Iron acquisition by plants

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In nongraminaceous plants, the Fe^{II}-transporter gene and ferric-chelate reductase gene have been cloned from *Arabidopsis thaliana*, whereas Fe^{III}-reductase has not. In graminaceous monocots, the genes for mugineic acids (MAs) synthesis, *nas* (nicotianamine synthase) and *naat* (nicotianamine aminotransferase), have been cloned from barley, whereas the Fe^{III}-MAs transporter gene is yet to be cloned. Transferrin absorption in *Dunaliella* has been reported, suggesting a phagocytotic (endocytotic) Fe-acquisition mechanism. Work to develop transgenic cultivars tolerant to Fe-deficiency in calcareous soils is now in progress.

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Current Opinion in Plant Biology 1999, **2**:250–253

http://biomednet.com/elecref/1369526600200250

Abbreviations

MA	mugineic acid
NAAT	nicotianamine aminotransferase
NAS	nicotianamine synthase
PETIS	positron emitting imaging system

Introduction

Iron is essential for the synthesis of chlorophyll and heme. Lime-induced chlorosis in calcareous soil (high pH), therefore, is a major agricultural problem resulting in reduced crop yields, because about 30% of the world's cultivated soils are calcareous. For example, plants demand ~10^{-4}–10^{-8} M Fe^{III} ions for normal growth, but, theoretically, only 10^{-17} M are soluble at pH 7. Plants have evolved two seperate mechanisms for the aquisition of iron, which, as I will discuss in more detail throughout the review, can be termed Strategy-I and Strategy-II. These working hypotheses, first proposed by Römheld and Marschner [1], still hold true today.

Strategy-I

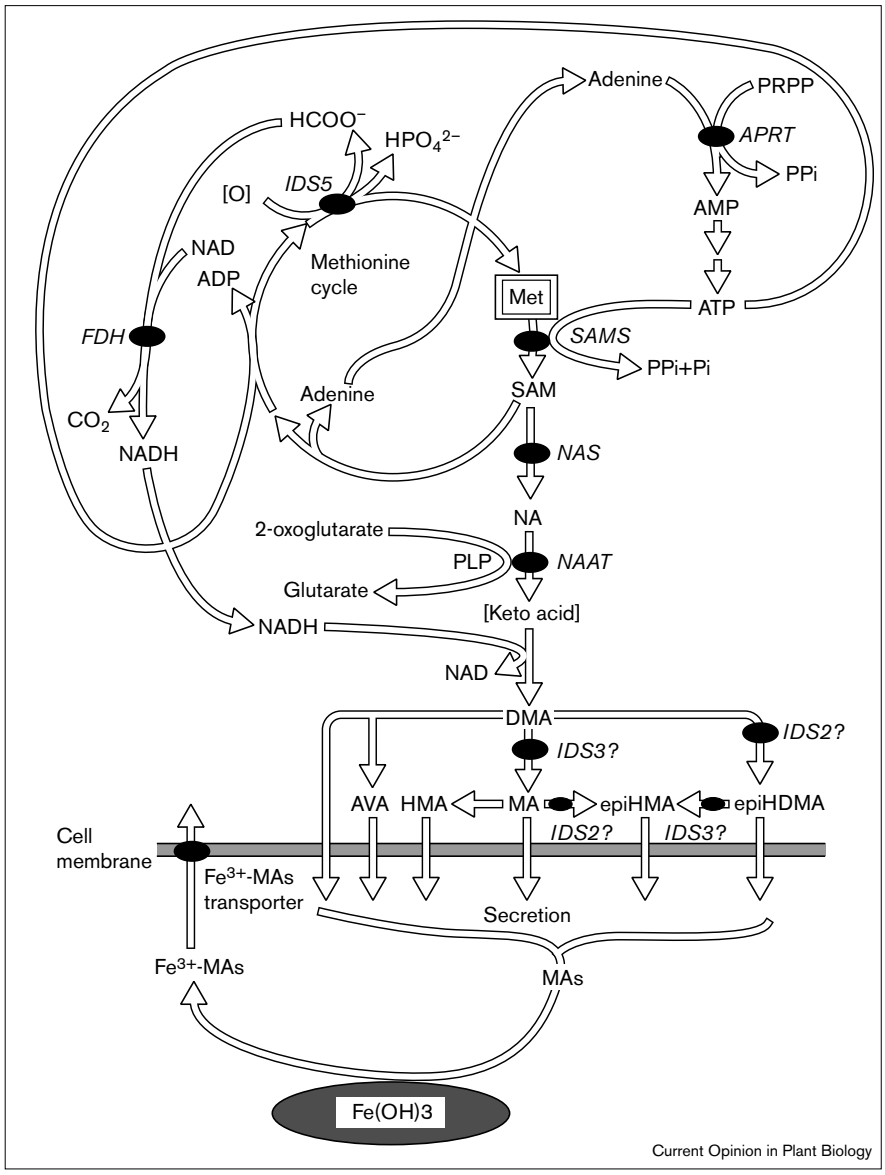
Strategy-I is an iron acquisition mechanism used by all higher plants except graminaceous monocots. Under Fe-deficient conditions, nongraminaceous plants release reductants or chelators in the rhizosphere, enhance proton excretion in the rhizosphere, increase their ferric reduction capacity at the root surface, and transport Fe^{II} ion through the Fe^{II}-transporter in the plasmamembrane. In most nongraminaceous plants, ferric reductase is an important enzyme that is closely tied to tolerance of Fe-deficiency. Ferric reductase, however, has never been purified sufficiently to determine its partial amino acid sequences, which would be one route to the cloning of ferric reductase genes. In an alternative strategy, Dancis *et al.* [2,3] cloned two ferric reductase genes (*FRE1* and *FRE2*) from *Saccharomyces cerevisiae* using yeast complementation. At last, however, Robinson *et al.* [4••] have cloned the Fe^{III}-reductase gene (*FR02*) from Fe-deficient roots of *Arabidopsis*, which is allelic to the *frd1* mutations that impair the activity of ferrric-chelate reductase. Many genes for proton extrusion ATPases have been cloned in plants; however, the successful cloning of the specific ATPase genes regulated by Fe-deficiency has yet to be reported. The cloning of Fe^{II}-transporter genes from plants has proved elusive for a long time, but two types of Fe^{II}-transporter cDNA have been isolated in yeast. Fet4 protein is proposed to be a low-affinity Fe^{II}-transporter [5] and Fet3 protein is a multicopper oxidase [6] which is regulated by copper and interacts with Ftr1 protein to form a complex that is a high-affinity Fe^{III}-transporter system. Recently, Eide *et al.* [7] successfully cloned a plant iron-regulated transporter cDNA (*IRT1*) by introducing an *Arabidopsis thaliana* cDNA library into a yeast double mutant (*fet3 fet4*). *IRT1* gene expression was only induced in the roots of Fe-deficient *Arabidopsis*. Introducing a high copy number of *IRT1* genes into *Saccharomyces cerevisiae* increased the uptake of Fe.

Strategy-II

After Takagi identified mugineic acid in the root-washings of Fe-deficient rice in 1976 [8], the Strategy-II iron acquisition mechanism was proposed. Graminaceous monocots release Fe-chelating substances, mugineic acid family phytosiderophores (MAs), in response to Fe-deficiency stress. These phytosiderophores solubilize inorganic Fe^{III}-compounds by chelation, and the Fe^{III}-MAs complexes are taken up through a specific transport system in the root plasmamembrane. From 1986 to date, most steps of the biosynthetic pathways from methionine to MAs in Fe-deficient barley roots have been deduced [9–13,14••]. These pathways are shown in Figure 1. Ma *et al.* [15] proposed that methionine from the methionine cycle (Yang cycle) was one of the sources of MAs. Nakanishi *et al.* [16••], however, recently followed ^{11}C-methionine transport in barley in real time using PETIS (positron emitting imaging system) and proved that methionine from a cut leaf of Fe-deficient barley did not move to the roots, but moved to other chlorotic leaves preferentially. This suggested that methionine from the leaves could not be used to synthesize MAs in the roots, but methionine for MAs synthesis was supplied from the methionine cycle operating in the roots.

Nicotianamine synthase (NAS) and nicotianamine aminotransferase (NAAT) are strongly induced in the roots of Fe-deficient barley. Characterization and purification of NAS have been performed by the parallel work of Higuchi *et al.* [17] and Herbrick *et al.* (abstract from the International Symposium on Iron Nutrition Interactions in Pants. Hohenheim University, 1997, p72). Based on the partial peptide sequences of the purified NAS, Higuchi *et al.* [18••] successfully isolated seven *nas*-cDNAs from a cDNA library constructed from mRNA of Fe-deficient barley roots

Figure 1



Biosynthetic pathway of mugineic acid family phytosiderophores in Fe-deficient barley roots (Strategy-II) and rhizosphere conditions. *APRT*, *adeninephosphoribosyltransferase*; *SAMS*, *S-adenosyl methionine synthetase*; *NAS*, *nicotianamine synthase*; *NAAT*, *nicotianamine aminotransferase*; *IDS3*, *iron deficiency specific clone 3* (putative mugineic acid synthetase); *FHD*, *formate dehydrogenase*; *IDS5*, see text; Met, methionine; SAM, S-adenosylmethionine; NA, nicotianamine; DMA, deoxymugineic acid; epiHDMA, epihydroxy-deoxymugineic acid; MA, mugineic acid; HMA, hydroxymugineic acid; epiHMA, epihydroxymugineic acid; AVA, avenic acid; MAs, mugineic acid family phytosiderophores; PLP, pyridoxal phosphate; PRPP, phosphoribosylpyrophosphate.

[19••]. After purification of NAAT, Takahashi *et al.* cloned two *naat* cDNAs from Fe-deficient barley roots on the basis of the partial peptide sequences of NAAT, and also cloned tandem arrayed genomic *naat-B•naat-A* gene (DDBJ, AB024006) from Fe-deficient barley roots [19••]. Northern analysis showed that Fe-deficiency specifically induced *naat-A* (DDBJ D88273) and *naat-B* (DDBJ AB005788), whereas the later had a different expression pattern.

By sequencing the proteins on 2D-SDS PAGE that specifically appeared from Fe-deficient barley roots, Itai *et al.*, (Plant Cell Physiol Abstract 1998, 39:s147) have cloned a barley adenine phosphoribosyl transferase gene, Hv *aprt* DDBJ ABO12046. Suzuki *et al.* [20••] cloned formate dehydrogenase (*Fdh*; DDBJ D88272). Nakanishi *et al.* [21] cloned an iron deficiency specific *Ids3* (DDBJ D37796) gene by differential hybridization and just recently Yamaguchi *et al.* have cloned the *Ids5* gene (ADBO 22597). APRT plays a role in scavenging adenine, which is released from the methionine cycle as AMP. IDS5 enzyme is a member of the methionine cycle which converts 1,2-dehydroxy-3-keto-methylthiopentene anion to 2-keto-4-methylthiobutylic acid and releases formate. Iron-deficiency induced formate dehydrogenase (FDH) is thought to detoxify the formate released by IDS5 from the methionine cycle, producing NADH which may be used for the synthesis of deoxymugineic acid from 3-keto acid (Figure 1). Because Fe is essential for synthesis of the porphyllin ring as a precursor of heme as well as chlorophyll, heme synthesis declines under Fe-deficient conditions in the roots, which means physiological anoxia is occurring in Fe-deficient barley roots even though oxygen is present [16••,18••]. Under these conditions, not only FDH but also alcohol dehydrogenase (ADH, DDBJ AB006592) were induced in the roots [20••,22] for energy production.

Cloning of the hypothetical 'Fe^{III}-MAs transporter' gene has been attempted, but complimentation of the yeast mutants (*ctr1*, *ftr1*, *fre1*, *ftr1 fre1 fet4*) by the introduction of cDNA from Fe-deficient barley has not yet been successful [23]. Purification of the root specific and Fe-deficiency specific proteins that are present in wild-type corn roots but absent in the *ys1* mutant has not yet been successful either [24].

Endocytotic iron acquisition mechanism?

Recently, a novel type of high-affinity Fe-transporter gene family (*Nramp*) has been cloned from mammals [25,26]. The products of these genes help to prevent engulfed microbes from biosynthesizing active defense enzymes that contain Fe^{II} or other metals as a cofactor by withdrawing those metals from the phagosome where microbes are engulfed. In this mechanism, Fe protein attatched to the plasmamembrane surface may be invaginated by an endocytotic mechanism. From the interior surface of the endocytotic vacuole, iron is split from the protein after digestion by proteases which are secreted from the endoplasmic reticulum surrounding the endocytotic vacuole, and then free Fe^{II} iron is absorbed into the cytoplasm through the *Nramp* type of Fe^{II}-transporter. Is this type of Fe-transport mechanism also present in the plant kingdom? One point indicating that this may indeed be the case comes from the report that "Fe uptake by the halotolerant Alga *Dunaliella* is mediated by a plasma membrane 150 kDa transferrin" [27••]. Perhaps the incorporation of transferrin into the algal cell occurs by the same mechanism as Fe-uptake in animal cells [28]. In fact, 20 years ago we reported that rice root cells, which were water-cultured with hemoglobin as the Fe source and sole source of nitrogen, possessed many endocytotic vacuoles that originated from the endocytosis of plasmamembrane-bound hemoglobin. This plant endocytotic mechanism has recently been reviewed by Nishizawa and Mori [29]. It seems possible that plant genomes include *Nramp* genes ([30] DDBJL 41217) for an endocytotic Fe acquisition mechanism, and this may function under the specific conditions where there are high levels of macromolecular organic Fe.

Transgenic plants tolerant to Fe-deficiency

In order to cure 'Fe-chlorosis', one alternative technology is to develop tolerant cultivars. This can be done both by conventional methods, including either crossing or grafting, and by using plant biotechnological means, for example the generation of transgenic plants.

Introducing the *refre1* gene into tobacco

As the ferric reductase gene from plants had yet to be cloned, (although it since has been [4••]), Ohki *et al.* [31••] introduced the *refre1* gene, which is a completely synthesised reconstructed *FRE1* gene, into tobacco plants (*Nicotiana tabacum* L cv. SRI) after the failure of direct introduction of *FRE1* into tobacco by Yamaguchi *et al.* (BioIron [ICBI] abstract, Ashville, 1995, p84). The transgenic plants expressed the full-length mRNA, and constitutive ferric reductase activity was observed under Fe-sufficient conditions [31••]. Samuelson *et al.* [32••] also produced transgenic tobacco with high *FRE2* expression. However, both authors' studies are still immature because the transgenic tobacco have less constitutive ferric reductase activity than that of the induced ferric reductase activity of the wild-type tobacco under Fe-deficient conditions. Although an Fe^{II}-transporter has been cloned [7], nobody has reported the introduction of this gene into dicot plants. It seems likely that the introduction of *FRO2* genes into dicots will be achieved in the near future.

Introduction of strategy-II genes into rice

The introduction of either the *nas* or *naat* gene, or both, into an Fe-deficiency susceptible cultivar of a graminaceous plant is an attractive concept for two reasons: first, because these genes are strongly induced by Fe-deficiency stress, and second because the activities of these enzymes are highly correlated with the total amount of MAs secreted. Takahashi *et al.* (*Plant Cell Physiol* Abst 1999, 40:s95) have introduced the *naat-A* gene with the 35S promoter into rice and have produced several rice lines tolerant to iron deficiency in calcereous soils. Work in my laboratory is now introducing an 11.2 kb segment of barley genomic DNA, which has the full length of the promoter region of *naat-A*, directly into the rice genome using a large capacity binary vector developed by Akiyama *et al.* (*Plant Cell Physiol* Abstract 1997, 39:s94). We are hoping to get the transgenic rice lines which show root-specific and iron-deficiency specific expression of *naat*. This vector is recommended for easy stabilization of the transgenic genome into the host genome because of genomic synteny between rice and barley.

Conclusions

Stemming from the Strategy-I and Strategy-II hypothesis for Fe acquisition mechanisms in plants, research has led to significant advances at both the practical and molecular levels in the last two decades. These advances have still to lead to the development of new transgenic cultivars that will remedy the problems of Fe-chlorosis on calcareous soils. In particular, intensive effort should focus on cloning the homologous genes of ferric reductase (*FR02*) and Fe^{II}-transporter (*ITR1*) of other species. The cloning of the FE^{II}-deficiency induced ATPase gene is also kept waiting as an important component of the Strategy-I mechanism. The genes involved in the Strategy-II mechanism should be much more closely examined at the genomic DNA level. The promoter region (*cis* elements) for iron deficiency-specific and root-specific regulation of *FR02* and *IRT1* genes in *Arabidopsis* and of genomic *naa* and *nas* genes in barley (Figure 1) should be clarified. The combination of the strongest Fe-deficiency specific and root specific promoter with the open reading frames of *FR02*, *IRT1*, *naat* or *naas* will hopefully make stronger transgenic plants that are more tolerant to Fe-deficiency. *trans*-acting factors that interact to Fe-deficiency specific *cis*-element(s) have not yet been cloned. Manipulation of such a transfactor may be another way to make transgenic plants tolerant to Fe-deficiency, just as overexpression of *CBF1*, a transfactor for cold

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References and recommended reading

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