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Time to pump iron: iron-deficiency-signaling mechanisms of higher plants

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Iron is an essential nutrient for plants, yet it often limits plant growth. On the contrary, overaccumulation of iron within plant cells leads to oxidative stress. As a consequence, iron-uptake systems are carefully regulated to ensure that iron homeostasis is maintained. In response to iron limitation, plants induce expression of sets of activities that function at the root–soil interface to solubilize iron and subsequently transfer it across the plasma membrane of root cells. Recent advances have revealed key players in the signaling pathways that function to induce these iron-uptake responses. Transcription factors belonging to the basic helix–loop–helix, ABI3/VP1(B3), and NAC families appear to function either directly or indirectly in the upregulation of iron deficiency responses.

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Introduction

Our current state of ignorance about many of the mechanisms involved in plant iron homeostasis is a major obstacle in devising approaches for biofortification: the genetic engineering of staple crops to accumulate additional bioavailable nutrients in edible parts. Biofortification is widely regarded as a sustainable means of improving the iron nutrition of the four to five billion people worldwide (World Health Organization; <http://www.who.int/nut/ida.htm>) whose inadequate diet causes iron deficiency anemia. Improving our understanding of the mechanisms controlling plant iron homeostasis is also crucial if we wish to improve growth of crops in marginal soils, where metal deficiencies — particularly iron deficiency — frequently limit crop growth. Here we review iron-uptake mechanisms in plants with a primary

focus on recently identified gene regulatory proteins that function in iron-deficiency-signaling pathways.

Iron deficiency responses

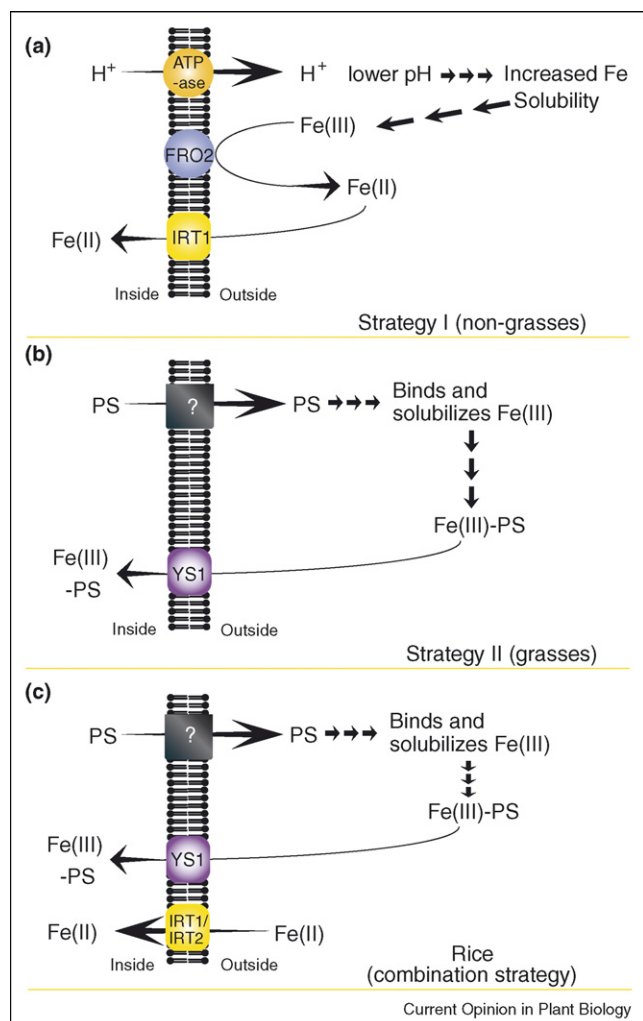
Upon sensing iron limitation, plants induce a coordinated set of responses that together allow the plant to maximize iron mobilization and uptake from the soil, utilize iron stores and prioritize the allocation of iron to crucial cellular processes. All plants except the grasses use the Strategy I response to solubilize and transport iron into roots when iron is limiting [1]. This response includes the induction of three activities localized at the plasma membrane of root cells: a proton pump acidifies the rhizosphere, thus driving more iron into solution, a ferric chelate reductase converts Fe(III)-chelates to Fe(II) and a Fe(II) transporter moves iron across the plasma membrane and into cells (Figure 1a). In *Arabidopsis thaliana*, the *Ferric Reductase Oxidase (FRO2)* gene encodes the iron-deficiency inducible ferric chelate reductase that reduces iron at the root–soil interface [2], and the *IRT1* gene encodes a high affinity ferrous iron transporter that transports iron into root cells [3–6]. Putative *FRO2* and *IRT1* orthologs have been identified in a number of Strategy I species [7–11]. The cucumber *CsHA1* gene most probably encodes a H⁺-ATPase that functions in iron-deficiency-induced rhizosphere acidification [12,13].

In grasses, a group that includes most of the world's staple grain crops, a distinct strategy for iron uptake has evolved. Grasses produce molecules of the mugineic acid family called phytosiderophores (PSs). PS form strong hexadentate chelates with Fe(III), the predominant form of iron found in aerobic soils. PS are secreted into the rhizosphere where they chelate and help to solubilize Fe(III). The Fe(III)–PS complex is then taken up into root cells through the action of Yellow Stripe1 (YS1) proteins [14–17] (Figure 1b).

Iron uptake in rice: a special case

Classically, the iron-uptake strategies used by grasses and nongrasses were thought to be completely distinct. The maize *ys1* mutant, which lacks the ability to take up Fe(III)–PS complexes, shows severe iron deficiency chlorosis (yellowing between the veins), and ultimately dies as a seedling, indicating that uptake of Fe(III)–PS is an essential process for this species [14,15,18]. Recently, there have been reports that, in contrast to other grasses, rice has an efficient Fe(II) uptake system. Rice plants not only produce PS, albeit in relatively low amounts [19], but

Figure 1



Strategies for iron uptake from the soil. **(a)** Strategy I. Acidification of the rhizosphere to solubilize Fe(III) provides the substrate for the enzyme ferric chelate reductase (FRO2), which in turn provides Fe(II) for transport into the cell by IRT1. **(b)** Strategy II. Phytosiderophores (PS) are produced by the root cells, and are released into the rhizosphere by an unknown mechanism. PS bind to Fe(III) in the soil, and the resulting Fe(III)-PS complexes are taken up into root cells by the transporter YS1. **(c)** Rice uses the typical grass Strategy II mechanism, but can also take up Fe(II) directly through OsIRT1 and OsIRT2.

also use PS efficiently, since rice engineered to produce higher amounts of PS are more tolerant to iron deficient, calcareous soils [20,21]. The rice genome encodes two proteins related to the Strategy I transporter IRT1 (OsIRT1 and OsIRT2) that are specifically upregulated in roots of iron-deficient plants, are localized in the plasma membrane of root epidermal cells, and confer iron uptake in a yeast functional complementation assay [22,23]. Thus the activity and expression pattern of these transporters is consistent with a role in primary uptake of iron from the soil. Ishimaru *et al.* [24] have also expressed high levels of a yeast ferric chelate reductase in rice.

These plants have enhanced Fe uptake and increased amounts of Fe in their tissues. Since Fe(III)-PS uptake should not be affected by enhancing the amount of reduced iron in the rhizosphere, the most straightforward way to explain these results is that rice possesses an Fe(II)-uptake system in addition to the PS system. In rice plants with a mutation in the nicotianamine amino-transferase (NAAT) gene, phytosiderophores are not synthesized [25^{••}]. These mutant plants show a strong growth defect when iron is supplied as Fe(III), but no growth defect on Fe(II). Fe(II)-grown *naat1* mutant plants are apparently not experiencing iron deficiency, since many iron deficiency inducible genes that are upregulated when mutant plants are grown on Fe(III) are not upregulated in mutant plants grown on Fe(II). WT plants maintain low expression of these genes when adequate iron is present, regardless of iron speciation. Thus rice seems to be able to take up both Fe(III)-PS, and Fe(II), when it is available. An important implication of the phenotype of the *naat1* mutant is that rice apparently cannot accomplish ferric reduction, as nongrass plants routinely do. Thus, even though rice can use Fe(II) readily when it is available, the full Strategy I acidification/reduction mechanism is not in place in this species (Figure 1c). This may be an adaptation to the soil conditions in flooded, and thus oxygen-poor, rice paddies, in which levels of soluble Fe(II) are expected to be high.

Transcription factors implicated in regulation of the Strategy I response

A putative transcription factor (FER) that functions in the iron-deficiency-signaling pathway was first identified in tomato [26]. The *fer* mutant is unable to induce the Strategy I response upon iron limitation and is severely chlorotic. Map-based cloning showed that *FER* encodes a basic helix-loop-helix (BHLH) transcription factor that is expressed in the roots [26]. Expression of *FER* is controlled by iron availability at multiple levels [27[•]]. *FER* transcripts are detected when plants are grown with either low (0.1 μM) or moderate (10 μM) amounts of iron [26,27[•]] but *FER* mRNA levels are depressed when plants are grown with 100 μM iron. In addition, analysis of *FER* protein levels in *35S-FER* plants showed that although *FER* mRNA is abundant in *35S-FER* plants irrespective of the iron status, *FER* protein is detectable only when iron levels are relatively low [27[•]], indicating that *FER* expression is controlled post-transcriptionally. *FER* expression was examined in the *chloronerva* background as well [27[•]]. *Chloronerva* lacks the metal chelator nicotianamine (NA) and shows defects in iron homeostasis. Because NA is required for proper trafficking of iron, *chloronerva* plants display iron-deficiency-induced chlorosis, yet accumulate excess iron because of constitutive upregulation of the Strategy I response [28]. *FER* protein levels are high in roots of *chloronerva* plants grown with 100 μM iron indicating that externally supplied iron is not sufficient to cause the downregulation of *FER*. This

suggests that a leaf-derived signal regulates FER in response to iron status [27*].

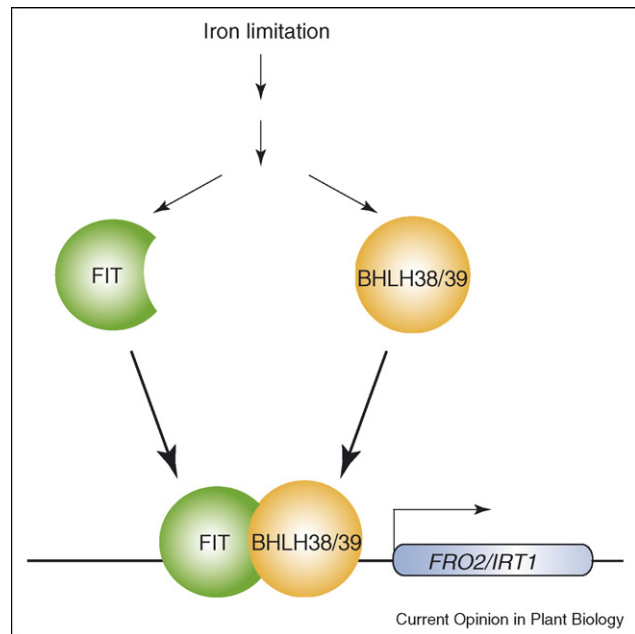
Studies in *Arabidopsis* identified FIT as the functional ortholog of FER [29**,30–32]. As was shown for *fer*, *Arabidopsis fit* is severely chlorotic, has reduced iron content and is unable to induce the Strategy I response [29**,30,31]. *FRO2* transcript abundance is dramatically reduced and ferric reductase activity is not induced by iron-deficiency in *fit*. However, although *IRT1* mRNA abundance is somewhat decreased in roots of iron-deficient *fit* plants [30,31], *IRT1* protein is not detectable in *fit* [29**]. These results suggested that FIT might function to control the Strategy I iron-uptake machinery at multiple levels and it was proposed that in addition to its role in induction of transcription of *FRO2* and *IRT1*, FIT might also act indirectly to prevent turnover of *IRT1* protein when iron is limiting [29**]. This model is appealing because *IRT1* is known to be subject to iron-induced protein turnover [33,34].

Constitutive high-level expression of *FIT* is not sufficient to induce high-level expression of *FRO2* and *IRT1* in roots under iron-sufficient conditions [29**,30,31], implying that FIT acts with a binding partner that is expressed only in response to iron limitation. Expression profiling experiments have implicated additional bHLH family members (BHLH38, BHLH39, BHLH100, and BHLH101) in the iron deficiency response [31,35,36*]. BHLH38 and BHLH39 physically interact with FIT and transgenic plants that constitutively coexpress either *bHLH38* or *bHLH39* with *FIT* show iron-independent high-level expression of *FRO2* and *IRT1* [36*], and accumulate more iron than wild-type plants [36*]. These data suggest that FIT functions together with either BHLH38 or BHLH39 to induce expression of the Strategy I iron-uptake machinery (Figure 2). Presumably, FIT and BHLH38/39 act directly to induce expression of *FRO2* and *IRT1* because coexpression of FIT with either BHLH38 or BHLH39 in yeast cells leads to the activation of GUS expression driven from the *IRT1* and *FRO2* promoters [36*].

Strategy II transcription factors

In rice, the bHLH protein OsIRO2 was identified as an iron-deficiency-induced gene in microarray expression profiling [37]. Although *IRO2* is well conserved in grasses, it is not closely related to *AtFIT* or *LtFER*, and, unlike these genes, *OsIRO2* is expressed in both roots and shoots. Plants overexpressing *OsIRO2* (*IRO2-OX*) exhibit improved growth (measured as plant height) under iron deficient conditions, while *OsIRO2* RNAi knockdown lines grown under iron deficiency show reduced biomass and chlorophyll content, and accumulate less Fe, Cu, Zn, and Mn [38*]. Expression of many genes involved in PS synthesis is enhanced in the *IRO2-OX* lines, and diminished in the corresponding RNAi lines. Expression of the

Figure 2



Model for the induction of *FRO2* and *IRT1* by FIT and BHLH38/BHLH39. Expression of FIT, BHLH38, and BHLH39 is increased under iron limitation. FIT heterodimerizes with either BHLH38 or BHLH39 to induce transcription of *FRO2* and *IRT1* in the outer layers of roots [36*].

putative rice *YS1* gene, *OsYSL15*, is markedly altered in the RNAi and OX lines, but interestingly, the expression of *OsIRT1* is unchanged, possibly indicating that OsIRO2 regulates the PS-mediated Fe uptake system of rice, but not the additional Fe(II) uptake mechanism. Interestingly, many of the genes regulated by OsIRO2 do not have the consensus IRO2 binding sequence (CTCGTGG) in their promoters, leading to the speculation that OsIRO2 may be acting by regulating other transcription factors [38*].

At least some of the factors regulating iron-deficiency-induced gene expression are conserved widely among land plants. *IDS2* is a dioxygenase involved in phyto siderophore biosynthesis [39]. Two cis-elements (IDE1 and IDE2) conferring iron regulated expression from the barley *IDS2* promoter were identified using promoter deletion analysis in tobacco [40]. Recently, trans-factors binding each of these elements were identified [41*,42**]. The rice *IDEF1* protein was identified using a candidate gene approach in which B3 DNA binding domain proteins of the ABI3/VP1 family were tested for the ability to bind to the IDE1 sequence. *IDEF1* mRNA is expressed in both roots and shoots, and is not itself regulated by iron. When *IDEF1* was coexpressed with an *IDE1* reporter construct in transgenic tobacco, iron-deficiency-induced gene expression was enhanced by ~2.5-fold. No enhanced expression was observed in

plants grown on sufficient iron, indicating that IDEF1 is specifically involved in iron regulated gene expression. In rice, overexpression of IDEF1 caused upregulation of the *OsIRT1* and *OsIRO2* genes, but failed to upregulate expression of a third iron-regulated gene, *OsNAS2*, even though this gene contains the IDE1 binding site in its promoter. Overexpression of IDEF1 in rice enhances the plants' ability to tolerate iron deficiency: both chlorophyll levels and plant height were improved in the transgenic lines. Iron content of the transgenic plants was not different from the untransformed controls, indicating that expression of IDEF1 primarily affects the efficiency of iron usage rather than the ability of the plant to take up additional iron during times of iron deficiency stress.

The *IDEF2* gene, which binds to the *IDE2* promoter element, was identified through yeast one hybrid assays, and encodes a member of the NAC family of transcription factors [42^{••}]. Like *IDEF1*, expression of the *IDEF2* transcript is not iron regulated, and *IDEF2* mRNA is present in both shoots and roots, although expression is substantially higher in shoots. IDEF2 function was blocked either with RNAi, or by fusing the repressive SRDX domain to the core DNA binding domain of IDEF2, and the effects were monitored using microarray analysis. Surprisingly, only one gene previously associated with iron uptake in rice was strongly affected in the transgenic lines: *OsYSL2*, which encodes a transporter of metal–nicotianamine complexes [43]. Expression of many other iron regulated genes was also affected, but genes with direct roles in PS synthesis or iron transport (e.g. *OsYSL15* or *OsIRT1*) were notably not affected in the transgenic lines. The transgenic lines exhibited increased accumulation of Fe in both shoots and roots of plants grown in iron sufficient conditions, while in iron deficient plants, lower than normal levels of iron were present, indicating an important role for IDEF2 in maintaining optimal levels of iron in tissues. Identification of the roles of the many uncharacterized genes regulated by IDEF2 can be expected to shed light on the processes that determine the total amount of iron that accumulates in tissues. Although no functional orthologs of IDEF1 have been identified in Strategy I plants [41[•]], genes that encode proteins with moderate homology to IDEF2 are present in the genomes of several Strategy I species [42^{••}]. It will be interesting to see if these putative IDEF2 orthologs play a role in the iron deficiency response.

Conclusions

In recent years, our understanding of the molecular mechanisms by which iron is transported into plant roots has improved dramatically. In addition, the functional characterization of a number of transcription factors that play roles in the induction of genes involved in iron uptake and utilization has provided much insight into iron-deficiency-signaling pathways, yet much remains to

be learned. For example, the identity of the iron sensor is not yet known. Although recent studies have revealed that ethylene and nitric oxide are involved in the upregulation of iron deficiency responses, it is not yet clear how these signals are integrated into the iron deficiency pathways [9,44–46]. The development of a comprehensive understanding of iron-deficiency signaling should enable genetic engineering strategies aimed at enhancing the iron content of staple crops and improving crop yield on marginal soils.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Römheld V: **Different strategies for iron acquisition in higher plants.** *Physiol Plant* 1987, **70**:231-234.
 2. Robinson NJ, Proctor CM, Connolly EL, Guerinot ML: **A ferric-chelate reductase for iron uptake from soils.** *Nature* 1999, **397**:694-697.
 3. Eide D, Broderius M, Fett J, Guerinot ML: **A novel iron-regulated metal transporter from plants identified by functional expression in yeast.** *Proc Natl Acad Sci U S A* 1996, **93**:5624-5628.
 4. Henriques R, Jasik J, Klein M, Martinoia E, Feller U, Schell J, Pais MS, Koncz C: **Knock-out of *Arabidopsis* metal transporter gene *IRT1* results in iron deficiency accompanied by cell differentiation defects.** *Plant Mol Biol* 2002, **50**:587-597.
 5. Vert G, Grotz N, Dedaldechamp F, Gaymard F, Guerinot ML, Briat J-F, Curie C: **IRT1, an *Arabidopsis* transporter essential for iron uptake from the soil and plant growth.** *The Plant Cell* 2002, **14**:1223-1233.
 6. Varotto C, Maiwald D, Pesaresi P, Jahns P, Francesco S, Leister D: **The metal ion transporter IRT1 is necessary for iron homeostasis and efficient photosynthesis in *Arabidopsis thaliana*.** *Plant J* 2002, **31**:589-599.
 7. Waters BM, Blevins DG, Eide DJ: **Characterization of FRO1, a pea ferric-chelate reductase involved in root iron acquisition.** *Plant Physiol* 2002, **129**:85-94.
 8. Li L, Cheng X, Ling HQ: **Isolation and characterization of Fe(III)-chelate reductase gene *LeFRO1* in tomato.** *Plant Mol Biol* 2004, **54**:125-136.
 9. Waters BM, Lucena C, Romera FJ, Jester GG, Wynn AN, Rojas CL, Alcantara E, Perez-Vincente R: **Ethylene involvement in the regulation of the H(+)-ATPase *CsHA1* gene and of the new isolated ferric reductase *CsFRO1* and iron transporter *CsIRT1* genes in cucumber plants.** *Plant Physiol Biochem* 2007, **45**:293-301.
 10. Eckhardt U, Marques AM, Buckhout TJ: **Two iron-regulated cation transporters from tomato complement metal uptake-deficient yeast mutants.** *Plant Mol Biol* 2001, **45**:437-448.
 11. Cohen CK, Garvin DF, Kochian LV: **Kinetic properties of a micronutrient transporter from *Pisum sativum* indicate a primary function in Fe uptake from the soil.** *Planta* 2004, **218**:784-792.
 12. Santi S, Cesco S, Varanini Z, Pinton R: **Two plasma membrane H(+)-ATPase genes are differentially expressed in iron-deficient cucumber plants.** *Plant Physiol Biochem* 2005, **43**:287-292.

13. Santi S, Schmidt W: **Laser-microdissection-assisted analysis of the functional fate of iron deficiency-induced root hairs in cucumber.** *J Exp Bot* 2008, **59**:697-704.
14. von Wirén N, Mori S, Marschner H, Römheld V: **Iron inefficiency in maize mutant *ys1* (*Zea mays* L. cv yellow-stripe) is caused by a defect in uptake of iron phytosiderophores.** *Plant Physiol* 1994, **106**:71-77.
15. Curie C, Panaviene Z, Loulergue C, Dellaporta SL, Briat J-F, Walker EL: **Maize *yellow stripe1* encodes a membrane protein directly involved in Fe(III) uptake.** *Nature* 2001, **409**:346-349.
16. Schaaf G, Ludewig U, Erenoglu BE, Mori S, Kitahara T, von Wirén N: **ZmYS1 functions as a proton-coupled symporter for phytosiderophore- and nicotianamine-chelated metals.** *J Biol Chem* 2004, **279**:9091-9096.
17. Murata Y, Ma JF, Yamaji N, Ueno D, Nomoto K, Iwashita T: **A specific transporter for iron(III)-phytosiderophore in barley roots.** *Plant J* 2006, **46**:563-572.
18. Bell WD, Bogorad LJM: **Yellow-stripe phenotype in maize. I. Effects of *ys1* locus on uptake and utilization of iron.** *Bot Gaz* 1962, **124**:1-8.
19. Mori S, Nishizawa N, Hayashi H, Chino E, Yoshimura E, Ishihara J: **Why are young rice plants highly susceptible to iron deficiency?** *Plant Soil* 1991, **130**:143-156.
20. Takahashi M, Nakanishi H, Kawasaki S, Nishizawa NK, Mori S: **Enhanced tolerance of rice to low iron availability in alkaline soils using barley nicotianamine aminotransferase genes.** *Nat Biotechnol* 2001, **19**:466-469.
21. Suzuki M, Morikawa KC, Nakanishi H, Takahashi M, Saigusa M, Mori S, Nishizawa NK: **Transgenic rice lines that include barley genes have increased tolerance to low iron availability in a calcareous paddy soil.** *Soil Sci Plant Nutr* 2008, **54**:77-85.
22. Bughio N, Yamaguchi H, Nishizawa NK, Nakanishi H, Mori S: **Cloning an iron-regulated metal transporter from rice.** *J Exp Bot* 2002, **53**:1677-1682.
23. Ishimaru Y, Suzuki M, Tsukamoto T, Sukuki K, Nakazono M, Kobayashi T, Wada Y, Watanabe S, Matsuhashi S, Takahashi M *et al.*: **Rice plants take up iron as an Fe³⁺-phytosiderophore and as Fe²⁺.** *Plant J* 2006, **45**:335-346.
24. Ishimaru Y, Kim S, Tsukamoto T, Oki H, Kobayashi T, Watanabe S, Matsuhashi S, Takahashi M, Nakanishi H, Mori S *et al.*: **Mutational reconstructed ferric chelate reductase confers enhanced tolerance in rice to iron deficiency in calcareous soil.** *Proc Natl Acad Sci U S A* 2007, **104**:7373-7378.
25. Cheng L, Wang F, Shou H, Huang F, Zheng L, He F, Li J, Zhao FJ, Ueno D, Ma JF *et al.*: **Mutation in nicotianamine aminotransferase stimulated the Fe(II) acquisition system and led to iron accumulation in rice.** *Plant Physiol* 2007, **145**:1647-1657.
- By preventing the synthesis of phytosiderophores in rice, the authors demonstrate that this species can use Fe(II) as the sole source of iron, and does not require Fe(III)-PS uptake as other grass species do.
26. Ling H-Q, Bauer P, Bereczky Z, Keller B, Ganai M: **The tomato *fer* gene encoding a bHLH protein controls iron-uptake responses in roots.** *Proc Natl Acad Sci U S A* 2002, **99**:13938-13943.
27. Brumbarova T, Bauer P: **Iron-mediated control of the basic helix-loop-helix protein FER, a regulator of iron uptake in tomato.** *Plant Physiol* 2005, **137**:1018-1026.
- The authors showed that iron status controls expression of FER at multiple levels. In addition, analysis of FER protein abundance in the *chloronerva* mutant indicated that FER protein levels are probably controlled by a leaf-derived iron signal as opposed to locally supplied iron.
28. Ling HQ, Koch G, Baulein H, Ganai MW: **Map-based cloning of *chloronerva*, a gene involved in iron uptake of higher plants encoding nicotianamine synthase.** *Proc Natl Acad Sci U S A* 1999, **96**:7098-7103.
29. Colangelo EP, Guerinot ML: **The essential basic helix-loop-helix protein FIT1 is required for the iron deficiency response.** *Plant Cell* 2004, **16**:3400-3412.
- Microarray expression profiling experiments led to the identification of *Arabidopsis* FIT1, a BHLH protein that is closely related to the tomato FER protein. Loss of FIT1 function results in severe chlorosis and seedling lethality that can be rescued by the application of exogenous iron. The authors showed that FIT1 is expressed in the outer layers of iron deficient roots and functions in the iron deficiency pathway to regulate FRO2 and IRT1.
30. Jakoby M, Wang HY, Reidt W, Weisshaar B, Bauer P: **FRU (BHLH029) is required for induction of iron mobilization genes in *Arabidopsis thaliana*.** *FEBS Lett* 2004, **577**:528-534.
31. Yuan YX, Zhang J, Wang DW, Ling HQ: **AtbHLH29 of *Arabidopsis thaliana* is a functional ortholog of tomato FER involved in controlling iron acquisition in Strategy I plants.** *Cell Res* 2005, **15**:613-621.
32. Bauer P, Ling HQ, Guerinot ML: **FIT, the FER-LIKE IRON DEFICIENCY INDUCED TRANSCRIPTION FACTOR in *Arabidopsis*.** *Plant Physiol Biochem* 2007, **45**:260-261.
33. Connolly EL, Fett JP, Guerinot ML: **Expression of the IRT1 metal transporter is controlled by metals at the levels of transcript and protein accumulation.** *Plant Cell* 2002, **14**:1347-1357.
34. Kerkeb L, Mukherjee I, Chatterjee I, Lahner B, Salt DE, Connolly EL: **Iron-induced turnover of the *Arabidopsis* IRON-REGULATED TRANSPORTER 1 metal transporter requires lysine residues.** *Plant Physiol* 2008, **146**:1964-1973.
35. Wang HY, Klatte M, Jakoby M, Baumlein H, Weisshaar B, Bauer P: **Iron deficiency-mediated stress regulation of four subgroup Ib BHLH genes in *Arabidopsis thaliana*.** *Planta* 2007, **226**:897-908.
36. Yuan Y, Wu H, Wang N, Li J, Zhao W, Du J, Wang D, Ling HQ: **FIT interacts with AtbHLH38 and AtbHLH39 in regulating iron uptake gene expression for iron homeostasis in *Arabidopsis*.** *Cell Res* 2008, **18**:385-397.
- The authors showed that BHLH38 and BHLH39 interact with FIT through yeast two hybrid and BiFC studies. Both genes are upregulated by iron deficiency and coexpression of FIT with BHLH38 or BHLH39 in plants resulted in constitutive expression of FRO2 and IRT1. This work shows that BHLH38 and BHLH39 can function as partners with FIT in the iron-deficiency-signaling pathway.
37. Ogo Y, Itai RN, Nakanishi H, Inoue H, Kobayashi T, Suzuki M, Takahashi M, Mori S, Nishizawa NK: **Isolation and characterization of IRO2, a novel iron-regulated bHLH transcription factor in graminaceous plants.** *J Exp Bot* 2006, **57**:2867-2878.
38. Ogo Y, Itai RN, Nakanishi H, Kobayashi T, Takahashi M, Mori S, Nishizawa NK: **The rice bHLH protein OsIRO2 is an essential regulator of the genes involved in Fe uptake under Fe-deficient conditions.** *Plant J* 2007, **51**:366-377.
- The rice BHLH transcription factor IRO2 was identified as a gene up-regulated by iron deficiency stress. IRO2 influences the expression of genes involved in PS synthesis and uptake in rice, but probably does this indirectly, through the action of additional, unknown factors.
39. Nakanishi H, Yamaguchi H, Sasakuma T, Nishizawa NK, Mori S: **Two dioxygenase genes, *Ids3* and *Ids2*, from *Hordeum vulgare* are involved in the biosynthesis of mugineic acid family phytosiderophores.** *Plant Mol Biol* 2000, **44**:199-207.
40. Kobayashi T, Nakayama Y, Itai RN, Nakanishi H, Yoshihara T, Mori S, Nishizawa NK: **Identification of novel cis-acting elements, *IDE1* and *IDE2*, of the barley *IDS2* gene promoter conferring iron-deficiency-inducible, root-specific expression in heterogeneous tobacco plants.** *Plant J* 2003, **36**:780-793.
41. Kobayashi T, Ogo Y, Itai RN, Nakanishi H, Takahashi M, Mori S, Nishizawa NK: **The transcription factor IDEF1 regulates the response to and tolerance of iron deficiency in plants.** *Proc Natl Acad Sci U S A* 2007, **104**:19150-19155.
- Using a candidate gene approach in rice, the B3 family transcription factor, IDEF1 was identified. Although plants overexpressing IDEF1 do not accumulate additional iron, they exhibit resistance to iron-deficiency-induced chlorosis, confirming that IDEF1 is able to mitigate iron-deficiency stress.
42. Ogo Y, Kobayashi T, Itai RN, Nakanishi H, Kakei Y, Takahashi M, Toki S, Mori S, Nishizawa NK: **A novel NAC transcription factor IDEF2 that recognizes the iron deficiency-responsive element**

- 2 regulates the genes involved in iron homeostasis in plants.** *J Biol Chem* 2008, **283**:13407-13417.
- Overexpression and underexpression analysis of IDEF2 demonstrates that this transcription factor has a strong influence on iron accumulation in rice. Since most of the genes typically associated with iron uptake are not mis-regulated, IDEF2 is likely to regulate a previously unrecognized set of genes involved in controlling the amount of iron that accumulates in tissues.
43. Koike S, Inoue H, Mizuno D, Takahashi M, Nakanishi H, Mori S, Nishizawa NK: **OsYSL2 is a rice metal-nicotianamine transporter that is regulated by iron and expressed in the phloem.** *Plant J* 2004, **39**:415-424.
 44. Graziano M, Lamattina L: **Nitric oxide accumulation is required for molecular and physiological responses to iron deficiency in tomato roots.** *Plant J* 2007, **52**:949-960.
 45. Graziano M, Lamattina L: **Nitric oxide and iron in plants: an emerging and converging story.** *Trends Plant Sci* 2005, **10**:4-8.
 46. Lucena C, Waters BM, Romera FJ, Garcia MJ, Morales M, Alcantara E, Perez-Vincente R: **Ethylene could influence ferric chelate reductase, iron transporter, and H⁺-ATPase gene expression by affecting FER (or FER-like) gene activity.** *J Exp Bot* 2006, **57**:4145-4154.