Searching iron sensors in plants by exploring the link among 2′-OG-dependent dioxygenases, the iron deficiency response and metabolic adjustments occurring under iron deficiency

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Knowledge accumulated on the regulation of iron (Fe) homeostasis, its intracellular trafficking and transport across various cellular compartments and organs in plants; storage proteins, transporters and transcription factors involved in Fe metabolism have been described in detail in recent years. However, the key sensor(s) of cellular plant “Fe status” triggering the long-distance shoot–root signaling and leading to the root Fe deficiency response is (are) still unknown. Local Fe sensing is also a major task for roots, for adjusting the internal Fe requirements to external Fe availability: how such sensing is achieved and how it leads to metabolic adjustments in case of nutrient shortage, is mostly unknown. Two proteins belonging to the 2′-OG-dependent dioxygenases family accumulate several folds among Fe-deficient Arabidopsis roots. Such proteins require Fe(l) as enzymatic cofactor; one of their subgroups, the HIF-PAH (hypoxia-inducible factor-prolyl 4-hydroxylase), is an effective oxygen sensor in animal cells. We envisage here the possibility that some members of the 2′-OG dioxygenase family may be involved in the Fe deficiency response and in the metabolic adjustments to Fe deficiency or even in sensing Fe, in plant cells.

Keywords: Arabidopsis thaliana, iron sensor, HIF (hypoxia-inducible factor), 2′-OG-dependent dioxygenase, prolyl 4-hydroxylase

INTRODUCTION
Iron is an essential micronutrient for plants although it is potentially toxic when present in a free, non-complexed form. A recent review on that subject (Kobayashi and Nishizawa, 2012) details the knowledge accumulated on the regulation of plant Fe homeostasis, its intracellular trafficking and transport across cellular compartments and organs under various conditions of Fe supply, unveiling a complex net of molecular interactions. Beside the intensification of Fe-uptake strategies activated by plants under Fe-limiting conditions and organs under various conditions of Fe supply, unveiling a complex net of molecular interactions. Beside the intensification of Fe-uptake strategies activated by plants under Fe-limiting conditions, root cells reprogram their metabolism to better cope with shortage of Fe (Vigani et al., 2012). Low Fe content triggers a high energy request to sustain the increased rate of Fe uptake from the soil, and at the same time it impairs the function of mitochondria and chloroplasts which provide energy to the cells. Thus, cells must increase the rate of alternative energy-providing pathways, such as glycolysis, Krebs cycle, or pentose phosphate pathway (López-Millán et al., 2000, 2012; Li et al., 2009; Vigani and Zocchi, 2009; Donnini et al., 2010; Bellarín-Araujo et al., 2010; Vigani, 2012a). To date, however, the sensors of plant “Fe status” triggering the signal transduction pathways, which eventually induce transcription factors such as the Arabidopsis FIT1, are still unknown and represent a challenging issue in plant science (Vigani et al., 2013). Efforts to fill up such gap of knowledge have been made by different research groups since years (Schmidt and Steinbach, 2000); recently, it has been demonstrated that localized Fe supply stimulates lateral root formation through the AUX1 auxin importer, which is proposed as a candidate for integrating the local Fe status in auxin signaling (Giehl et al., 2012).

2′-OG Fe(l)-DEPENDENT DIOXYGENASES AND PROLYL 4-HYDROXYLASES
It has been recently observed that some similarities might exist between the metabolic reprogramming occurring in Fe-deficient roots and that one occurring in tumor cells (Vigani, 2012b). In tumor cells, such reprogramming is known as “Warburg-effect” in which glucose is preferentially converted to lactate by enhancing glycolysis and fermentative reactions rather than completely oxidized by oxidative phosphorylation (OXPHOS; Brahim-Horn et al., 2007). Also in root cells a low Fe availability causes a decrease of OXPHOS activity and induction of glycolysis and anaerobic reactions (Vigani, 2012b). The Warburg-effect in animal cells is mediated by hypoxia-inducible factor (HIF1), a heterodimeric complex whose α-subunit is inducible by hypoxia. Under normoxic conditions, HIFα is post-translationally modified via the hydroxylation of proline residues by prolyl 4-hydroxylases (P4H); such modification leads to the proteasome-mediated degradation of HIFα. Under hypoxic conditions, however, such hydroxylation cannot occur because P4H enzymes belong to the 2-oxoglutarate Fe(l)-dependent dioxygenase family which have molecular oxygen as co-substrates; in other words, the lack of oxygen inhibits the P4H enzymatic activity. HIFα escapes degradation, it translocates to the nucleus where it can therefore form a complex whose...
dimer with HiFi subunit; the complex then activates the cascade of hypoxia-responsive gene expression pathways (Myllyharju, 2003; Ken and Costa, 2006; Semenza, 2007).

Prolyl 4-hydroxylases are present in animal as well as in plant cells. In animal cells, P4Hs are classified into two categories: the collagen-type-P4H and the above cited HiFi-P4H. The first class is localized within the lumen of the endoplasmic reticulum and it catalyzes the hydroxylation of proline residues within X-Pro-Gly sequences in collagen and in collagen-type proteins (Myllyharju, 2003), thus stabilizing their triple helical structure at body temperature (Myllyharju, 2003). These P4Hs are αβ γ tetramers and their catalytic site is located in the α subunit (Myllyharju, 2003; Tiiainen et al., 2005). Three αα residues, His-412, Asp-414, and His-483, are the binding sites for Fe(II) in the human αα subunit (Myllyharju, 2003). The second class of P4H is localized in cytoplasm and it is responsible for hydroxylation of a proline residue in the HiFiα subunit, under normoxic conditions, as described above. The KM values of HiFi-P4Hs for O2 are slightly above atmospheric concentration, making such proteins effective O2 sensors (Hirsilä et al., 2003). A novel role has also been uncovered for a human collagen-type-P4H, as regulator of Argonaute2 stability with consequent influence on RNA interference mechanisms (Qi et al., 2008).

Several genes similar to P4H are present in plants; for instance, 13 P4H have been identified in Arabidopsis and named AP4H1–AP4H13 (Vlad et al., 2007a,b); with the exception of temperature (Myllyharju, 2003), thus stabilizing their triple helical structure at body temperature (Myllyharju, 2003). These P4Hs are αβ γ tetramers and their catalytic site is located in the α subunit (Myllyharju, 2003; Tiiainen et al., 2005). Three αα residues, His-412, Asp-414, and His-483, are the binding sites for Fe(II) in the human αα subunit (Myllyharju, 2003). The second class of P4H is localized in cytoplasm and it is responsible for hydroxylation of a proline residue in the HiFiα subunit, under normoxic conditions, as described above. The KM values of HiFi-P4Hs for O2 are slightly above atmospheric concentration, making such proteins effective O2 sensors (Hirsilä et al., 2003). A novel role has also been uncovered for a human collagen-type-P4H, as regulator of Argonaute2 stability with consequent influence on RNA interference mechanisms (Qi et al., 2008).

Cloning and biochemical characterization of two of them, i.e., AP4H1, encoded by At3g03800 gene (Herta and Myllyharju, 2002) and AP4H2, encoded by At3g03800 gene (Tiiainen et al., 2005) show that substrate specificity varies: recombinant AP4H1I effectively hydroxylates poly(L-proline) and other synthetic peptides with KM values lower than those for AP4H2, thus suggesting different physiological roles between the two. Recombinant AP4H1I can also effectively hydroxylate human HiFi-like peptides and collagen-like peptides, whereas recombinant AP4H2 cannot (Herta and Myllyharju, 2002; Tiiainen et al., 2005). Their KM for Fe(II) are 16 and 5 μM, respectively (Herta and Myllyharju, 2002; Tiiainen et al., 2005).

Two proteins belonging to the 2′-OG dioxygenase family, encoded by At3g12900 and At3g13610 genes, accumulate several folds in Fe-sufficient roots, when compared to Fe-sufficient ones (Lan et al., 2011). The protein encoded by At3g13610 gene, named FeH1, is involved in the synthesis of coumarins via the phenylpropanoid pathway, as it catalyzes the ortho-hydroxylation of feruloyl CoA, which is the precursor of scopoletin (Kai et al., 2008). Scopoletin and its β-glucoside glucoside accumulate in Arabidopsis roots and, at lower levels, also in shoots (Kai et al., 2006). Phenolics can facilitate the reutilization of siderophores as well as auxin-like compounds (Jin et al., 2006, 2008, 2010).

Plant 2′-OG dioxygenases are also involved in synthesis of phenylpropanoids such as Ids3 from barley, which is induced by Fe deficiency and it catalyzes the hydroxylation step from 2′-deoxymugenic acid (DMA) to mugenic acid (MA; Kobayashi et al., 2001).

2′-OG Fe(II)-DEPENDENT DIOXYGENASES, Fe DEFICIENCY RESPONSE AND METABOLIC REPROGRAMMING: IS THERE A COMMON LINK?

Given the above premises, it is possible that a link among P4H activity, and more generally among 2′-OG Fe(II)-dependent dioxygenase activities, the Fe deficiency responses and the metabolic reprogramming occurring during Fe deficiency exists in higher plants. If such a link exists for a given 2′-OG Fe(II)-dependent dioxygenase, at least two possible scenarios could be predicted for such enzyme (Figure 1.): (a) If, for a given sub-cellular localization, the KM of such enzyme for Fe is close to the physiological concentration of the LIP (labile iron pool, consisting of free redox-active Fe ions), then the enzyme activity is strongly affected by Fe fluctuations, similarly to the above described HiFi-P4H, which is an effective sensor for O2 (Hirsilä et al., 2003). Upon reduction of Fe availability below the physiological LIP, its enzymatic activity should be indeed drastically reduced or fully inhibited; reduction or complete lack of enzymatic product might, in turn, triggers the "Fe deficiency" signaling. The enzyme might therefore act as true Fe sensor (Figure 1, upper panel, right). Although the Fe-dependent transcriptional regulation of such an Fe sensor enzyme might be not expected, it cannot be excluded a priori: for example, chitin recognition is dependent not only on the presence of specific receptors, but also on the expression of extracellular chitinases, which are essential for the production of smaller chito-oligosaccharides from chitin hydrolysates, in animal (Gestrzaleny et al., 2010, Vega and Kalkum, 2012) as well as in plant systems (Shibuya and Minami, 2001; Wan et al., 2008). These smaller, diffusible molecules induce, in turn, the expression of several defense protein, among which also chitinase activities. Chitinase is thus both an example of a crucial enzyme for the signal production but also an integral part of the response. (b) If, for a given sub-cellular localization, the KM for Fe of such an enzyme is instead far below the physiological LIP, the enzyme might still be active under Fe deficiency. Additionally, if transcriptional/translational up-regulation occurs under Fe deficiency, accumulation of protein and increased total enzymatic activity might be observed. The enzyme might be involved in the Fe response/metabolic adjustment occurring under Fe deficiency, without being itself a Fe sensor (Figure 1, lower panel, right).
This second scenario is supported by the evidence that the Arabidopsis 2′-OG dioxygenase F6'H1 (described in previous paragraph) which accumulates in Fe-deficient roots (Lan et al., 2011) is indeed possibly involved in the Fe response/metabolic adjustment occurring under Fe deficiency. Arabidopsis mutants KO for the At3g13610 gene (coding for F6'H1) have indeed altered root phenotype under Fe deficiency (I. Murgia, unpublished observations).

Such a link among 2′-OG Fe(II)-dependent dioxygenase activity, the Fe deficiency responses and the metabolic reprogramming during Fe deficiency can be explored first by analyzing the transcriptional co-regulation of 2′-OG-dependent dioxygenase genes with genes involved in the Fe deficiency response or in the metabolic reprogramming. The bioinformatic approach of our choice was already described (Beekwilder et al., 2008; Menges et al., 2008; Berri et al., 2009; Murgia et al., 2011) and successfully applied in Arabidopsis and rice. Such analysis identifies genes which are co-regulated in large microarray datasets; in this case, it provides candidate genes potentially involved in the Fe deficiency response or in the metabolic reprogramming. As pivot bioinformatic analysis, we analyzed the correlation of such AtP4H subclass with two gene groups. The first group consisted of a list of 25 Fe-homeostasis/trafficking/transport related genes, described in recent reviews on this subject (Conte and Walker, 2011; Kobayashi and Nishizawa, 2012). The second group consisted of an equal number of genes coding for enzymes possibly involved in the metabolic adjustments under Fe deficiency, such as those catalyzing the synthesis of pyruvate (Pyr). It is indeed known that several glycolytic genes are overexpressed in roots of Fe-deficient
plants (Thimm et al., 2001; different isoforms of hexokinase (HXK), phosphoglyceratekinase, enolase (ENO), phosphoglycerate mutase (iPGAM), were therefore considered. Also, genes coding for enzymes involved in the consumption of Pyr by non-OXOPHOS reactions and whose expression is affected by Fe deficiency, such as alcohol dehydrogenase (ADH), lactate dehydrogenase (LDH), and malate dehydrogenase, were also considered (Thimm et al., 2001). Last, the genes coding for the four isoforms of Arabidopsis phosphophenylpyruvate carboxylase (PEPC; PPC1, 2, 3, 4; Sanchez et al., 2006) were also included in the second group, since PPC is strongly induced in several dicotyledonous plants under Fe deficiency (Vigani, 2012a) and PEPC is supposed to play a central role in the metabolic reprogramming occurring in Fe-deficient root cells (Zocchi, 2006).

As positive controls, the two Z′-OG-dioxigenases encoded by At3g12900 and At3g13610 and accumulating in Fe-deficient roots (Luo et al., 2011) whereas, as negative control, the ferritin gene whose expression is known to be repressed under Fe deficiency (Murgia et al., 2002), were included. The full list of genes for which the correlation analysis has been performed, is reported in Table 1.

The resulting Pearson’s correlation coefficients, calculated by using either linear or logarithmic expression values (Menges et al., 2008; Murgia et al., 2011) are reported in Table 2, if above a defined threshold (≥0.60 or ≤−0.60); genes for which none of the Pearson’s coefficient fulfilled this condition, were not included in Table 2 (At4g19690, At4g29310, At4g19680, At4g29313, At4g29310, At4g29313, At4g29310, At4g29313).

In accordance with results obtained by TRAQ (inosic acid tags for relative and absolute quantitation) analysis of Fe-deficient roots (Luo et al., 2011), both At3g12900 and At3g13610 show positive correlation with genes actively involved in the Fe deficiency response, such as iron-regulated transporter 1 (IRT1; Vert et al., 2002), ferric-chelate oxidase reductase (FRO2; Connolly et al., 2003) CYP82C4 (Murgia et al., 2011), are reported in Table 2 (At3g12900 and At3g13610) whereas genes in class 3 might contain significant correlation with the ferritin genes since their correlation values fall within the [−0.60 or 0.60] range (data not shown).

According to such results, the AtP4H genes could be divided into three classes:

Class 1: positive or negative correlation with metabolic genes only (At3g28490, At2g43080, and At5g18900).

Class 2: positive correlation with Fe-related genes and positive or negative correlation with metabolic genes (At2g17720 and At3g13610) besides the positive control At3g13610.

Class 3: no significant correlation (positive or negative) with any of the genes tested (At1g20270, At4g33910, At5g66060, At4g55820).

Genes in class 1 might not be involved in the plant response to improve Fe uptake and trafficking in order to alleviate Fe deficiency symptoms.

Genes in class 2 might be the ones linking the stimulation of Fe deficiency response with the metabolic adaptations triggered by Fe deficiency (Figure 1) whereas genes in class 3 might contain the candidate Fe sensor(s) (Figure 1).

Regarding the genes in class 2, it is interesting to notice that beside with the Fe-related genes, the positive control At3g13610 is positively correlated with PPC3 and ENO1, the At3g06300 gene coding for PPC1, PPC3, ENO1, and also negatively correlated with a malate dehydratase family (MTP3), Arrivault et al., 2006) (Table 2); vicesversa, they show no significant correlation with the ferritin genes since their correlation values fall within the [−0.3 or +0.3] range (data not shown).
Table 2 | Correlation analysis of Arabidopsis thaliana 2′-OG dioxygenase genes with genes involved in Fe deficiency response or with genes possibly involved in metabolic reprogramming during Fe deficiency.

| AGI code | 2′-OG-diox | 2′-OG-diox | P4H-1 | P4H-2 | P4H-4 | P4H-5 | P4H-6 |
|----------|------------|------------|-------|-------|-------|-------|-------|
|           | log        | log        | log   | log   | log   | log   | log   |
| IRT1     | 0.69       | 0.23       | 0.74  | 0.69  | 0.21  | 0.21  | 0.54  |
| At3g12900 | 0.54       | 0.50       | 0.10  | 0.11  | 0.36  | 0.53  | −0.02 |
| 0.06      |            |            |       |       |       |       |       |
| AHA2     | 0.72       | 0.09       | 0.69  | 0.69  | 0.35  | 0.31  | 0.76  |
| At4g30190 | 0.70       | 0.18       | 0.18  | 0.22  | 0.76  | 0.79  | −0.07 |
| 0.07      |            |            |       |       |       |       |       |
| CYP92C4  | 0.54       | 0.47       | 0.61  | 0.56  | 0.30  | 0.33  | 0.40  |
| At4g31940 | 0.40       | 0.43       | 0.16  | 0.22  | 0.27  | 0.27  | 0.37  |
| 0.04      |            |            |       |       |       |       | −0.13 |
| IREG2    | 0.73       | 0.42       | 0.79  | 0.59  | 0.26  | 0.22  | 0.64  |
| At5g03570 | 0.66       | 0.55       | 0.23  | 0.31  | 0.41  | 0.51  | −0.02 |
| 0.08      |            |            |       |       |       |       |       |
| MTPJ     | 0.77       | 0.27       | 0.83  | 0.70  | 0.30  | 0.37  | 0.62  |
| At5g68110 | 0.66       | 0.16       | 0.23  | 0.40  | 0.58  | 0.08  | −0.04 |
| 0.10      |            |            |       |       |       |       |       |
| HXK4     | 0.25       | 0.22       | 0.32  | 0.25  | 0.24  | 0.27  | 0.29  |
| At1g20040 | 0.37       | 0.36       | 0.02  | 0.17  | 0.26  | 0.72  | 0.06  |
| HXL3     | 0.01       | 0.05       | 0.06  | 0.03  | 0.13  | 0.05  | −0.08 |
| At4g79640 | 0.06       | 0.06       | 0.32  | 0.31  | 0.11  | 0.05  | −0.06 |
| 0.06      |            |            |       |       |       |       |       |
| PPC1     | 0.03       | −0.06      | 0.41  | 0.55  | 0.19  | 0.22  | 0.60  |
| At1g53310 | 0.63       | 0.03       | 0.00  | 0.10  | 0.73  | 0.70  | −0.06 |
| 0.06      |            |            |       |       |       |       |       |
| PPC3     | 0.23       | 0.21       | 0.78  | 0.73  | 0.35  | 0.27  | 0.69  |
| At2g14940 | 0.58       | 0.19       | 0.23  | 0.58  | 0.58  | 0.02  | 0.03  |
| 0.03      |            |            |       |       |       |       |       |
| PPC4     | 0.01       | 0.08       | 0.04  | 0.10  | 0.13  | 0.05  | −0.05 |
| At1g68750 | 0.08       | 0.08       | 0.35  | 0.08  | 0.00  | 0.13  | 0.78  |
| 0.21      |            |            |       |       |       |       |       |
| PDK1     | −0.13      | −0.11      | −0.50 | −0.41 | −0.67 | −0.69 | −0.49 |
| At3g12780 | −0.46      | −0.66      | −0.74 | −0.33 | −0.30 | −0.09 | −0.03 |
| 0.03      |            |            |       |       |       |       |       |
| ENO1     | 0.14       | 0.03       | 0.72  | 0.59  | 0.37  | 0.31  | 0.67  |
| At1g94200 | 0.59       | 0.12       | 0.06  | 0.54  | 0.49  | 0.05  | −0.01 |
| 0.01      |            |            |       |       |       |       |       |
| PGAM     | 0.07       | 0.03       | 0.47  | 0.51  | 0.04  | 0.10  | 0.54  |
| At1g09780 | 0.61       | 0.62       | 0.07  | 0.03  | 0.06  | 0.54  | 0.49  |
| Mal d. fam. | 0.00 | 0.13 | 0.78 | 0.21 | 0.66 | 0.62 | 0.07 | 0.03 |
| At5g68330 | −0.19      | −0.23      | −0.56 | −0.49 | −0.70 | −0.67 | −0.61 |
| −0.59     | −0.60      | −0.47     | −0.47 | −0.08 | 0.02  | 0.00  | 0.02 |

For each gene pair, the Pearson’s correlation coefficient, from logarithmic or linear analysis, is reported. Coefficients with values ≥0.60 or ≤−0.60 are highlighted in grey.

for P4H5 is positively correlated with PPC1 and iPGAM (Zhao and Assmann, 2011).

Interestingly, PPC1 and PPC3 are mainly expressed in root tissues and their expression is affected by abiotic stress when compared with PPC2, which is considered to cover an housekeeping role (Sanchez et al., 2016), whereas ENO1 encodes the plastid-localized isoform of phosphoenolpyruvate (PEP)-ENO (Prabhakar et al., 2009). PEP is further metabolized to Pyr by pyruvate kinase (PK). PEP and Pyr represent essential precursors for anaerobic reaction. PEP is fed into the shikimate pathway, which is localized within the plastid stroma (Herrmann and Voordeckers, 2009) and more active transceptors, whose feature is that transport and sensing activity can be uncoupled, have been described in animals and yeasts (Thevelein and Voordecker, 2009; Kriel et al., 2011) and more active transceptors have been postulated also in plants (Gojon et al., 2011). Three major global challenges faced by agriculture are food and energy production as well as environmental compatibility (Ehrhardt and Frommer, 2012). Advancements in the area of nutrient sensing and signaling can positively contribute solutions to all these three challenges and the extensive analysis of the complete 2′-OG-dioxygenase gene family, based on pilot analysis described in the present perspective, could be a novel way to pursue these advancements.

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