

A novel iron-regulated metal transporter from plants identified by functional expression in yeast

[Fe(II) transport/iron deficiency/strategy I response]

DAVID EIDE*[†], MARGARET BRODERIUS*, JANETTE FETT[‡], AND MARY LOU GUERINOT[‡]

*Department of Biochemistry and Molecular Biology, University of Minnesota School of Medicine, Duluth, MN 55812; and [‡]Department of Biological Sciences, Dartmouth College, Hanover, NH 03755

Communicated by Richard D. Klausner, National Cancer Institute, Bethesda, MD, February 9, 1996 (received for review January 2, 1996)

ABSTRACT Iron is an essential nutrient for virtually all organisms. The *IRT1* (iron-regulated transporter) gene of the plant *Arabidopsis thaliana*, encoding a probable Fe(II) transporter, was cloned by functional expression in a yeast strain defective for iron uptake. Yeast expressing *IRT1* possess a novel Fe(II) uptake activity that is strongly inhibited by Cd. *IRT1* is predicted to be an integral membrane protein with a metal-binding domain. Data base comparisons and Southern blot analysis indicated that *IRT1* is a member of a gene family in *Arabidopsis*. Related sequences were also found in the genomes of rice, yeast, nematodes, and humans. In *Arabidopsis*, *IRT1* is expressed in roots, is induced by iron deficiency, and has altered regulation in plant lines bearing mutations that affect the iron uptake system. These results provide the first molecular insight into iron transport by plants.

Iron deficiency is one of the most common human nutritional disorders in the world today (1). Indeed, iron is an essential nutrient for virtually all organisms because it plays a critical role in important biochemical processes such as respiration and photosynthesis. Although abundant in nature, iron is often available in limited amounts because the oxidized form, Fe(III), is extremely insoluble at neutral or basic pH. This fact is of particular importance to agriculture because approximately one-third of the world's soils are classified as iron-deficient (2). Many "iron-efficient" plant varieties have iron uptake strategies (designated strategy I or strategy II) that, not surprisingly, are directed at solubilizing iron (3). Strategy II plants, which includes all of the grasses, release Fe(III)-binding compounds called "phytosiderophores" into the surrounding soil that bind iron and are then taken up into the roots. Most other iron-efficient plants use strategy I and respond to iron deprivation by inducing the activity of membrane-bound Fe(III) chelate reductases that reduce Fe(III) to the more soluble Fe(II) form. The Fe(II) product is then taken up into the roots by an Fe(II)-specific transport system that is also induced by iron-limiting growth conditions (4). Furthermore, the roots of strategy I plants release more protons when iron-deficient, lowering the rhizosphere pH and thereby increasing the solubility of Fe(III).

We have used functional expression in yeast to identify a gene that encodes a probable Fe(II) transporter expressed in the roots of the strategy I plant *Arabidopsis thaliana*. There is striking similarity between iron uptake in strategy I plants and the mechanism of iron uptake in *Saccharomyces cerevisiae* (2). In *S. cerevisiae*, Fe(III) reductases in the plasma membrane reduce extracellular Fe(III) to Fe(II) (5–7). The Fe(II) product is then taken up by either of two uptake systems. One system, with low affinity for substrate, requires the Fe(II) transporter encoded by the *FET4* gene (8). The second system has high affinity for Fe(II) and is induced under conditions of iron

limitation. The high affinity system requires the FET3 multicopper oxidase for activity (9, 10). It has been proposed that FET3, as one component of a multisubunit transporter complex, is responsible for oxidizing Fe(II) back to Fe(III) during the transport process. A *fet3 fet4* double mutant, although viable, is extremely sensitive to iron limitation (8). In this report, we describe the successful isolation and characterization of a gene from *A. thaliana*, *IRT1*, that suppresses the growth defect of a *fet3 fet4* strain on iron-limited media. To our knowledge, *IRT1* is the first gene encoding an Fe(II) transporter to be cloned from plants or animals.

MATERIALS AND METHODS

Yeast Growth Conditions and Library Screening. Yeast cells were grown in 1% yeast extract, 2% peptone supplemented with 2% glucose (YPD). The pH of liquid YPD medium was lowered to pH 4.0 with HCl to aid growth of *fet3 fet4* double mutants. YPD medium was made iron-limiting by adding 80 μ M bathophenanthroline disulfonate (BPS; Sigma). Cells were also grown in synthetic defined medium (SD, 6.7 g/liter of yeast nitrogen base without amino acids) supplemented with 20 g/liter of glucose and necessary auxotrophic supplements. This medium was also supplemented with 10 μ M FeCl₃ and the pH was lowered to 3.5 to aid growth of the *fet3 fet4* strain. DEY1453 (*MATa/MATa ade2/+ can1/can1 his3/his3 leu2/leu2 trp1/trp1 ura3/ura3 fet3-2::HIS3/fet3-2::HIS3 fet4-1::LEU2/fet4-1::LEU2*) was transformed using standard procedures (11) with a plasmid library containing *A. thaliana* cDNAs inserted under the control of the phosphoglycerate kinase promoter in pFL61 (12). The poly(A)⁺ RNA used to construct this library was isolated from whole young seedlings (stage two leaves) grown on an iron-sufficient medium. Ura⁺ transformants were isolated, pooled into 100 groups of 30,000 transformants each (i.e., 3×10^6 total transformants), and 1×10^6 cells from each pool were inoculated onto 100 YPD plus 80 μ M BPS plates. Cells plated from six pools of transformants gave rise to several large colonies on this medium and a single colony was selected from each pool for further analysis. Plasmids were selectively removed from transformants using 5-fluoroorotic acid (13).

Yeast DNA Manipulations. *Escherichia coli* TOP10F' cells (Stratagene) were used for all recombinant DNA procedures. The plasmid pZH1 was constructed by inserting the 1.4 kb *NotI* insert fragment from one isolate, pIRT-1, into the *NotI* site of pBluescript SK (+) (Stratagene). Sequence analysis of the insert in pZH1 was performed by LARK Sequencing Technologies (Houston). Computer database comparisons were performed using BLAST software (conducted Nov. 1995) (14);

Abbreviations: BPS, bathophenanthroline disulfonate; EST, expressed sequence tag.

Data deposition: The sequence reported in this paper has been deposited in the GenBank data base (accession no. U27590).

[†]To whom reprint requests should be addressed.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

hydropathy analysis was performed and potential transmembrane segments were identified using the TOP-PREDII program (15).

Iron Uptake Assays. Iron uptake assays using $^{55}\text{FeCl}_3$ (Amersham) were performed as described (7) except that MGN (10 mM Mes/2% glucose/1 mM nitrilotriacetic acid, pH 6.1) was used for the assay buffer. Where noted, 1 mM sodium ascorbate was added to reduce Fe(III) to Fe(II). Stock solutions of the chloride salt of each metal (except for iron) were prepared in water at a concentration of 100 mM and diluted into MGN to a final concentration of 10 μM before addition of the cells. The $^{56}\text{FeCl}_3$ stock was 50 mM prepared in 0.1 M HCl. The statistical significance of differences in values relative to controls was determined using STATVIEW software (Abacus Concepts, Berkeley, CA). Data was subjected to one-way analysis of variance (ANOVA) followed by a Scheffe's test.

Plant Growth Conditions. Seeds of *A. thaliana* (Columbia ecotype) WT, *frd1*, and *frd3* (16) were surface-sterilized and sown on plates of Gamborg's B5 medium (Sigma) with 2% sucrose, 0.5 g/liter Mes, and 0.7% agar (final pH 5.8). Plates were stored for 2 days in the dark at 4°C and then incubated at 21°C under constant illumination (65 $\mu\text{E m}^{-2}\text{s}^{-1}$) for 11 days. A 3-mm thick yellow acrylic filter (acrylic yellow-2208, Cadillac Plastic and Chemical, Pittsburgh) was placed between the light source and the plates to prevent the photochemical degradation of Fe(III)-EDTA (17). Seedlings were then transferred to either iron-sufficient or iron-deficient nutrient plates. The medium contained macro- and micronutrients (18) plus 0.7% agar and 0.5 g/liter of Mes (final pH 6.0). The iron-sufficient medium contained 50 μM Fe(III)-EDTA and the iron-deficient medium contained 300 μM FerroZine [3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine sulfonate, HACH Chemical (Ames, IA)]. Plates were incubated for 3 days in the growth chamber described above.

Arabidopsis Nucleic Acid Analysis. For Southern blot analysis, 15- μg samples of *Arabidopsis* genomic DNA (19) were digested overnight with the appropriate restriction enzymes, separated by electrophoresis on a 0.8% agarose gel, transferred to a nitrocellulose membrane (Schleicher & Schuell), and bound to the membrane by UV crosslinking (Stratalinker; Stratagene). Standard procedures were used for prehybridization and hybridization (20). Membranes were then washed twice at room temperature for 15 min in 5 \times SSPE, 0.1% SDS, followed by two 15 min washes in 0.1 \times SSPE, 0.1% SDS at 50°C (high stringency) or at room temperature (low stringen-

cy). Membranes were stripped for reprobings with a boiling solution of 1 \times SSC, 0.1% SDS. Southern blot analysis of genomic DNA from Columbia and Landsberg ecotypes digested with *SaI* and probed with a labeled *IRT1* fragment revealed a restriction fragment length polymorphism between these lines. To map *IRT1*, Southern blots of genomic DNA from 30 recombinant inbred lines (21) were then analyzed for segregation of the polymorphism. The *IRT1* segregation data were compared with the segregation patterns of other markers and the *IRT1* map position was determined using MAPMAKER software (22). RNA was extracted (23) from root and shoot fractions of plants that had been grown axenically on either iron-sufficient or iron-deficient plates. Samples (10 μg) of RNA were denatured and electrophoresed on a 0.8% agarose, 6.2% formaldehyde gel and then transferred to a nylon membrane (BioTrans; ICN). RNA was bound to the membrane by UV crosslinking (Stratalinker; Stratagene). The membrane was prehybridized, hybridized, washed, and stripped as described by Pilgrim and McClung (24). DNA fragments used as hybridization probes were radiolabeled by the random primer method (25). For Southern blot analysis, the 1.4-kb *NotI* insert fragment of pIRT-1 and the 1.3-kb *EcoRI/XbaI* insert fragment of expressed sequence tag (EST) 37F12T7 were used as probes for *IRT1* and *IRT2*, respectively. The same *IRT1* DNA fragment was used as a probe for Northern blot analysis as well as the 2.5-kb *EcoRI* insert fragment of pARR16 encoding rRNA (26).

RESULTS AND DISCUSSION

Isolation and Sequence Analysis of the *IRT1* Gene. An *A. thaliana* cDNA library was screened for clones that, when expressed in *S. cerevisiae*, could restore iron-limited growth to a yeast strain defective for iron uptake. A *fet3 fet4* double mutant is sensitive to iron limitation due to its reliance on additional and apparently less efficient uptake mechanisms. This mutant strain was transformed with an *Arabidopsis* cDNA library constructed in a yeast expression vector, and approximately 3 \times 10⁶ independent transformants were screened on a rich medium made iron-limiting by adding the Fe(II) chelator, BPS. Six independent transformants that formed larger colonies on this medium were isolated. The plasmids carried by these transformants were required for the improved growth; this ability was lost when the plasmid was removed from each strain. Restriction endonuclease mapping indicated that all six

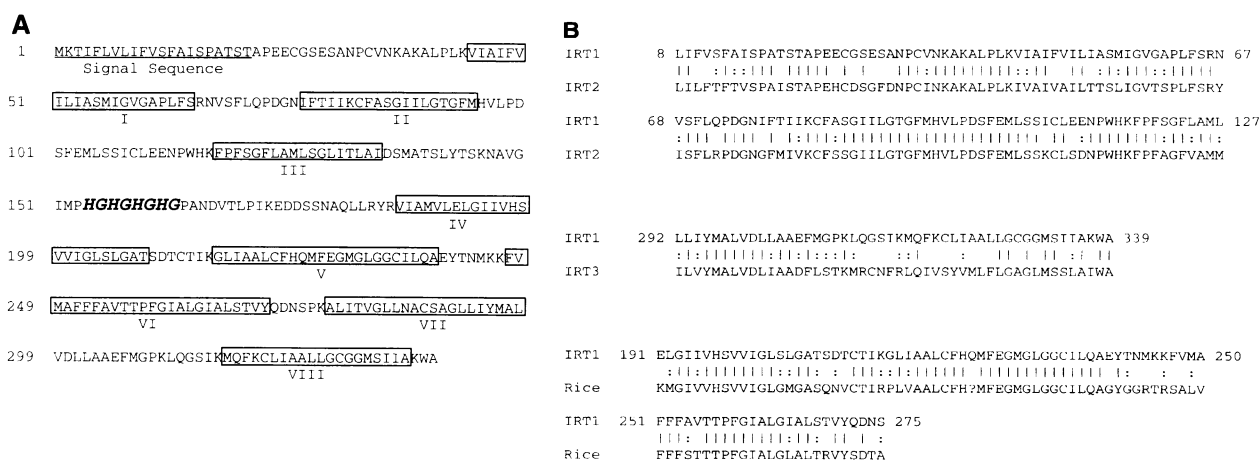


FIG. 1. Predicted amino acid sequence of the *IRT1* protein. (A) Amino acids are numbered on the left beginning with the initiator methionine residue. The potential signal sequence is underlined, the histidine-glycine repeats that are proposed to form a metal-binding domain are in boldface and italic, and the putative membrane-spanning domains detected by the TOP PRED II program (15) are boxed and numbered I-VIII. (B) Similarity of the *IRT1* amino acid sequence to other plant sequences in the current sequence databases. The GenBank data base accession numbers for *IRT2*, *IRT3*, and the rice EST are T04324, M35868, and D49213, respectively. The numbers refer to the *IRT1* amino acid sequence, bars indicate positions of amino acid identity, and positions of conservative substitutions are indicated by the colons. Conservative substitutions are based on the following groupings of amino acids: (L, I, V, M), (A, G, P, S, T), (R, K, H), (Q, D, E, N), and (F, Y, W) (27).

plasmids contain inserts derived from the same gene. We have designated this gene *IRT1* for iron-regulated transporter. *IRT1* was mapped to chromosome 4 by restriction fragment length polymorphism analysis (21).

The entire cDNA insert of one of the six plasmids, pIRT-1, was sequenced and found to be 1348 bp in length and to contain a single 1017 bp open reading frame capable of encoding a polypeptide of 339 amino acids (Fig. 1A). The predicted amino acid sequence of IRT1 suggested that it is an integral membrane protein. Greater than 60% of the amino acids are nonpolar and these are arrayed in eight regions longer than 20 amino acids. These eight regions may form transmembrane domains. The hydrophobic nature of the IRT1 amino acid sequence and the arrangement of potential transmembrane domains, coupled with the biochemical analysis described later in this report, suggest that IRT1 is an Fe(II) transport protein. Therefore, the IRT1 amino acid sequence was examined for potential metal-binding domains. IRT1 has four histidine-glycine repeats located at amino acids 154–161 in the region between transmembrane domains 3 and 4. This histidine-rich domain may be important in substrate binding or regulation of this transporter. Several metal-binding proteins use the imidazole ring nitrogen of histidine as a coordinating ligand for metal ions (28, 29). Moreover, similar domains [i.e., (-His-X)₃₋₆] are found in analogous positions in the amino acid sequences of four other proteins thought to play a role in metal transport (30–32).

IRT1 is a Member of a Gene Family. The predicted amino acid sequence of IRT1 has no detectable similarity to FET3 (8), FET4 (9), or COPT1, a putative copper transporter from *A. thaliana* (33). Also, although they share the same number of potential transmembrane domains, there is no detectable similarity between IRT1 and the *E. coli* Fe(II) transporter protein encoded by the *feoB* gene (34). The lack of similarity among these proteins suggests that each may transport the substrate by a different biochemical mechanism. However, comparison of the IRT1 amino acid sequence with GenBank, EMBL, and SWISS-PROT databases identifies two closely related sequences in *Arabidopsis*. Amino acids 8 through 127 of IRT1 are 72% (86 of 119) identical and 86% similar (i.e., identities plus conservative substitutions) to the predicted amino acid sequence of a cDNA partially sequenced as an EST (Fig. 1B). Because of this high degree of similarity to *IRT1*, we have designated this gene *IRT2*. Furthermore, the carboxyl-terminal 47 amino acids of IRT1 are 45% (21 of 47) identical and 68% similar to the sequence of a partially sequenced open reading frame located downstream of the ferredoxin-encoding *FEDA* gene (35). We refer to this gene as *IRT3*.

A low stringency Southern blot using *IRT1* as the probe confirmed that *IRT1* is a member of a small gene family (Fig. 2). A comparison of the hybridization patterns seen on Southern blots using *IRT1* and *IRT2* as probes indicates that some of the bands seen on the low stringency Southern blot probed with *IRT1* can be attributed to *IRT2*. When *A. thaliana* DNA was digested with *EcoRI*, *IRT1* and *IRT2* hybridized strongly to 4.2- and 9.6-kb fragments, respectively. The same fragments showed weak (but visible) hybridization with the opposite probes, i.e., *IRT1* weakly hybridized to the 9.6-kb fragment and *IRT2* weakly hybridized to the 4.2-kb band. Digestion with the enzymes *HincII* and *AvaI* generated a 1.2-kb fragment that hybridized strongly to *IRT1* and a 1.8-kb fragment that strongly hybridized to *IRT2*. Again, both fragments showed weak hybridization to the opposite probes. With both digestions, other weakly hybridizing fragments were visible that could not be attributed to either *IRT1* or *IRT2*. These fragments likely represent additional members of the *IRT1* gene family, such as *IRT3*, present in the *A. thaliana* genome. Furthermore, DNA sequences similar to *IRT1* were detected by low stringency hybridization of the *IRT1* cDNA to DNA isolated from several other dicots including tomato, broccoli, and mustard (data not shown).

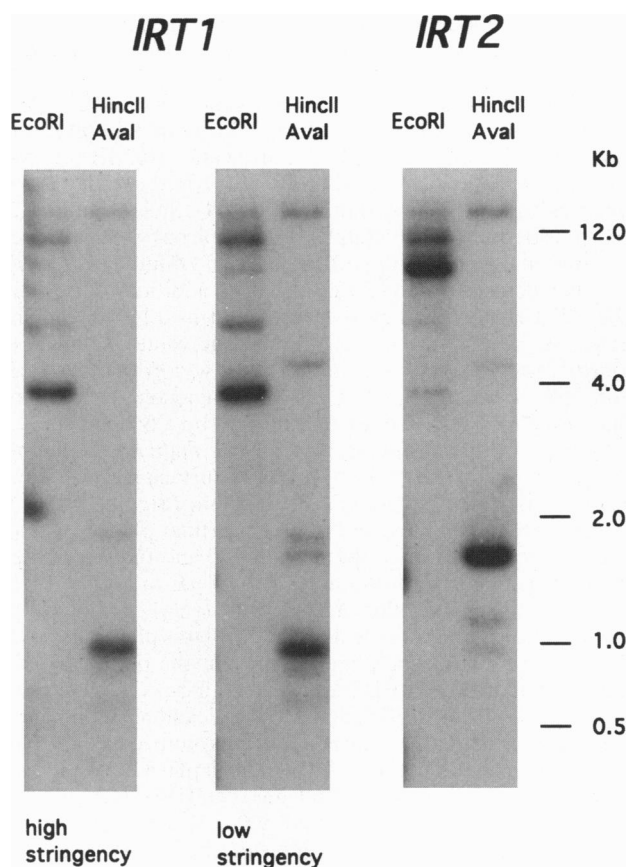


Fig. 2. Southern blot analysis of *A. thaliana* genomic DNA using *IRT1* and *IRT2* as gene probes. Each lane contains 15 μ g of *A. thaliana* (Columbia ecotype) genomic DNA digested with either *EcoRI* or a combination of *HincII* and *AvaI*. (Left) The blot was hybridized to *IRT1* and washed under high stringency conditions. (Center) The blot hybridized to *IRT1* and washed under low stringency conditions. (Right) The blot used with *IRT1* at low stringency was stripped, hybridized to *IRT2*, and washed under low stringency conditions.

Database comparisons also identified *IRT1*-related genes in the genomes of rice (a strategy II plant) (Fig. 1B), yeast, nematodes, and humans (data not shown). The rice gene was identified as an EST and has 64% identity and 82% similarity to *IRT1* over an 84-aa region. Two related *S. cerevisiae* genes (GenBank accession nos. P32804 and X91258) were identified. Both of these genes encode proteins that are similar in length to *IRT1* (376 and 422 amino acids) and are \approx 30% identical and 60% similar to *IRT1*. These genes were identified as open reading frames in the course of genomic sequencing and their functions are currently being investigated. The nematode sequence (GenBank accession no. U28944) was also identified by genomic sequencing and has 23% identity and 47% similarity to *IRT1* over an 84 amino acid stretch. Finally, a human EST (GenBank accession no. H20615) was identified with 31% identity and 43% similarity to *IRT1* over 82 amino acids. Given their close similarity to *IRT1*, we propose that these related genes may encode metal transporters in the organisms in which they are found.

IRT1 Expression in Yeast Confers Iron Uptake Activity. To determine if *IRT1* encodes an iron transporter, we examined ^{55}Fe uptake rates in a *fet3 fet4* strain expressing *IRT1*. Little or no uptake was detected at 0°C for either *IRT1*-expressing or untransformed control cells (Fig. 3A). At 30°C, *IRT1* expression resulted in an increased uptake rate for the first 10 min of the assay, after which the rate dropped to the control level. The *IRT1*-dependent rate was \approx 3-fold higher than the control uptake rate. No increased uptake was apparent in strains

bearing either of two randomly selected clones from the library (data not shown), indicating the dependence of these uptake effects on expression of IRT1. The iron uptake activity dependent on IRT1 expression was also concentration-dependent and saturable (Fig. 3B). The concentration dependence of IRT1-mediated uptake was found to generate a linear Eadie-Hofstee plot (Fig. 3B, *Inset*) with an apparent K_m of $6 \pm 1 \mu\text{M}$ and a V_{max} of 1.9 ± 0.4 pmol per min per 10^6 cells. Taken together, these results indicate that IRT1 expression in yeast produces a time-, temperature-, and concentration-dependent system of iron uptake.

The experiments described above were conducted with iron supplied as Fe(II), i.e., in the presence of ascorbate, an agent capable of reducing Fe(III) to Fe(II). To determine if Fe(II) is the preferred substrate over Fe(III), assays were carried out in the absence of ascorbate where iron is supplied to the cells as Fe(III). We found that the iron uptake rate in the absence of ascorbate was $\approx 10\%$ of the rate when ascorbate was present (Fig. 4A). This result suggests that Fe(II) is preferred over Fe(III) as substrate for the IRT1 transporter. Although yeast are capable of reducing Fe(III) to Fe(II) through the action of plasma membrane Fe(III) reductases, this rate of cell-

mediated reduction is slower than reduction by ascorbate (unpublished observations) and therefore may be rate-limiting for IRT1-dependent uptake. To assess if metals other than iron are potential substrates for IRT1, we tested several transition metals for their ability to inhibit accumulation of iron in IRT1-expressing cells (Fig. 4B). Iron was supplied as Fe(II) in these assays (i.e., in the presence of ascorbate) and the concentration of the metals tested was 10 times higher than the concentration of radiolabeled iron. The addition of Sr, Ni, Cu, Co, Zn, and Mn had no significant effect on the rate of iron uptake by IRT1. Cd and nonradiolabeled Fe(II) proved to be potent inhibitors of iron uptake. At 100-fold excess, Co, Mn, and Zn were also found to inhibit IRT1-dependent iron uptake (data not shown). The observed decreases in iron uptake rate were not due to toxicity of any of these metals because control experiments detected no loss of cell viability resulting from metal exposure (data not shown). Therefore, although the mechanism of this inhibition is not yet known, these data suggest that IRT1 is relatively specific for Fe(II) but may also be capable of transporting Cd, Co, Mn, and/or Zn.

Regulation of IRT1 in Wild-Type and Mutant Plant Lines in Response to Iron. IRT1 mRNA is expressed at a high level in roots of iron-deficient plants (Fig. 5); no signal was detected on a Northern blot with total RNA prepared from roots of iron-sufficient plants or from shoots of iron-sufficient or iron-deficient plants. The signal detected on the Northern blot is specific for IRT1; using gene-specific probes for IRT1 and IRT2, no hybridization was detected with the IRT2 probe (data not shown). Thus, IRT1 has a pattern of expression similar to Fe(III) chelate reductase activity, showing increased expression under iron deficiency. We have also examined the pattern of IRT1 expression in two different Fe(III) chelate reductase mutants, *frd1* and *frd3*. Plants carrying the *frd1* mutation do not show an increase in Fe(III) chelate reductase activity in response to iron deficiency whereas *frd3* mutants express reductase activity under both iron-sufficient and iron-deficient growth conditions (16). The *frd1* mutant showed some expression of IRT1 in roots from plants grown on iron-sufficient plates, indicating that these plants may actually be iron-deficient. This is consistent with the chlorosis observed in this line. *frd3* plants showed equally high levels of IRT1 mRNA in

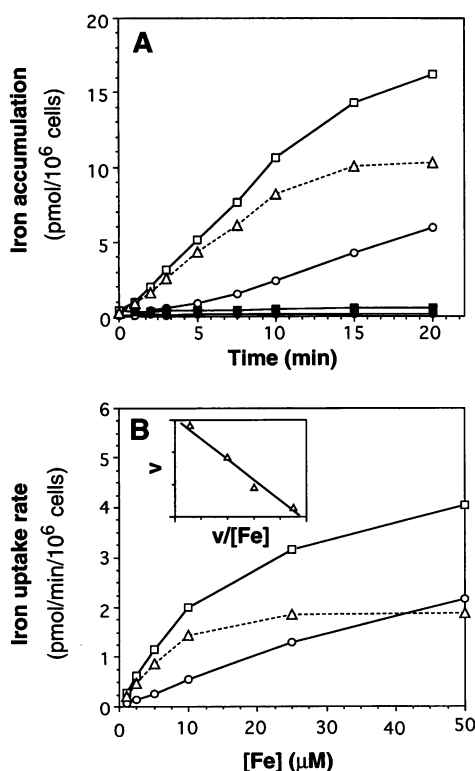


FIG. 3. Effect of IRT1 expression on iron uptake in yeast. The *fet3 fet4* mutant strain DEY1453 (circles) and DEY1453 transformed with pIRT-1 (squares) were grown to exponential phase in SD glucose and assayed for iron uptake with ⁵⁵Fe. (A) Time- and temperature-dependence of iron accumulation assayed in MGN with 1 mM ascorbate and 5 μM ⁵⁵FeCl₃ assayed at 30°C (open symbols) or 0°C (solid symbols). The dashed line marked with open triangles represents the IRT1-dependent accumulation, i.e., the accumulation of iron by the untransformed strain at 30°C subtracted from the accumulation of the pIRT-1-bearing strain at 30°C. (B) Concentration-dependence of IRT1-dependent uptake. The same strains as in A were assayed for iron uptake rates for 10 min over a range of concentrations. The dashed lines marked with open triangles represents the IRT1-dependent uptake rate, i.e., background uptake rate of the untransformed strain subtracted from the corresponding rate of the pIRT-1-bearing strain. (*Inset*) Eadie-Hofstee plot of the IRT1-dependent uptake data. Each point represents the mean of three experiments each performed in duplicate. The standard deviation within each experiment was less than 20% of the corresponding mean.

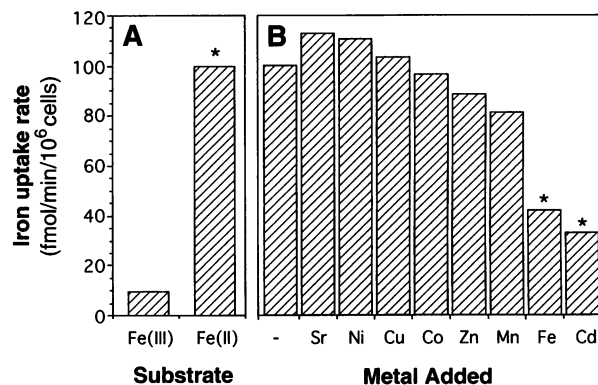


FIG. 4. Inhibition of IRT1-dependent uptake in yeast by other metals. The *fet3 fet4* mutant strain DEY1453 and DEY1453 transformed with pIRT-1 were grown to exponential phase in SD glucose and assayed for iron uptake with 1 μM ⁵⁵FeCl₃ in MGN for 10 min. The values shown are the IRT1-dependent rates, i.e., the untransformed strain control values were subtracted from the DEY1453 pIRT-1 values and represent the means of four replicates. The asterisks indicate significant differences from the control values ($P < 0.05$). (A) Preference of IRT1-dependent transport for Fe(II) over Fe(III). Assays were performed in the absence [Fe(III)] or presence [Fe(II)] of 1 mM ascorbate. (B) Inhibition of IRT1-dependent uptake by other transition metals. Assays were conducted in the absence (-) or presence of 10 μM metal. Radioactive iron was supplied as Fe(II) in the presence of 1 mM ascorbate.

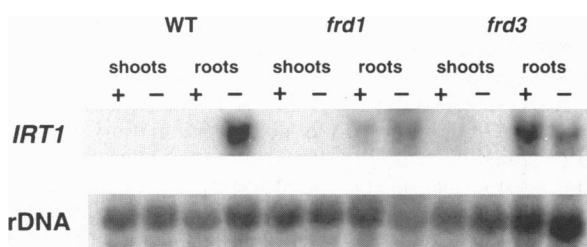


FIG. 5. Regulation of *IRT1* mRNA levels by iron availability in wild-type and mutant lines of *A. thaliana*. *IRT1* was hybridized to a Northern blot containing 10 μ g of total RNA prepared from either roots or shoots of plants 3 days after transfer of the plants to iron-sufficient (+) or iron-deficient (-) growth conditions. The blot was stripped and hybridized to an rDNA fragment to serve as a loading control.

the roots of iron-sufficient and iron-deficient plants. This pattern of regulation is similar to that of the Fe(III) chelate reductase in this mutant and suggests that reductase activity and *IRT1* expression are controlled by iron availability through a shared regulatory system.

What, then, is the role of *IRT1* in *A. thaliana* iron metabolism? The ability of *IRT1* to suppress the mutant phenotype of a yeast strain defective for plasma membrane Fe(II) transport, together with the increased Fe(II) uptake observed in yeast expressing *IRT1*, implies a role for this gene in uptake of iron across the plasma membrane of plant cells. Also, given the observations that *IRT1* mRNA is expressed in roots, is induced by iron deprivation, and is coregulated with the plasma membrane Fe(III)-chelate reductase in wild-type and *frd3* plants, we propose that the physiological role of *IRT1* is the uptake of iron from the rhizosphere across the plasma membrane in the root epidermal cell layer. However, we recognize that alternative hypotheses exist that explain our results and further studies will address this question.

Does *IRT1* play a role in the uptake of other physiologically relevant substrates? Our studies demonstrate that some other transition metals (Cd, Co, Mn, and Zn) are inhibitors of *IRT1*-mediated Fe(II) uptake in yeast and, therefore, may be substrates for this transporter. Thus, our ongoing studies on the *IRT* gene family may have possible applications for phytoremediation, i.e., the use of plants to remove toxic pollutants such as heavy metals from the environment. Although the physiological limitations of metal accumulation by plants are as yet only poorly understood (36–38), transport of metals into plant roots is certainly a necessary step in bio-accumulation. A comprehensive understanding of metal transport in plants will be essential to developing schemes to genetically engineer plants that accumulate specific metals, either to aid ecosystem restoration or to improve human nutrition.

Note added in proof. We have recently determined that one of the two *IRT1*-like yeast genes referred to in this report (GenBank accession no. P32804) encodes the transporter protein of a high affinity zinc uptake system (39). We have designated this gene *ZRT1*.

The authors thank Min Wei and Hui Zhao for technical assistance, Francois Lacroute for providing the cDNA library, Claire Lister for analyzing the *IRT1* restriction fragment length polymorphism data using MAPMAKER, and Ann Thering and Rob McClung for critical reading of the manuscript. This work was supported by Grants MCB-9405200 (to D.E.) and IBN-9318093 (to M.L.G.) from the National Science Foundation.

1. Yip, R. (1994) *J. Nutr.* **124**, 1479S–1490S.
2. Yi, Y. & Guerinot, M. L. (1994) *Plant Physiol.* **104**, 815–820.
3. Römheld, V. (1987) *Physiol. Plant.* **70**, 231–234.
4. Fox, T. C., Shaff, J. E., Grusak, M. A., Norvell, W. A. & Kochian, L. V. (1996) *Plant Physiol.*, in press.
5. Lesuisse, E. & Labbe, P. (1989) *J. Gen. Microbiol.* **135**, 257–263.
6. Dancis, A., Klausner, R. D., Hinnebusch, A. G. & Barriocanal, J. G. (1990) *Mol. Cell. Biol.* **10**, 2294–2301.
7. Eide, D., Davis-Kaplan, S., Jordan, I., Sipe, D. & Kaplan, J. (1992) *J. Biol. Chem.* **267**, 20774–20781.
8. Dix, D. R., Bridgham, J. T., Broderius, M. A., Byersdorfer, C. A. & Eide, D. (1994) *J. Biol. Chem.* **269**, 26092–26099.
9. Askwith, C., Eide, D., Van Ho, A., Bernard, P. S., Li, L., Davis-Kaplan, S., Sipe, D. & Kaplan, J. (1994) *Cell* **76**, 403–410.
10. Dancis, A., Yuan, D. S., Haile, D., Askwith, C., Eide, D., Moehle, C., Kaplan, J. & Klausner, R. D. (1994) *Cell* **76**, 393–402.
11. Schiestl, R. H. & Gietz, R. D. (1989) *Curr. Genet.* **16**, 339–346.
12. Minet, M., Dufour, M. E. & Lacroute, F. (1992) *Plant J.* **2**, 417–422.
13. Boeke, J. D., Trueheart, J., Natsoulis, G. & Fink, G. R. (1987) *Methods Enzymol.* **154**, 164–175.
14. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990) *J. Mol. Biol.* **215**, 403–410.
15. Claros, M. G. & von Heijne, G. (1994) *Comput. Appl. Biol. Sci.* **10**, 685–686.
16. Yi, Y. (1995) Ph. D. thesis (Dartmouth College, Hanover, NH).
17. Hangarter, R. P. & Stasinopoulos, T. C. (1991) *Plant Physiol.* **96**, 843–847.
18. Marschner, H., Römheld, V. & Ossenberg-Neuhaus, O. (1982) *Z. Pflanzenphysiol.* **105**, 407–416.
19. Dellaporta, S. L., Wood, J. & Hicks, J. B. (1983) *Plant Mol. Biol. Rep.* **1**, 19–21.
20. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1995) *Current Protocols in Molecular Biology* (Wiley, New York).
21. Lister, C. & Dean, C. (1993) *Plant J.* **4**, 745–750.
22. Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Day, M. J., Lincoln, S. E. & Newberg, L. (1987) *Genomics* **1**, 174–181.
23. Verwoerd, T. C., Dekker, B. M. M. & Hoekema, A. (1989) *Nucleic Acids Res.* **17**, 2362.
24. Pilgrim, M. L. & McClung, R. (1993) *Plant Physiol.* **103**, 553–564.
25. Feinberg, A. P. & Vogelstein, B. (1984) *Anal. Biochem.* **137**, 266–267.
26. Richards, E. & Ausubel, F. M. (1988) *Cell* **53**, 127–136.
27. Dayhoff, M. O., Schwartz, R. M. & Orcutt, B. C. (1978) in *Atlas of Protein Sequence and Structure* (Natl. Biomed. Res. Found., Silver Spring, MD), pp. 345–352.
28. Karlin, K. D. (1993) *Science* **261**, 701–708.
29. O'Halloran, T. V. (1993) *Science* **261**, 715–725.
30. Kamizono, A., Nishizawa, M., Teranishi, Y., Murata, K. & Kimura, A. (1989) *Mol. Gen. Genet.* **219**, 161–167.
31. Conklin, D. S., McMaster, J. A., Culbertson, M. R. & Kung, C. (1992) *Mol. Cell. Biol.* **12**, 3678–3688.
32. Palmiter, R. D. & Findley, S. D. (1995) *EMBO J.* **14**, 639–649.
33. Kampfenkel, K., Kushnir, S., Babiychuk, E., Inze, D. & Montagu, M. V. (1995) *J. Biol. Chem.* **270**, 28479–28486.
34. Kammler, M., Schon, C. & Hantke, K. (1993) *J. Bacteriol.* **175**, 6212–6219.
35. Somers, D. E., Caspar, T. & Quail, P. H. (1990) *Plant Physiol.* **93**, 572–577.
36. Raskin, I., Kumar, N., Dushenkov, S. & Salt, D. E. (1994) *Curr. Opin. Biotechnol.* **5**, 285–290.
37. Benemann, J., Rabson, R., Tavares, J. & Levine, R. (1994) *Summary Report of a Workshop on Phytoremediation Research Needs* (U. S. Department of Energy, Washington, DC).
38. Cunningham, S. C. & Berti, W. R. (1993) *In Vitro Cell Dev. Biol.* **29P**, 207–212.
39. Zhao, H. & Eide, D. (1996) *Proc. Natl. Acad. Sci. USA* **93**, 2454–2458.