AtFer1* expression under con-

ditions of iron deficiency [54,55]. A transcription factor (TF) stably interacts with IDRS and, in conditions of iron deficiency, a still unidentified proteic repressor binds to such IDRS–TF complex, thus preventing *AtFer1* transcription; on the contrary, in conditions of iron excess, such binding cannot occur and *AtFer1* gene can be therefore transcribed [56]. Challenging hypothesis is that the chloroplastic NO burst observed few minutes after cell treatment with iron excess [56] is responsible for the production of a retrograde signal that, in turn, would up-regulate the expression of the nuclear encoded *AtFer1* gene through the proteasomal-dependent degradation of the repressor [40,56].

In mammals, the expression of different genes involved in iron uptake, storage and export is regulated at the post-transcriptional level [57] and it is accomplished through the interactions of iron responsive elements (IREs), present in either their 5′-UTR or 3′-UTR (untranslated regions), with iron regulatory proteins (IRPs). Under normal iron availability, cytoplasmic aconitase possesses a functional [4Fe–4S] cluster in its catalytic site [58]. However, in iron deficient cells such aconitase activity goes down because its [Fe–S] cluster is disassembled and the protein thus turns into the mRNA-binding IRP1, able to bind to IREs. In macrophages, the increase of IRE binding activity and loss of aconitase activity were correlated and dependent on NO synthesis [59]. Some aspects of the translational control of mammalian ferritin through the IRE/IRP system [60,61] are common with the control of *AtFer1* expression by NO. The inhibitory effect of the NO donor SNAP on *AtFer1* suggests that in plants, ferritin regulation is sensitive to the predominant NO redox form [62], similarly to what observed in mammalian cells [63,64]. Nevertheless, it should be added that the IRE/IRP1 interaction does not operate in plants [65].

It is known that NO can act as signalling molecule in different pathways, such as the cGMP dependent one [60,66]. In plants, the NO dependent regulation of *AtFer1* expression seems to be cGMP independent, since the guanylate cyclase inhibitor ODQ (1H-[1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one) is unable to block SNP-mediated induction of *AtFer1* expression and the 8Br-cGMP, the membrane-permeable analog of cGMP, is unable to potentiate *AtFer1* expression (I. Murgia, unpublished observations).

NO is also involved in the regulation of *AtFer3* and *AtFer4* Arabidopsis ferritin isoforms. The NO donor SNP triggers *AtFer3* and *AtFer4* transcript accumulation but not that of *AtFer2* (I. Murgia, unpublished data); GSNO, another NO donor, can also induce *AtFer4* expression [67]; although IDRS-like sequences have been identified in *AtFer3-4* promoters, their functionality has not been established yet [40] suggesting that NO might regulate expression of the other Arabidopsis ferritin isoforms differently from what observed for *AtFer1*.

5. Frataxin-nitric oxide crosstalk further contributes to keep cellular iron homeostasis and compartmentalization

Mitochondria from eukaryotic cells are an essential and dynamic component of cellular metabolism [68,69]. Iron plays a crucial role in mitochondrial biochemistry. Several enzymatic activities and the function of mitochondrial, as well as of chloroplastic electron transport chains depend on the availability of iron.

Frataxin is a mitochondrial protein highly conserved in mammals, yeast, bacteria and plants. This strongly suggests that frataxin is an essential protein that could play similar roles in many unrelated organisms. In humans, mutations in the frataxin gene are responsible for the cardio-neuro degenerative disease Friedreich's ataxia [70]. Several functions have been proposed for frataxin including iron homeostasis [70,71], Fe–S cluster assembly [72–74], regulation of respiration and oxidative phosphorylation [75,76], control of antioxidant defences [77] and iron chaperone modulating mitochondrial aconitase activity [78], among others. In many organisms, frataxin deficiency is associated with iron accumulation in mitochondria and oxidative stress [79]. Iron-overload triggers oxidative stress and iron detoxification seems to be an important function of frataxin relevant in anti-oxidant defence. In that sense, frataxin deficiency has been shown to increase the sensitivity of yeast cells to oxidative stress [79,80].

Frataxin-like genes are present in all plant genomes investigated so far [81]; in Arabidopsis, a single gene *AtFH* homologous to human frataxin has been identified [82], which is able to complement null mutant frataxin yeast (Δ*yfh*). Arabidopsis *AtFH* frataxin can rescue frataxin-deficient RNAi *Trypanosoma brucei* cells restoring activities of Fe–S proteins [83] strongly indicating functional similarities of frataxin in several organisms. Two frataxin knock-outs and one knock-down T-DNA insertional mutants have been recently analysed in Arabidopsis. The knock-out mutants (*atfh-2* and *atfh-3*) are embryo lethal, indicating an essential role of frataxin at this early stage of development [84,85]. The knock-down mutant (*atfh-1*) plant has reduced levels of frataxin mRNA and protein. In this mutant, the activity of two mitochondrial Fe–S containing enzymes, aconitase and succinate dehydrogenase, is reduced whereas malate dehydrogenase which does not contain Fe–S moiety remains almost unaltered, indicating a role of frataxin in Fe–S cluster assembly and/or insertion [84]. These results indicate that frataxin is an essential protein in plants, required for full activity of mitochondrial Fe–S proteins. Frataxin deficiency causes iron accumulation in root cells which seems to be concentrated in mitochondria and plastids [67]. Since the expression of *AtFer4* is increased in *atfh-1* roots, it was hypothesized that iron could be also accumulated in plastids of frataxin mutant [67]. Furthermore, a strong decrease in cytoplasmic iron level was observed in *atfh-1* root cells compared to the wild type by using the iron sensitive fluorescent probe calcein (Zabaleta et al., unpublished data). Calcein reacts with the chelatable iron pool upon internalization to the cytoplasm [86]. This may be suggesting that frataxin strongly influences the subcellular iron compartmentalization and that iron accumulation in specific organelles seems to be most likely at the expense of cytoplasmic iron. Since cytoplasmic Fe–S cluster containing proteins depend on cluster synthesis or assembly in mitochondria [87], iron is apparently not efficiently exported back to the cytoplasm in frataxin deficient *atfh-1* mutant causing cytoplasmic iron depletion. Consistent with this idea, *STA-1/ATM3* gene coding for an ABC transporter possibly implicated in Fe–S clusters exportation from mitochondria and whose product is located at the mitochondrial inner membrane, is down-regulated in *atfh-1* roots [88,89] [Zabaleta et al., unpublished data]. Thus, frataxin deficiency affects mitochondrial Fe–S cluster assembly and trafficking and contributes to the iron accumulation inside the organelle. Since plastids appear not to have an Fe exportation system like mitochondria [89], all Fe–S clusters of cytoplasmic proteins would depend on mitochondrial supply. It remains to be determined if the predicted lower Fe–S exportation from the mitochondria and the lower cytosolic iron concentrations in *atfh-1* plants result in diminished activities of cytosolic enzymes containing Fe–S clusters.

The excess of iron inside mitochondria and plastids triggers oxidative stress [67] in many organisms. As mentioned above, excess of free iron produces hydroxyl radicals (•OH) via the Fenton's reaction [90]. Indeed, it was reported that frataxin-deficient plants exhibit increased ROS formation and induction of detoxifying enzymes involved in oxidative stress response indicating that *atfh-1* plants are under oxidative stress [84]. The abnormally high iron content and ROS production in mitochondria occur in parallel to an increase of NO production in both, frataxin-deficient plants and yeast [67]. This increased NO production has shown to be essential to maintain low levels of oxidative damage in root cells [67]. As was already stated, it has been shown that accumulation of

NO may protect from oxidative stress by two ways: (i) directly by scavenging $O_2^{-\bullet}$ [91] and, (ii) indirectly by NO-mediated induction of ferritin genes (*AtFer1* and *AtFer4*) [54,67]. Thus, NO positively contributes to diminish free iron levels within the organelles and controls ROS formation with consequent protection from oxidative stress and cell death. It was then suggested [67] that high NO production in *atfh-1* plants might be part of the response to oxidative stress caused by frataxin deficiency-mediated altered cellular iron compartmentalization. Peroxinitrite ($ONOO^-$) is rapidly formed after the reaction between $O_2^{-\bullet}$ and NO, and could be also responsible of the inhibition of aconitase activity by disrupting its Fe–S cluster.

Frataxin-deficient Arabidopsis plants exhibit a hairy root phenotype when grown under normal conditions, similar to what observed in wild type roots grown under iron-deficiency [67]. This phenotype, together with the high NO production in *atfh-1* plants, is consistent with the hairy root phenotype associated with NO accumulation observed in wild type roots exposed to iron deficiency [11]. Furthermore, the increased number of root hairs in *atfh-1* is abolished by the NO scavenger cPTIO. However, the NO-dependent up-regulation of *FIT*, *IRT1* and *FRO2* expression normally observed in both tomato [11] and Arabidopsis roots ([16], Ramirez et al., unpublished) exposed to iron deficiency was not observed in *atfh-1* plants but, instead, a slight reduction of *IRT1* and *FRO2* expression as well as of the enzymatic activity of FRO was observed [67]. Another important difference between *atfh-1* and wt plants was found in plants growing on iron-deficient medium. While *atfh-1* mutant plants grow green and healthy, extensive chlorosis is always seen in wt plants [Zabaleta et al., unpublished data]. Even if NO-mediated responses seem to be dependent on iron concentration and compartmentalization, further experiments are necessary to explain these differences.

Chloroplasts contain a membrane permease involved in transport of iron and copper termed PIC1 (Permease In Chloroplasts1) [92]. The *pic1* KO mutant grows only heterotrophically and is characterized by a chlorotic and dwarfish phenotype reminiscent of iron-deficient plants. Additionally, *AtFer1* and *AtFer4* transcripts are accumulated in *pic1* mutant as well as transcripts of stress-related genes indicating an impaired metal homeostasis at the cellular level. Roots from *pic1* mutant are smaller than in wild-type plants showing a down-regulation of *IRT1* transcript. Thus, *pic1* phenotype is somehow similar to *atfh-1* phenotype. It would be interesting to evaluate the NO levels as well as to analyse the subcellular iron distribution and content in *pic1* plants. Such experiments, however, seem not to be straightforward due to the difficulties encountered in isolating intact chloroplast from *pic1* plants [92].

Mitochondria and plastids possess their own iron–sulfur cluster (ISC) assembly proteins including members of the SUF (sulfur mobilization), the NFU family, ISC system and the corresponding scaffold proteins [93–97]. Assembly of Fe–S proteins in the eukaryotic cytosol and nuclei requires the assistance of both the mitochondrial ISC assembly machinery and a mitochondrial ISC export system [98]. Additionally, maturation of these Fe–S proteins is dependent on the essential cytosolic Fe–S-protein assembly (CIA) machinery [99].

The normal green *atfh-1* phenotype suggests that mitochondrial frataxin deficiency does not affect the chloroplastic Fe–S system although the mitochondrial system is compromised [84]. Conversely, mitochondrial Fe–S proteins and respiration were not affected in inducible RNAi silenced CpNifS plants (an important factor for Fe–S cluster synthesis in chloroplasts), suggesting that mitochondrial and chloroplastic Fe–S assembly operate independently [100].

Fig. 1 represents a model to explain changes in root cells of the *atfh-1* mutant. While mitochondrial homeostasis is kept through a coordinated regulation of their components in wild type Arabidopsis plants (Fig. 1a), frataxin deficiency leads to iron accumulation in mitochondria as well as in plastids and to an increased ROS and NO production. Part of the observed mitochondrial iron increase could be due to reduced mitochondrial Fe–S cluster exportation system (*STA1* down-regulation) affecting iron concentration and activity of the Fe–S containing enzymes in the cytoplasm (Fig. 1b).

In this scenario two different compartments, one with excess of iron and consequently increased ROS and NO formation (organelles) and one with a decrease of iron (cytoplasm) are produced in *atfh-1*. As a result, a contradictory output could be expected: hairy root in green plants. Evidences indicate that frataxin and NO seem to be important components to preserve cellular iron homeostasis in plants through the control of mitochondrial, plastidial and cytosolic iron homeostasis.

6. Nitric oxide, labile iron and nitrosyl iron complexes in plants: detection of MNICs and DNICs

As stated, NO has a central role in iron homeostasis in plants. Beyond the regulation of genes related to iron acquisition [11,16], a key role for NO in the availability of bioactive iron has been suggested. Since NO donors could revert iron-deficiency symptoms in maize, rice and tomato, and iron deficiency phenotype in *ys*1 and *ys*3 maize mutants without changes in total iron content [6,11], these effects have been linked with the formation of low molecular weight complexes between iron and NO [20]; such complexes could represent a mechanism for transporting not only iron but also NO among plant organs [101].

DNICs with thiolate ligands were found first in animal tissues and yeast cells, and more recently in plants, where they exhibit a characteristic electron paramagnetic resonance (EPR) signal [102]. Similar to S-nitrosothiols, DNIC formed in living systems represent NO-stabilizing species. Based on the nature of thiolate ligand, these complexes could be protein-bound or low-molecular weight compounds, when including cysteine or glutathione (GSH).

Solutions of S-nitrosocysteine (an S-nitrosothiol) in the presence of Fe(II)-citrate lead to the formation of MNICs with cysteine as the thiol ligand. These compounds are paramagnetic and exhibit a triplet signal when detected by EPR. The ratio between Fe(II) and S-nitrosothiols concentrations seems to be determinant in the formation of MNIC or DNIC as dominant species [103]. Even if DNICs are paramagnetic, the detection by EPR faces two problems: the high detection limit of this technique and the signal given by Mn^{2+} (present in plants, and with paramagnetic properties) which appeared at the same magnetic field [104].

In plants, detection of DNICs in vivo employing endogenous iron and thiol-containing ligands was demonstrated in leaves from China rose (*Hibiscus rosa sinensis*) after incubation with 10 mM nitrite [104]. Under these conditions, leaves generated sufficient NO such that EPR could be used to detect its production using endogenous iron and thiol ligands.

As stated, iron is an essential mineral but it is also associated with toxic effects if present in excess. The toxicity of iron is believed to be originated from the Fenton reaction which proceeds when peroxide is bound to iron and leads to formation of strong oxidants like hydroxyl radical or an oxoiron$^{(2+)}$ compound. NO reacts with Fe(II) with high rate constants (10^5–10^8 M^{-1} s^{-1}) which are higher than the reaction between iron and peroxide. As a result, NO through the formation of an iron nitrosyl complex can inhibit the Fenton reaction to a large extent [105].

The concept of the existence of an intracellular transit iron pool, also named as “labile” or “chelated” or even “free” iron, is employed to refer the cellular redox-active iron that can participate in the activation of free-radical generation processes [106]. Such pool is a small (0.2–3%) proportion of total cellular iron and is called “iron in transit” because it serves as a dynamic reservoir. Operationally,

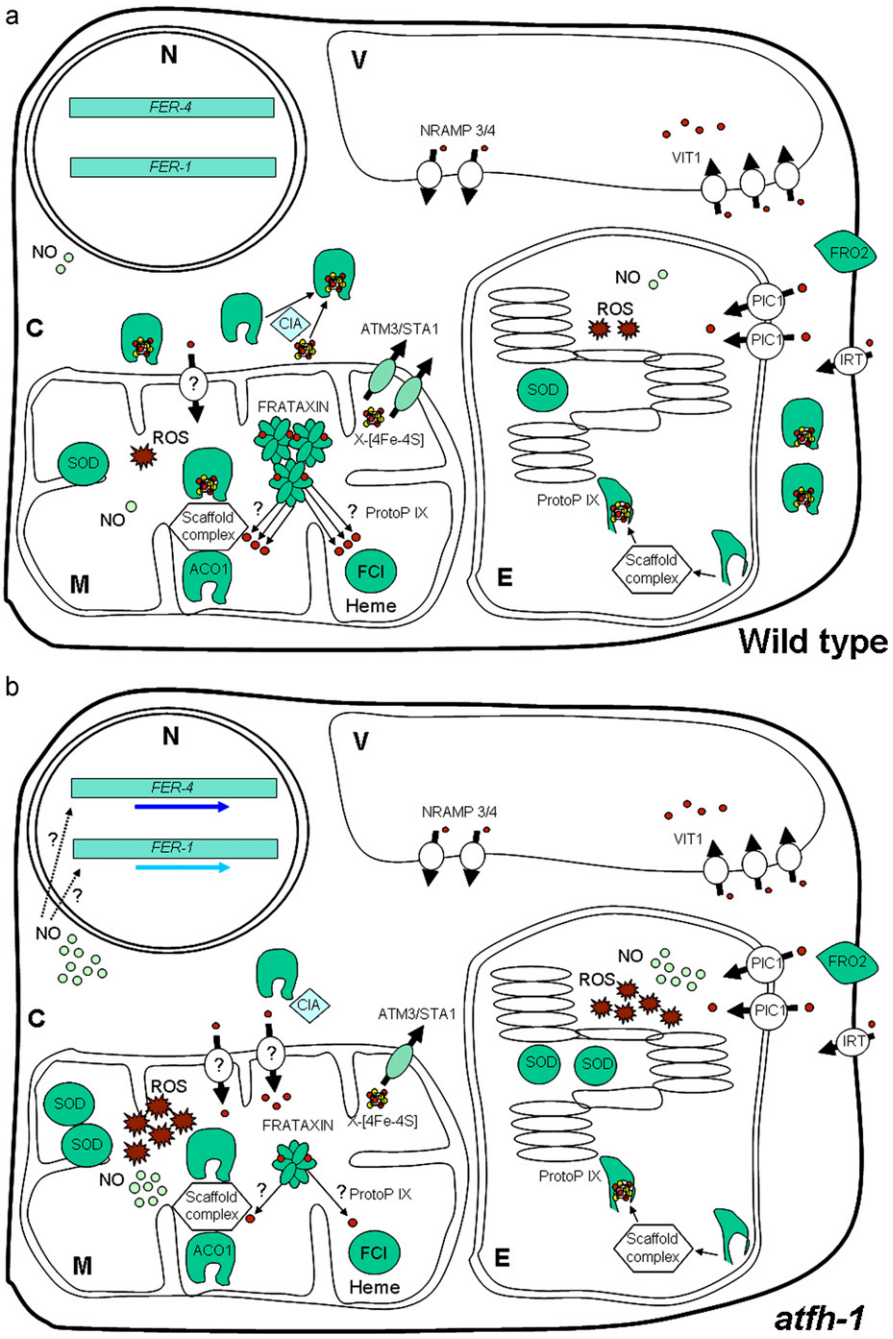

Fig. 1. Model representing the biological function of frataxin in plant iron metabolism. Schematic representation of the iron metabolism in root cells from *Arabidopsis* wild type (a) and *afth-1* knockdown mutant (b). Frataxin is a mitochondrial protein supposed to provide iron (red circles) to the synthesis of the mitochondrial heme and in the acquisition of Fe–S clusters by mitochondrial aconitase (ACO-1) by means of a Scaffold complex. Basal levels of superoxide dismutase (SOD) and mitochondrial ferritin maintain normal levels of NO (green circles) and ROS (red star circles). As yet unknown transporter (?) would participate in the importation of iron inside mitochondria; ATM3/STA1 is probed to be responsible for the exportation of Fe–S clusters to the cytosol (C). The incorporation of Fe–S clusters to the corresponding cytoplasmic apo-proteins takes place in the cytosol by means of a CIA complex. Iron is imported from the soil by using the IRT1/FRO2 system. Etioplasts contain their own Fe–S protein synthesis and SOD to maintain normal levels of iron, NO and ROS. VIT1 and NRAMP3/4 transporters participate in the movement of iron from the vacuole. Knockdown of frataxin causes increments of free iron, NO and ROS levels in mitochondria (M) and etioplasts (E). An as yet unknown retrograde regulation to the nucleus (N) in which NO participates, Ferritin (FER-1 and FER-4) and SOD genes are induced. ATM3/STA1 is reduced and it is supposed that the unknown mitochondrial and plastid (PIC1) iron transporters could be induced to explain the observed iron increment in organelles. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

it is defined as a cell chelatable iron pool that comprises both ionic forms of iron (Fe^{2+} and Fe^{3+}) associated with a heterogeneous population of ligands [107].

NO increases the size of the labile iron pool in animal cells [108,109], as well as in sorghum embryonic axes [10]. In animal cells NO can act as a chelator, binding iron and inducing its intracellular release from the Fe-storage molecule ferritin [110,111]. A previous work showed that both desferrioxamine (another strong iron chelator employed to trap iron) and a complex formed by NO, bound the same free endogenous iron [106].

In rat hepatocytes cell culture, NO was shown to be involved in the inhibition of oxidative stress induced by iron or ethanol, through the reaction with low molecular weight complex of iron to form nitrosyl-iron complexes unable to promote oxidative stress

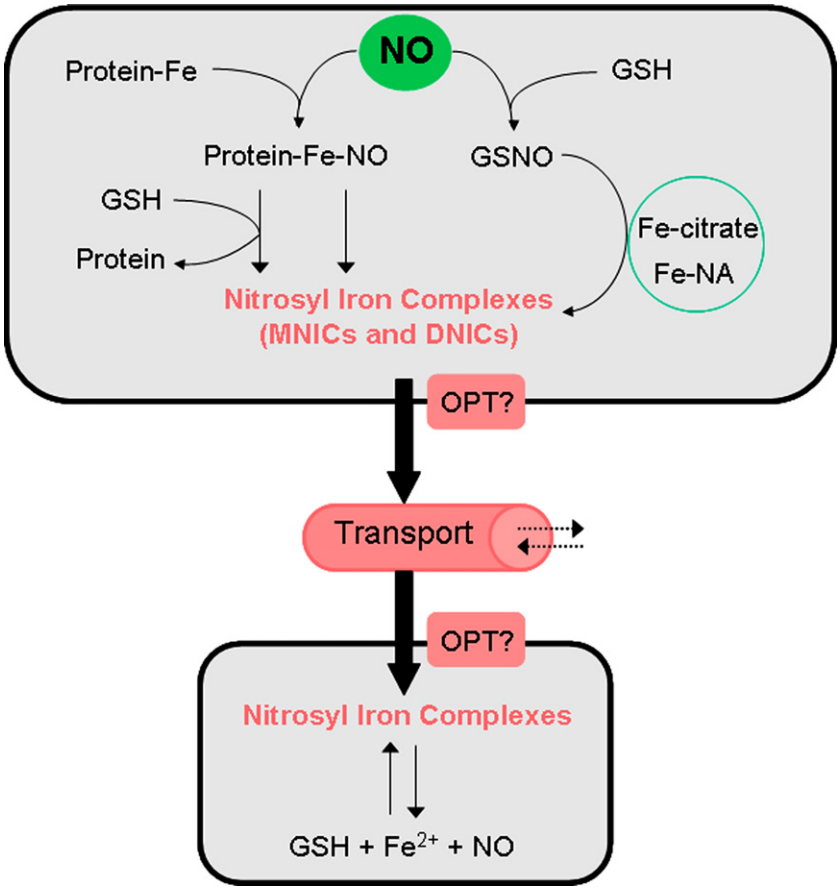

Fig. 2. Mechanism proposed for NO-mediated iron mobilization in plant cells. NO is generated endogenously in cells or diffuses from either other cells or from the external medium. Once in the cell, NO reacts with thiols (e.g. GSH, the major intracellular thiol) forming nitrosothiols (e.g. GSNO, S-nitrosoglutathione). Nitrosothiols decompose in the presence of iron leading to the generation of nitrosyl iron complexes MNICs and DNICs [100]. NO also binds to proteins containing iron (protein nitrosylation). Iron can be bound to physiologically available Fe–chelators as citrate or NA (nicotinamine). These iron complexes of low molecular weight are part of labile iron pool. The later reaction of NO–Fe–protein with GSH could lead to the release of iron from proteins as proposed in animal cells [106]. Nitrosyl iron complexes could be transported between cells probably through oligopeptide transporters family (OPT). In cells of other tissues, nitrosyl iron complexes transported via phloem can release iron and NO.

[112]. In summary, chelation of iron is a central process in iron homeostasis because it enhances its cellular availability by helping its solubilization and it also scavenges iron in a non-Fenton active form.

7. Formation of nitrosyl iron compounds

One of the most important biological activities of NO is accomplished through its reaction with transition metals. The reaction of NO with iron plays a central role in physiology and biochemistry, since many regulatory functions of NO are due to its ability to bind iron within the haem group of proteins.

The interaction between iron, thiols and NO lead to the formation of DNICs or MNICs with the general formula $(RS)_2Fe(NO)_2$ or $(RS)_2Fe–NO$, respectively [103]. In these coordination complexes the iron atom binds one or two NO molecules, and two thiols (e.g. GSH). Vanin and co-workers proposed a mechanism of nitrosyl-iron complex formation through the decomposition of S-nitrosothiol in the presence of iron [103]. Recently, a pathway leading to the formation of nitrosyl-glutathionyl-iron complex has been proposed in animals. That complex is formed through the reaction of NO with a protein-bound iron followed by the addition of GSH, resulting in the release of iron from the protein and the formation of a GS–Fe–NO complex [110].

It is important, however, to consider where the iron came from and what the physiological consequences of its removal are during the formation of nitrosyl-iron compounds. For instance, NO has been reported to release iron from proteins such as aconitase and respiration complexes I and II in mitochondria [113]. Evidence has also shown that the chelatable iron pool, in addition to protein-bound iron, may be the source of iron incorporated into DNICs and MNICs. In fact, EPR measurements of the chelatable iron pool using desferrioxamine show quantitative conversion of chelatable iron pool into DNIC after exposure to NO [114,115]. The conversion of the labile iron pool into stable DNIC mediated by NO, lead to a saturation of the metal coordination sphere and prevents its ability to reduce H_2O_2 through the Fenton reaction.

8. Transport of nitrosyl iron complexes

Iron is highly reactive and in the cells exists as stable complexes with organic ligands or inorganic phosphate. The information about the nature of the different Fe-ligand species in different cell compartments is scarce. Circulation of iron throughout the plant and distribution to tissues and organelles relies on transmembrane transporters whose nature is probably associated to the type of the iron complex to be transported. Yellow-stripe-like (YSL) transporters belong to the oligopeptide transporters (OPT) family and mediate long-distance transport of iron within the plant. It has been strongly suggested a role for YSL in the transport of iron-nicotianamine complex [116]. OPT family is a relatively poorly characterized family of proteins involved in the transport of tri-, tetra-, penta- and hexapeptides as well as amino acids-derived compounds found in archebacteria, bacteria, fungi

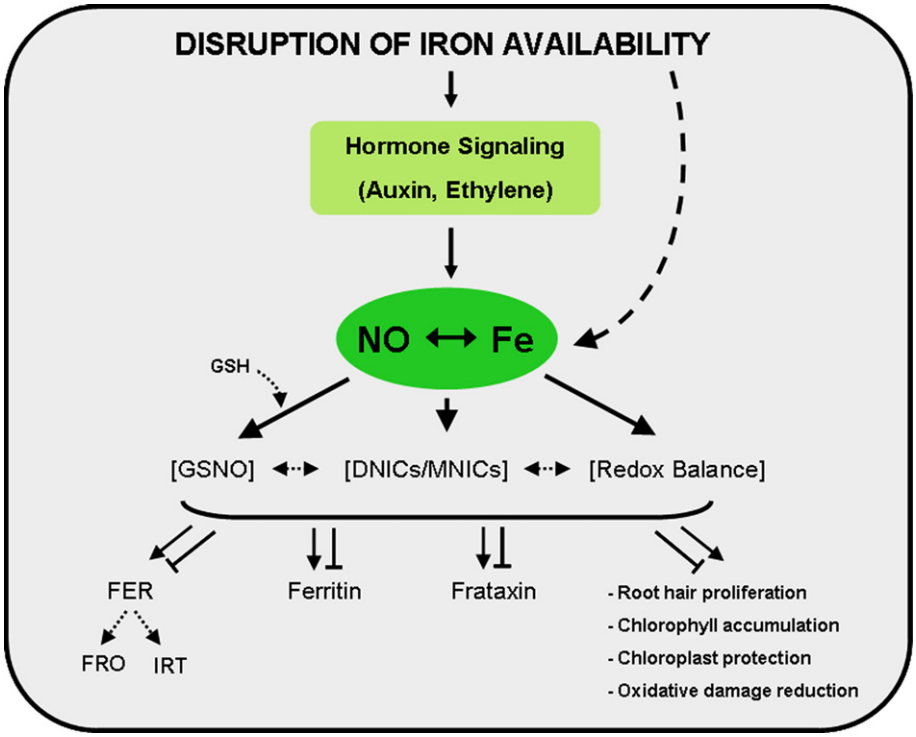

Fig. 3. Schematic model highlighting NO interactions in plant cells stressed by disruption in iron availability. Once plant cells sense disruption of iron availability, a hormone-mediated response is triggered. Different counterbalance responses involving NO, GSH, ferritin, frataxin and nitrosyl iron complexes, i.e. mononitrosyl iron complexes (MNIC) and dinitrosyl iron complexes (DNIC) are operating in coordination to moderate pronounced imbalances in redox changes and to modulate gene expression and physiological adaptations. FER, transcription factor; FRO, iron-chelate reductase; IRT, iron transporter; →, induction; ⊣, repression; ↔, interaction.

and plants. In yeast, OPTs mediate the transport of the modified peptide GSH (γ-Glu-Cys-Gly) [117]. In Arabidopsis, the member of OPT family AtOPT3 plays a critical role in maintenance of whole-plant iron homeostasis and iron nutrition of developing seeds [118]. AtOPT3 might mediate the distribution of peptides/modified peptides-Fe chelator or Fe–chelator complexes that could be critical for long-distance signalling of whole-plant iron status. It would be interesting to study the involvement of the OPT family, including YSL, in the transport of small iron-nitrosyl complexed with GSH.

On the other hand, it was demonstrated the role of the GSH-conjugate transporter multidrug resistance-associated protein 1 (MRP1) in NO-mediated iron efflux in tumor mammalian cells [119]. MRP1 is a member of the ATP-binding cassette (ABC) family. In plants, ATP-binding cassette (ABC) proteins constitute a large superfamily that encodes membrane-bound proteins which participate directly in the transport of a wide range of molecules across membranes [120]. It has been suggested that GSH participates in the export of the iron from mammalian cells by either acting as a reducing agent or by completing the coordination shell of a Fe–NO complex [110]. This complex (GSH–Fe–NO) may be released from the cells by an active process through a transporter identified as a multi-drug resistance-associated protein 1 (MRP1) [110]. The authors have suggested a NO-mediated iron release from endothelial cells in the form of nitrosyl-iron complex, which could reach smooth muscle cells where NO exerts its biological action. In animal tissues it has been also shown that DNIC-(GSH) has excellent membrane permeability and has a relatively high affinity for the liver and kidney where it can donate iron [121].

As the OPT family members, it would be interesting to analyze the presence of MRP1 homologous in the plant ABC superfamily and test their functional homology in transporting nitrosyl iron complexes. Fig. 2 describes the interaction of cellular components involved in nitrosyl iron complexes formation, their putative cellular transporters and their role to support iron and NO delivery in a safe form.

9. Biological functions of nitrosyl iron complex

The biological relevance of nitrosyl iron complexes may be awarded the ability to act as NO stabilizing compounds in which nitrosonium ions present in DNIC confer them high nitrosylating activity, e.g., the ability to induce S-nitrosylation of thiols [102]. In parallel, nitrosyl iron complexes represent a way to transport iron in a safe form. Additionally, DNICs with thiol ligands were shown to be able to activate soluble guanylate cyclase (sGC) [122], a typical NO-mediated signalling process.

In animals, the formation of protein-bound DNIC species has been shown to be present in inflammatory responses in various cells/tissues. However, both the subcellular localization and the origin of NO-reactive nonheme iron that is incorporated into DNIC have not been elucidated precisely. The role of DNIC in NO-related apoptosis is also controversial. The generation of DNIC was associated with cell death [123]; however, the formation of DNICs could be an intermediate of S-nitrosation-dependent inhibition of caspases-3 [124], thus explaining the protective role of intracellular free iron against NO-induced apoptosis. It has been postulated that low-molecular DNIC with thiolate ligands and low-molecular S-nitrosothiols are mutually interconvertible and form a self regulatory system in the presence of NO and thiols [102].

The nitrosylated iron pool could be sufficiently high that they may represent a biological strategy for maintaining bioavailable iron levels compatible with cell requirements even under iron starvation, thus explaining the previously observed effect of NO in preventing iron deficiency symptoms [6,104].

10. Concluding remarks—perspectives

Even if considerable advances have been attained on the role of novel cellular components like NO and frataxin to understand plant iron nutrition, metabolism and homeostasis in the last decade, our knowledge on their coordinating actions with ferritin, free iron status and redox control is still scarce and remains preliminary in comparison with the state of the art in animal systems.

Researches are aimed to go deep in studying how iron moves and its traffic is regulated between different cellular compartments to counteract iron excess or deficiency, and how iron is interchangeable between iron-containing proteins including iron-storage proteins like ferritin and low molecular iron-containing compounds. The molecular mechanisms implicated in iron transportation between cells and tissues and its delivery are also requiring attention. Developing novel strategies to understand all these important topics remain a big challenge to face iron-generated disruptive conditions in plant cell homeostasis.

Interesting questions could serve as a guide to focus and mobilize efforts on critical aspects of plant iron nutrition and metabolism.

1. Could the biological functions of iron as an active redox form be replaced by another metal, or even by NO, taking into consideration the three redox forms NO, NO^- and NO^+ that can be interconverted between them? If this occurs physiologically, then plants with higher NO production would need less iron requirements.
2. Is the frataxin deficiency-mediated disruption of iron homeostasis indicating that [Fe–S] formation and correct assemble in cell proteins is an essential step that determines cell iron distribution and compartmentalization?
3. How ferritin and iron nitrosyl complexes interact at the molecular level to modulate with a fine tuned control the size of labile iron pool and allow a rapid and dynamic iron availability for basic cellular reactions?
4. Are MNICs and DNICs differentially influencing the physiology of the plant? Is any enzymatic activity involved in the biosynthesis and degradation of DNICs in plants?

Fig. 3 proposes a model that describes the cellular components operating to preserve iron homeostasis under disruptive-generating conditions. It is postulated that a permanent counterbalance results from the coordinated and synchronized interaction between NO generation systems, nitrosyl iron complexes formation and redox control.

This is an exciting time for plant biologists since novel pharmacological, molecular and genetic approaches will help to understand the network of interactions operating in plant adaptive physiological responses to the consequences of a changing environment.

References

[1] A. Laxalt, M.V. Beligni, L. Lamattina, Nitric oxide preserves the level of chlorophyll in potato leaves infected by *Phytophthora infestans*, Eur. J. Plant Pathol. 73 (1997) 643–651.
[2] M. Delledonne, Y. Xia, R.A. Dixon, C. Lamb, Nitric oxide functions as a signal in plant disease resistance, Nature 394 (1998) 585–588.
[3] L. Lamattina, C. Garcia-Mata, M. Graziano, G. Pagnussat, Nitric oxide: the versatility of an extensive signal molecule, Annu. Rev. Plant Biol. 54 (2003) 109–136.
[4] A. Besson-Bard, A. Pugin, D. Wendehenne, New insights into nitric oxide signaling in plants, Annu. Rev. Plant Biol. 59 (2008) 21–39.
[5] M. Moreau, C. Lindermayr, J. Durner, D.F. Klessig, NO synthesis and signalling in plants: where do we stand? Physiol. Plant. 138 (2010) 372–383.
[6] M. Graziano, M.V. Beligni, L. Lamattina, Nitric oxide improves internal iron availability in plants, Plant Physiol. 130 (2002) 1852–1859.
[7] S. Spiller, N. Terry, Limiting factors in photosynthesis. II. Iron stress diminishes photochemical capacity by reducing the number of photosynthetic units, Plant Physiol. 65 (1980) 121–125.
[8] H. Inoue, T. Kobayashi, T. Nozoye, M. Takahashi, Y. Kakei, K. Suzuki, M. Nakazono, H. Nakanishi, S. Mori, N.K. Nishizawa, Rice OsYSL15 is an iron-regulated iron(III)-deoxymugineic acid transporter expressed in the roots and is essential for iron uptake in early growth of the seedlings, J. Biol. Chem. 284 (2009) 3470–3479.
[9] S. Lee, J.C. Chiecko, S.A. Kim, E.L. Walker, Y. Lee, M.L. Guerinot, G. An, Disruption of OsYSL15 leads to iron inefficiency in rice plants, Plant Physiol. 150 (2009) 786–800.
[10] S. Jasid, M. Simontacchi, S. Puntarulo, Exposure to nitric oxide protects against oxidative damage but increases the labile iron pool in sorghum embryonic axes, J. Exp. Bot. 5 (2008) 3953–3962.
[11] M. Graziano, L. Lamattina, Nitric oxide accumulation is required for molecular and physiological responses to iron deficiency in tomato roots, Plant J. 52 (2007) 949–960.
[12] H.Q. Ling, P. Bauer, Z. Bereczky, B. Keller, M. Ganal, The tomato fer gene encoding a bHLH protein controls iron-uptake responses in roots, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 13938–13943.
[13] A. Besson-Bard, A. Gravot, P. Richaud, P. Auroy, C. Duc, F. Gaymard, L. Taconnat, J.P. Renou, A. Pugin, D. Wendehenne, Nitric oxide contributes to cadmium toxicity in *Arabidopsis* by promoting cadmium accumulation in roots and by up-regulating genes related to iron uptake, Plant Physiol. 149 (2009) 1302–1315.
[14] J. Xu, H. Yin, Y. Li, X. Liu, Nitric oxide is associated with long-term Zn tolerance in *Solanum nigrum*, Plant Physiol. 154 (2010) 1319–1334.
[15] C.W. Jin, S.T. Du, W.W. Chen, G.X. Li, Y.S. Zhang, S.J. Zheng, Elevated carbon dioxide improves plant iron nutrition through enhancing the iron-deficiency-induced responses under iron-limited conditions in tomato, Plant Physiol. 150 (2009) 272–280.
[16] M.J. García, C. Lucena, F.J. Romera, E. Alcántara, R. Pérez-Vicente, Ethylene and nitric oxide involvement in the up-regulation of key genes related to iron acquisition and homeostasis in *Arabidopsis*, J. Exp. Bot. 61 (2010) 3885–3899.
[17] W.W. Chen, J.L. Yang, C. Qin, C.W. Jin, J.H. Mo, T. Ye, S.J. Zheng, Nitric oxide acts downstream of auxin to trigger root ferric-chelate reductase activity in response to iron deficiency in *Arabidopsis thaliana*, Plant Physiol. 10 (2010) 810–819.
[18] L. Ramirez, M. Graziano, L. Lamattina, Decoding plant responses to iron deficiency: is nitric oxide a central player? Plant Signal. Behav. 3 (2008) 795–797.
[19] M. Leitner, E. Vandelle, F. Gaupels, D. Bellin, M. Delledonne, Signals in the haze: nitric oxide signalling in plant defence, Curr. Opin. Plant Biol. 12 (2009) 451–458.
[20] M. Graziano, L. Lamattina, Nitric oxide and iron in plants: an emerging and converging story, Trends Plant Sci. 10 (2005) 4–8.
[21] G.J. Quinlan, T.W. Evans, J.M. Gutteridge, Iron and the redox status of the lungs, Free Radical Biol. Med. 33 (2002) 1306–1313.
[22] M. Becana, M.A. Matamoros, M. Udvardi M, D.A. Dalton, Recent insights into antioxidant defenses of legume root nodules, New Phytol. 188 (2010) 960–976.
[23] D.A. Wink, J.B. Mitchell, Chemical biology of nitric oxide: insights into regulatory, cytotoxic, and cytoprotective mechanisms of nitric oxide, Free Radical Biol. Med. 25 (1998) 434–456.
[24] M.D. Bartberger, W. Liu, E. Ford, K.M. Miranda, C. Switzer, J.M. Fukuto, P.J. Farmer, D.A. Wink, K.N. Houk, The reduction potential of nitric oxide (NO) and its importance to NO biochemistry, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 10958–10963.
[25] M.N. Hughes, Relationships between nitric oxide, nitroxyl ion, nitrosonium cation and peroxynitrite, Biochim. Biophys. Acta. 1411 (1999) 263–272.
[26] C. Curie, J.F. Briat, Iron transport and signaling in plants, Annu. Rev. Plant Biol. 54 (2003) 183–206.
[27] S. Susin, A. Abadia, J.A. González-Reyes, J.J. Lucena, J. Abadia, The pH requirement for in vivo activity of the iron-deficiency-induced "turbo" ferric chelate reductase (a comparison of the iron-deficiency-induced iron reductase activities of intact plants and isolated plasma membrane fractions in sugar beet), Plant Physiol. 110 (1996) 111–123.
[28] W. Brüggemann, K. Maas-Kantel, P.R. Moog, Iron uptake by leaf mesophyll cells: the role of the plasma membrane-bound ferric-chelate reductase, Planta 190 (1993) 151–155.
[29] L. Ramirez, E.J. Zabaleta, L. Lamattina, Nitric oxide and frataxin: two players contributing to maintain cellular iron homeostasis, Ann. Bot. 105 (2010) 801–810.
[30] O. Thimm, B. Essigmann, S. Kloska, T. Altmann, T.J. Buckhout, Response of *Arabidopsis* to iron deficiency stress as revealed by microarray analysis, Plant Physiol. 127 (2001) 1030–1043.
[31] V.B. Tognetti, J.F. Palatnik, M.F. Fillat, M. Melzer, M.R. Hajirezaei, E.M. Valle, N. Carrillo, Functional replacement of ferredoxin by a cyanobacterial flavodoxin in tobacco confers broad-range stress tolerance, Plant Cell 18 (2006) 2035–2050.
[32] V.B. Tognetti, M.D. Zurbriggen, E.N. Morandi, M.F. Fillat, E.M. Valle, M.R. Hajirezaei, N. Carrillo, Enhanced plant tolerance to iron starvation by functional substitution of chloroplast ferredoxin with a bacterial flavodoxin, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 11495–11500.
[33] A. Aliverti, V. Pandini, A. Pennati, M. de Rosa, G. Zanetti, Structural and functional diversity of ferredoxin-NADP(+) reductases, Arch. Biochem. Biophys. 474 (2008) 283–291.

[34] M.D. Zurbriggen, V.B. Tognetti, M.F. Fillat, M.R. Hajirezaei, E.M. Valle, N. Carrillo, Combating stress with flavodoxin: a promising route for crop improvement, Trends Biotechnol. 26 (2008) 531–537.
[35] C. Lindermayr, G. Saalbach, J. Durner, Proteomic identification of S-nitrosylated proteins in *Arabidopsis*, Plant Physiol. 137 (2005) 921–930.
[36] M.V. Beligni, L. Lamattina, Nitric oxide interferes with plant photo-oxidative stress by detoxifying reactive oxygen species, Plant Cell Environ. 25 (2002) 737–748.
[37] B. Sun, Y. Jing, K. Chen, L. Song, F. Chen, L. Zhang, Protective effect of nitric oxide on iron deficiency-induced oxidative stress in maize (*Zea mays*), J. Plant Physiol. 164 (2007) 536–543.
[38] S. Recalcati, P. Invernizzi, P. Arosio, G. Cairo, New functions for an iron storage protein: the role of ferritin in immunity and autoimmunity, J. Autoimmun. 30 (2008) 84–89.
[39] P. Arosio, R. Ingrassia, P. Cavadini, Ferritins: a family of molecules for iron storage, antioxidation and more, Biochim. Biophys. Acta 1790 (2009) 589–599.
[40] J.F. Briat, N. Arnaud, C. Duc, J. Boucherez, B. Touraine, F. Cellier, F. Gaymard, New insights into ferritin synthesis and function highlight a link between iron homeostasis and oxidative stress in plants, Ann. Bot. 105 (2010) 811–822.
[41] J.F. Briat, C. Duc, K. Ravet, F. Gaymard, Ferritins and iron storage in plants, Biochim. Biophys. Acta 1800 (2010) 806–814.
[42] S. Levi, B. Corsi, M. Bosisio, R. Invernizzi, A. Volz, D. Sanford, P. Arosio, J. Drysdale, A human mitochondrial ferritin encoded by an intronless gene, J. Biol. Chem. 276 (2001) 24437–24440.
[43] S. Levi, E. Rovida, The role of iron in mitochondrial function, Biochim. Biophys. Acta 1790 (2009) 629–636.
[44] M. Zancani, M. Peresson, A. Biroccio, G. Federici, A. Urbani, I. Murgia, C. Soave, F. Micali, A. Vianello, F. Macrì, Evidence for the presence of ferritin in plant mitochondria, Eur. J. Biochem. 271 (2004) 3657–3664.
[45] D. Tarantino, F. Casagrande, C. Soave, I. Murgia, Knocking out of the mitochondrial AtFer4 ferritin does not alter response of *Arabidopsis* plants to abiotic stresses, J. Plant Physiol. 167 (2010) 453–460.
[46] D. Tarantino, N. Santo, P. Morandini, F. Casagrande, H.P. Braun, J. Heinemeyer, G. Vigani, C. Soave, I. Murgia, AtFer4 ferritin is a determinant of iron homeostasis in *Arabidopsis thaliana* heterotrophic cells, J. Plant Physiol. 167 (2010) 1598–1605.
[47] J.M. Petit, J.F. Briat, S. Lobreaux, Structure and differential expression of the four members of the *Arabidopsis thaliana* ferritin gene family, Biochem. J. 359 (2001) 575–582.
[48] I. Murgia, J.F. Briat, D. Tarantino, C. Soave, Plant ferritin accumulates in response to photoinhibition but its ectopic overexpression does not protect against photoinhibition, Plant Physiol. Biochem. 39 (2001) 1–10.
[49] D. Tarantino, J.M. Petit, S. Lobreaux, J.F. Briat, C. Soave, I. Murgia, Differential involvement of the IDRS cis-element in the developmental and environmental regulation of the AtFer1 ferritin gene from *Arabidopsis*, Planta 217 (2003) 709–716.
[50] I. Murgia, V. Vazzola, D. Tarantino, F. Cellier, K. Ravet, J.F. Briat, C. Soave, Knock-out of the ferritin AtFer1 causes earlier onset of age-dependent leaf senescence in *Arabidopsis*, Plant Physiol. Biochem. 45 (2007) 898–907.
[51] K. Ravet, B. Touraine, J. Boucherez, J.F. Briat, F. Gaymard, F. Cellier, Ferritins control interaction between iron homeostasis and oxidative stress in *Arabidopsis*, Plant J. 57 (2009) 400–412.
[52] K. Ravet, B. Touraine, S.A. Kim, F. Cellier, S. Thomine, M.L. Guerinot, J.F. Briat, F. Gaymard, Post-translational regulation of AtFER2 ferritin in response to intracellular iron trafficking during fruit development in *Arabidopsis*, Mol. Plant 2 (2009) 1095–1106.
[53] P. Lipinski, J.C. Drapier, Interplay between ferritin metabolism, reactive oxygen species and nitric oxide, J. Biol. Inorg. Chem. 2 (1997) 559–566.
[54] I. Murgia, M. Delledonne, C. Soave, Nitric oxide mediates iron-induced ferritin accumulation in *Arabidopsis*, Plant J. 30 (2002) 521–528.
[55] J.M. Petit, O. van Wuytswinkel, J.F. Briat, S. Lobreaux, Characterization of an iron-dependent regulatory sequence involved in the transcriptional control of *AtFer1* and *ZmFer1* plant ferritin genes by iron, J. Biol. Chem. 276 (2001) 5584–5590.
[56] N. Arnaud, I. Murgia, J. Boucherez, J.F. Briat, F. Cellier, F. Gaymard, An iron-induced nitric oxide burst precedes ubiquitin-dependent protein degradation for *Arabidopsis AtFer1* ferritin gene expression, J. Biol. Chem. 281 (2006) 23579–23588.
[57] S. Recalcati, G. Minotti, G. Cairo, Iron regulatory proteins: from molecular mechanisms to drug development, Antioxid. Redox Signal. 13 (2010) 1593–1616.
[58] H. Beinert, M.C. Kennedy, Aconitase, a two-faced protein: Enzyme and iron regulatory factor, FASEB J. 7 (1993) 1442–1449.
[59] J.C Drapier, H. Hirling, J. Wietzerbin, P. Kaldy, L.C. Kühn, Biosynthesis of nitric oxide activates iron regulatory factor in macrophages, EMBO J. 12 (1993) 3643–3649.
[60] M. Delledonne, A. Polverari, I. Murgia, The functions of nitric oxide-mediated signaling and changes in genes expression during the plant hypersensitive response, Antioxid. Redox Signal. 5 (2003) 33–42.
[61] M.W. Hentze, M.U. Muckenthaler, B. Galy, C. Camaschella, Two to tango: regulation of mammalian metabolism, Cell 142 (2010) 24–38.
[62] I. Murgia, M.C. de Pinto, M. Delledonne, C. Soave, L. De Gara, Comparative effects of various nitric oxide donors on ferritin regulation, programmed cell death and cell redox state in plant cells, J. Plant Physiol. 161 (2004) 777–783.
[63] S. Kim, S.S. Wing, P. Ponka, S-nitrosylation of IRP2 regulates its stability via the ubiquitin-proteasome pathway, Mol. Cell Biol. 24 (2004) 330–337.
[64] J. Wang, C. Fillebeen, G. Chen, B. Andriopoulos, K. Pantopoulos, Sodium nitroprusside promotes IRP2 degradation via an increase in intracellular iron and in the absence of S-nitrosylation at C178, Mol. Cell Biol. 26 (2006) 1948–1954.
[65] N. Arnaud, K. Ravet, A. Borlotti, B. Touraine, J. Boucherez, C. Fizames, J.F. Briat, F. Cellier, F. Gaymard, The iron-responsive element (IRE)/iron-regulatory protein 1 (IRP1)-cytosolic aconitase iron-regulatory switch does not operate in plants, Biochem. J. 405 (2007) 523–531.
[66] A. de Montaigu, E. Sanz-Luque, A. Galvan, E. Fernandez, A soluble guanylate cyclase mediates negative signaling by ammonium on expression of nitrate reductase in *Chlamydomonas*, Plant Cell 22 (2010) 1532–1548.
[67] M. Martin, M.J. Rodriguez-Colman, D.F. Gómez-Casati, L. Lamattina, E.J. Zabaleta, Nitric oxide accumulation is required to protect against iron-mediated oxidative stress in frataxin-deficient *Arabidopsis* plants, FEBS Lett. 583 (2009) 542–548.
[68] E.L. Mackenzie, K. Iwasaki, Y. Tsuji, Intracellular iron transport and storage: from molecular mechanisms to health implications, Antioxid. Redox Signal. 10 (2008) 997–1030.
[69] G. Noctor, R. De Paepe, C.H. Foyer, Mitochondrial redox biology and homeostasis in plants, Trends Plant Sci. 12 (2007) 125–134.
[70] I. Napier, P. Ponka, D.R. Richardson, Iron trafficking in the mitochondrion: novel pathways revealed by disease, Blood 105 (2005) 1867–1874.
[71] F. Foury, D. Talibi, Mitochondrial control of iron homeostasis. A genome wide analysis of gene expression in a yeast frataxin-deficient strain, J. Biol. Chem. 276 (2001) 7762–7768.
[72] O.S. Chen, S. Hemenway, J. Kaplan, Genetic analysis of iron citrate toxicity in yeast: implications for mammalian iron homeostasis, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 16922–16927.
[73] G. Duby, F. Foury, A. Ramazzotti, J. Herrmann, T. Lutz, A nonessential function for yeast frataxin in iron–sulfur cluster assembly, Hum. Mol. Genet. 11 (2002) 2635–2643.
[74] R. Lill, U. Muhlenhoff, Iron–sulfur-protein biogenesis in eukaryotes, Trends Biochem. Sci. 30 (2005) 133–141.
[75] M. Ristow, M.F. Pfister, A.J. Yee, M. Schubert, L. Michael, C.Y. Zhang, K. Ueki, M.D. Michael, B.B. Lowell, C.R. Kahn, Frataxin activates mitochondrial energy conversion and oxidative phosphorylation, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 12239–12243.
[76] R. Santos, N. Buisson, S.A. Knight, A. Dancis, J.M. Camadro, E. Lesuisse, *Candida albicans* lacking the frataxin homologue: a relevant yeast model for studying the role of frataxin, Mol. Microbiol. 54 (2004) 507–519.
[77] O. Gakh, S. Park, G. Liu, L. Macomber, J.A. Imlay, G.C. Ferreira, G. Isaya, Mitochondrial iron detoxification is a primary function of frataxin that limits oxidative damage and preserves cell longevity, Hum. Mol. Genet. 15 (2006) 467–479.
[78] A.L. Bulteau, H.A. O'Neill, M.C. Kennedy, M. Ikeda-Saito, G. Isaya, L.I. Szweda, Frataxin acts as an iron chaperone protein to modulate mitochondrial aconitase activity, Science 305 (2004) 242–245.
[79] M. Babcock, D. de Silva, R. Oaks, S. Davis-Kaplan, S. Jiralerspong, L. Montermini, M. Pandolfo, J. Kaplan, Regulation of mitochondrial iron accumulation by *Yfh1p*, a putative homolog of frataxin, Science 276 (1997) 1709–1712.
[80] F Foury, O. Cazzalini, Deletion of the yeast homologue of the human gene associated with Friedreich's ataxia elicits iron accumulation in mitochondria, FEBS Lett. 411 (1997) 373–377.
[81] I. Murgia, D. Tarantino, C. Soave, Mitochondrial iron metabolism in plants: frataxin comes into play, Plant Soil 325 (2009) 5–14.
[82] M.V. Busi, E.J. Zabaleta, A. Araya, D.F. Gomez-Casati, Functional and molecular characterization of the frataxin homolog from *Arabidopsis thaliana*, FEBS Lett. 576 (2004) 141–144.
[83] S. Long, Z. Vávrová, J. Lukeš, The import and function of diatom and plant frataxins in the mitochondrion of *Trypanosoma brucei*, Mol. Biochem. Parasitol. 162 (2008) 100–104.
[84] M.V. Busi, M.V. Maliandi, H. Valdez, M. Clemente, E.J. Zabaleta, A. Araya, D.F. Gomez-Casati, Deficiency of *Arabidopsis thaliana* frataxin alters activity of mitochondrial Fe–S proteins and induces oxidative stress, Plant J. 48 (2006) 873–882.
[85] V. Vazzola, A. Losa, C. Soave, I. Murgia, Knockout of frataxin gene causes embryo lethality in *Arabidopsis*, FEBS Lett. 581 (2007) 667–672.
[86] S. Epsztejn, O. Kakhlon, H. Glickstein, B. Breuer, Z.I. Cabantchik, Fluorescence analysis of the labile iron pool of mammalian cells, Anal. Biochem. 248 (1997) 31–40.
[87] R. Lill, Function and biogenesis of iron–sulphur proteins, Nature 460 (2010) 831–838.
[88] S. Kushnir, E. Babiychuk, S. Storozhenko S, M.W. Davey, J. Papenbrock, R. De Rycke, G. Engler, U.W. Stephan, H. Lange, G. Kispal, R. Lill, M. Van Montagu, A mutation of the mitochondrial ABC transporter Sta1 leads to dwarfism and chlorosis in the *Arabidopsis* mutant starik, Plant Cell 13 (2001) 89–100.
[89] D.G. Bernard, Y. Cheng, Y. Zhao, J. Balk, An allelic mutant series of ATM3 reveals its key role in the biogenesis of cytosolic iron–sulfur proteins in *Arabidopsis*, Plant Physiol. 151 (2009) 590–602.
[90] B. Halliwell, J.M. Gutteridge, Biologically relevant metal ion-dependent hydroxyl radical generation. An update, FEBS Lett. 307 (1992) 108–112.
[91] M.V. Beligni, L. Lamattina, Nitric oxide counteracts cytotoxic processes mediated by reactive oxygen species in plant tissues, Planta 208 (1999) 337–344.
[92] D. Duy, G. Wanner, A.R. Meda, N. von Wirén, J. Soll, K. Philippar, PIC1, an ancient permease in *Arabidopsis* chloroplasts, mediates iron transport, Plant Cell 19 (2007) 986–1006.

[93] S Léon, B. Touraine, J.F. Briat, S. Lobréaux, Mitochondrial localization of *Arabidopsis thaliana* Isu Fe–S scaffold proteins, FEBS Lett. 579 (2005) 1930–1934.
[94] H. Ye, M. Pilon, E.A. Pilon-Smits, CpNifS-dependent iron–sulfur cluster biogenesis in chloroplasts, New Phytol. 171 (2006) 285–292.
[95] A.P. Frazzon, M.V. Ramirez, U. Warek, J. Balk, J. Frazzon, D.R. Dean, B.S. Winkel, Functional analysis of *Arabidopsis* genes involved in mitochondrial iron–sulfur cluster assembly, Plant Mol. Biol. 64 (2007) 225–240.
[96] T. Yabe, E. Yamashita, A. Kikuchi, K. Morimoto, A. Nakagawa, T. Tsukihara, M. Nakai, Structural analysis of *Arabidopsis* CnfU protein: an iron–sulfur cluster biosynthetic scaffold in chloroplasts, J. Mol. Biol. 381 (2008) 160–173.
[97] S. Schwenkert, D.J. Netz, J. Frazzon, A.J. Pierik, E. Bill, J. Gross, R. Lill, J. Meurer, Chloroplast HCF101 is a scaffold protein for [4Fe–4S] cluster assembly, Biochem. J. 425 (2009) 207–214.
[98] G. Kispal, P. Csere, C. Prohl, R. Lill, The mitochondrial proteins Atm1p and Nfs1p are required for biogenesis of cytosolic Fe/S proteins, EMBO J. 18 (1999) 3981–3989.
[99] R. Lill R, U. Mühlenhoff, Maturation of iron–sulfur proteins in eukaryotes: mechanisms, connected processes, and diseases, Annu. Rev. Biochem. 77 (2008) 669–700.
[100] D. Van Hoewyk, S.E. Abdel-Ghany, C.M. Cohu, S.K. Herbert, P. Kugrens, M. Pilon, E.A. Pilon-Smits, Chloroplast iron–sulfur cluster protein maturation requires the essential cysteine desulfurase CpNifS, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 5686–5691.
[101] M. Graziano, L. Lamattina, Nitric oxide and dinitrosyl iron complexes: roles in plant iron sensing and metabolism, in: E. van Faassen, A. Vanin (Eds.), Radicals for Life: The Various forms of Nitric Oxide, Elsevier, 2007, pp. 161–169.
[102] A.F. Vanin, Dinitrosyl iron complexes with thiolate ligands: physicochemistry, biochemistry and physiology, Nitric Oxide 21 (2009) 1–13.
[103] A.F Vanin, A.A. Papina, V.A. Serezhenkov, W.H. Koppenol, The mechanisms of S-nitrosothiol decomposition catalyzed by iron, Nitric Oxide 10 (2004) 60–73.
[104] A.F. Vanin, D.A. Svistunenko, V.D. Mikoyan, V.A. Serezhenkov, M.J. Fryer, N.R. Baker, C.E. Cooper, Endogenous superoxide production and the nitrite/nitrate ratio control the concentration of bioavailable free nitric oxide in leaves, J. Biol. Chem. 279 (2004) 24100–24107.
[105] C. Lu, W.H. Koppenol, Inhibition of the Fenton reaction by nitrogen monoxide, J. Biol. Inorg. Chem. 10 (2005) 732–738.
[106] A.V. Kozlov, D.Y. Yegorov, Y.A. Vladimirov, O.A. Azizova, Intracellular free iron in liver tissue and liver homogenate: studies with electron paramagnetic resonance on the formation of paramagnetic complexes with desferal and nitric oxide, Free Radical Biol. Med. 13 (1992) 9–16.
[107] O. Kakhlon, Z.I. Cabantchik, The labile iron pool: characterization, measurement, and participation in cellular processes, Free Radical Biol. Med. 33 (2002) 1037–1046.
[108] A. Ramachandran, E. Ceaser, V.M. Darley-Usmar, Chronic exposure to nitric oxide alters the free iron pool in endothelial cells: role of mitochondrial respiratory complexes and heat shock proteins, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 384–389.
[109] P. Lipinski, R.R. Starzynski, J.C. Drapier, C. Bouton, T. Bartlomiejczyk, B. Sochanowicz, E. Smuda, A. Gajkowska, M. Kruszewski, Induction of iron regulatory protein 1 RNA-binding activity by nitric oxide is associated with a concomitant increase in the labile iron pool: implications for DNA damage, Biochem. Biophys. Res. Commun. 327 (2005) 349–355.
[110] D.R. Richardson, H.C. Lok, The nitric oxide-iron interplay in mammalian cells: transport and storage of dinitrosyl iron complexes, Biochim. Biophys. Acta 1780 (2008) 638–651.
[111] R.N. Watts, D.R. Richardson, The mechanism of nitrogen monoxide (NO)-mediated iron mobilization from cells. NO intercepts iron before incorporation into ferritin and indirectly mobilizes iron from ferritin in a glutathione-dependent manner, Eur. J. Biochem. 269 (2002) 3383–3392.
[112] O. Sergent, B. Griffon, I. Morel, M. Chevanne, M. Dubos, P. Cillard, J. Cillard, Effect of nitric oxide on iron-mediated oxidative stress in primary rat hepatocyte culture, Hepatol. 25 (1997) 122–127.
[113] S.S. Gross, M.S. Wolin, Nitric oxide: pathophysiological mechanisms, Annu. Rev. Physiol. 57 (1995) 737–769.
[114] K. Asanuma, K. Iijima, N. Ara, T. Koike, J. Yoshitake, S. Ohara, T. Shimosegawa, T. Yoshimura, Fe–S cluster proteins are intracellular targets for nitric oxide generated luminally at the gastro-oesophageal junction, Nitric Oxide Biol. Chem. 16 (2007) 395–402.
[115] G.C. Brown, V. Borutaite, Inhibition of mitochondrial respiratory complex I by nitric oxide, peroxynitrite and S-nitrosothiols, Biochim. Biophys. Acta 1658 (2004) 44–49.
[116] C. Curie, G. Cassin, D. Couch, F. Divol, K. Higuchi, M. Le Jean, J. Misson, A. Schikora, P. Czernic, S. Mari, Metal movement within the plant: contribution of nicotianamine and yellow stripe 1-like transporters, Ann. Bot. 103 (2008) 1–11.
[117] A. Bourbouloux, P. Shahi, A. Chakladar, S. Delrot, A.K. Bachhawat, Hgt1p, a high affinity glutathione transporter from the yeast *Saccharomyces cerevisiae*, J. Biol. Chem. 275 (2000) 13259–13265.
[118] M.G. Stacey, A. Patel, W.E. McClain, M. Mathieu, M. Remley, E.E. Rogers, W. Gassmann, D.G. Blevins, G. Stacey, The *Arabidopsis* AtOPT3 protein functions in metal homeostasis and movement of iron to developing seeds, Plant Physiol. 146 (2008) 589–601.
[119] R.N. Watts, C. Hawkins, P. Ponka, D.R. Richardson, Nitrogen monoxide (NO)-mediated iron release from cells is linked to NO-induced glutathione efflux via multidrug resistance-associated protein 1, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 7670–7675.
[120] P.J. Verrier, D. Bird, B. Burla, E. Dassa, C. Forestier, M. Geisler, M. Klein, U. Kolukisaoglu, Y. Lee, E. Martinoia, A. Murphy, P.A. Rea, L. Samuels, B. Schulz, E.J. Spalding, K. Yazaki, F.L. Theodoulou, Plant ABC proteins—a unified nomenclature and updated inventory, Trends Plant Sci. 13 (2008) 151–159.
[121] T. Ueno, Y. Suzuki, S. Fujii, A.F. Vanin, T. Yoshimura, In Vivo distribution and behavior of paramagnetic dinitrosyl dithiolato iron complex in the abdomen of mouse, Free Radical Res. 31 (1999) 525–534.
[122] I.S. Severina, O.G. Bussygina, N.V. Pyatakova, I.V. Malenkova, A.F. Vanin, Activation of soluble guanylate cyclase by NO donors—S-nitrosothiols, and dinitrosyl-iron complexes with thiol-containing ligands, Nitric Oxide 8 (2003) 155–163.
[123] A.L Kleschyov, S. Strand, S. Schmitt, D. Gottfried, M. Skatchkov, N. Sjakste, A. Daiber, V. Umansky, T. Munzel, Dinitrosyl-iron triggers apoptosis in Jurkat cells despite overexpression of Bcl-2, Free Radical Biol. Med. 40 (2006) 1340–1348.
[124] Y.M. Kim, H.T. Chung, R.L. Simmons, T.R. Billiar, Cellular non-heme iron content is a determinant of nitric oxide-mediated apoptosis, necrosis, and caspase inhibition, J, Biol. Chem. 275 (2000) 10954–10961.