Characterization of an Iron-dependent Regulatory Sequence Involved in the Transcriptional Control of \textit{AtFer1} and \textit{ZmFer1} Plant Ferritin Genes by Iron*

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In eukaryotic cells, ferritin synthesis is controlled by the intracellular iron status. In mammalian cells, iron derepresses ferritin mRNA translation, whereas it induces ferritin gene transcription in plants. Promoter deletion and site-directed mutagenesis analysis, combined with gel shift assays, has allowed identification of a new cis-regulatory element in the promoter region of the \textit{ZmFer1} maize ferritin gene. This Iron-Dependent Regulatory Sequence (IDRS) is responsible for transcriptional repression of \textit{ZmFer1} under low iron supply conditions. The IDRS is specific to the \textit{ZmFer1} iron-dependent regulation and does not mediate the antioxidant response that we have previously reported (Savino et al. (1997) J. Biol. Chem. 272, 33319–33326). In addition, we have cloned \textit{AtFer1}, the \textit{Arabidopsis thaliana} \textit{ZmFer1} orthologue. The IDRS element is conserved in the \textit{AtFer1} promoter region and is functional as shown by transient assay in \textit{A. thaliana} cells and stable transformation in \textit{A. thaliana} transgenic plants, demonstrating its ubiquity in the plant kingdom.

Metals are essential for all living organisms but are toxic at high concentrations. Therefore, metal homeostasis requires regulatory circuits to coordinate metal uptake and storage (1). Such mechanisms involve metal sensing proteins that transduce the signal within the cell to modulate gene expression. These proteins often act as regulatory factors, binding RNA or DNA cis-elements to promote or repress gene expression (1, 2). Among these metals, iron is one of the most abundant and is highly reactive as a pro-oxidant in Haber-Weiss reactions to generate free radicals (3). Furthermore, aerobic organisms have to overcome iron insolubility and then maintain iron in a bioavailable form (4). Plants, due to their immobility, need to tightly regulate iron homeostasis to prevent both iron toxicity or deficiency (5). Recently, genes involved in iron uptake in plants have been cloned: \textit{fro2}, encoding a root ferric reductase (6), and \textit{irt1}, encoding a putative ferrous transporter (7). Inside the plant cell, a particular class of plastid-localized proteins, the ferritins, are able to store iron in a safe and bioavailable form (8). Ferritins are ubiquitous proteins found in bacteria, animals, and plants, and, upon assembly, able to store up to 4500 iron atoms (4). Ferritin gene regulation has become a model to study iron-regulated expression both in the plant and animal kingdoms. In animal cells, ferritin synthesis is mainly regulated at the translational level in response to iron (2), whereas in plants transcriptional control has been demonstrated in soybean cells (9). In maize, iron overload induces accumulation of both ferritin mRNA and protein subunits (10, 11). Two nuclear maize ferritin genes have been characterized and named \textit{ZmFer1} and 2 (12). The \textit{ZmFer2} gene is regulated by a cellular pathway involving the plant hormone abscisic acid (ABA), whereas \textit{ZmFer1} is regulated by an ABA-independent pathway (13). Using derooted plantlets and maize cell suspension cultures, we have shown that this gene is regulated by both iron and redox signals (13). Indeed, \textit{ZmFer1} mRNA increase of abundance is induced by hydrogen peroxide treatments, and the iron-induced accumulation of this transcript is abolished in the presence of N-acetylcysteine (NAC), an antioxidant agent. The accumulation of \textit{ZmFer1} mRNA induced by both H$_2$O$_2$ and iron treatments were inhibited in the presence of the Ser/Thr phosphatase inhibitors okadaic acid and calyculin A (13), suggesting the involvement of dephosphorylation events in the transduction pathways regulating \textit{ZmFer1} gene expression. To further characterize the regulation of the \textit{ZmFer1} maize ferritin gene, we have developed a transient expression assay in maize cells. Using this system, a 2.2-kbp DNA fragment of the \textit{ZmFer1} gene was shown to be sufficient to regulate reporter gene expression in response to iron, with the same characteristics as observed for the endogenous \textit{ZmFer1} gene regulation (13).

In this paper, this transient expression assay was used to identify an Iron-Dependent Regulatory Sequence (IDRS) essential for the regulation by iron of the maize \textit{ZmFer1} and the \textit{Arabidopsis thaliana} \textit{AtFer1} ferritin genes. Stable transformation of \textit{A. thaliana} plants was performed to demonstrate the functionality of this element in planta.

\section*{EXPERIMENTAL PROCEDURES}

\subsection*{Cell Cultures and Transient Expression Assays—Maize BMS (Black Mexican Sweet) cells were cultivated as described (13). \textit{A. thaliana} cells (gift from Dr. Scheel) were cultivated at 24 °C under constant shaking at 170 rpm, in 100 ml of MS medium (14), containing 50 μM iron-EDTA. Subcultures were made every 2 weeks using 7.5 ml of cell suspension. For iron-starved cell cultures, 10 ml of 14-day-old cell culture were

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\end{itemize}

\begin{footnotesize}
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    \item 1 The abbreviations used are: ABA, abscisic acid; NAC, N-acetylcysteine; OA, okadaic acid; BMS, Black Mexican Sweet; MS, Murashige and Skoog medium; GUS, β-glucuronidase; IRE, iron-responsive element; IRF, iron-responsive protein; IDRS, iron-dependent regulatory sequence; CAMV, cauliflower mosaic virus; bp, base pair(s); PCR, polymerase chain reaction; SHS, soybean homologous sequence.
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transferred to 90 ml iron-free medium. Iron treatments were performed as described (13).

Eight-day-old iron-starved BMS or Arabidopsis cells were transformed by particle bombardment as previously described (13). For Arabidopsis cells the gun vacuum was set to 30 mbar and the helium pressure was 3.5 bars. The pRD109 and pAHCh18 plasmids were used as internal standards as described previously (13).

**Gel Shift Assays—** Maize cell nuclear extracts were prepared as described by Zhou et al. (15). Oligonucleotide sequences used in these experiments are described below in Fig. 3B. The DNA probe was end-labeled, and gel shift assays were performed as described in Villain et al. (16).

Cloning of the AtFer1 Gene—AtFer1 cDNA (17) was used to screen an *A. thaliana* genomic library (18). Positive bacteriophage DNA was prepared by polyethylene glycol precipitation (19), and the genomic DNA fragment was subcloned in pUC18 and sequenced.

**Plasmid Constructs—** All the constructs for transient expression assays were made using standard recombinant DNA techniques (19) and sequenced. Oligonucleotide sequences used in these experiments are described below in Fig. 3B. The DNA probe was end-labeled, and gel shift assays were performed as described in Villain et al. (16).

Analysis of ZmFer1 Promoter Deletions in Response to Iron—In a previous study (13), we have set up a transient expression assay, using particle bombardment-mediated transformation, to study ZmFer1 maize ferritin gene regulation. This system enabled us to show that a 2.2-kbp fragment of the *ZmFer1* gene fused to the β-glucuronidase (GUS) reporter gene mimics the regulation of the endogenous gene. An 8-fold increase of GUS activity was measured in response to iron overload (13), see pSL5 in Fig. 1. Furthermore, iron-induced expression of the ZmFer1-GUS fusion gene was inhibited by the antioxidant agent NAC, and the Ser7Thr phosphatase inhibitor OA. These results are in agreement with data obtained by Northern blot analysis of the ZmFer1 gene expression. The ZmFer1-GUS fusion pSL5 contained 1.6 kbp of the promoter and the region spanning from exon 1 to 3 (13). To localize cis-acting elements involved in the iron-dependent regulation of the ZmFer1 ferritin gene, we have performed a deletion analysis of the 2.2-kbp *ZmFer1* fragment mentioned above. Intron sequences were required to observe a significant decrease in expression activity.
level of expression of the construct in maize cells (13). This was in agreement with our general knowledge that introns are essential for the expression of many genes from monocotyledonous plants (26). These intron sequences act mainly by stimulating the basal level of gene expression. However, it could not be ruled out that intron sequences present in the pSL5 construct play a role in the iron-dependent regulation of the fusion gene. To clarify this point, ZmFer1 intron sequences were substituted by an intronic region of the rice actin gene, which has been shown to strongly enhance reporter gene expression when present in a chimeric construct (27). Furthermore, we have previously demonstrated that this rice actin gene DNA fragment did not contain iron-regulated sequences (13). This switch from maize ferritin to rice actin intron sequences results in a strong increase of expression of the construct in the absence of iron, although the promoter sequence was the same (pSL5 and p1600 in Fig. 1). Iron treatment of the cells lead to a 4.5-fold increase in GUS activity (Fig. 1, p1600). This slight decrease from 8- to 4.5-fold of the induction factor appears to be a result of the intron sequence replacement. This phenomenon could be linked, in part, to the strong stimulation of gene expression in the presence of the rice intron sequences. However, the expression of the construct remains inducible by iron overload, suggesting that essential sequences for this regulation are not located within introns. The p1600 construct still contains 61 bp of the ZmFer1 5′-untranslated region. Deletion of this sequence up to the previously identified transcription initiation sites (12) did not affect the pattern of expression of the construct (data not shown). Therefore, a promoter deletion analysis was performed, starting from the p1600 fusion gene containing 1600 bp of ZmFer1 promoter sequence (Fig. 1). Reduction of the promoter length from 1600 to 495 or 124 bp results in a decrease from 4.5- to 2.5-fold induction in response to iron (Fig. 1). Such a decrease could be linked to the presence of regulatory sequences participating to the iron-dependent regulation of ZmFer1 gene. These deletions had no effect on the level of expression in low iron conditions and demonstrate that essential iron response elements are localized upstream of −124 bp. The reduction of promoter size from −124 bp to −64 bp lead to a more drastic effect. The expression level in low iron conditions increased to the iron-induced level of the −124 bp construct, and stimulation by iron was abolished. A further deletion from −64 to −27 bp did not change this pattern of expression. The promoterless construct (pPL) does not support reporter gene expression. Therefore, the 60-bp region spanning from −124 to −64 appears to contain essential regulatory sequences for iron-induced expression of ZmFer1 maize ferritin gene. Furthermore, our results suggest that this region would be involved in the repression of ZmFer1 expression in low iron conditions.

Site-directed Mutagenesis of the ZmFer1 Promoter Region—To further analyze and localize the cis-acting elements implicated in this regulation, a site-directed mutagenesis approach was used. This approach was performed on a −124-bp ZmFer1 promoter, which represents the minimal promoter to maintain an iron-induced expression of the chimeric gene. This fragment was fused to rice actin introns as mentioned above and to the luciferase reporter gene. A 3.3-fold increase in luciferase activity was measured in response to iron treatment, when this construct was analyzed by transient expression in BMS cells (Fig. 2B, p124Luc). This confirms the results obtained with the GUS gene as reporter (Fig. 1).

Within the 124 bp of ZmFer1 promoter, some putative regulatory sequences were identified by sequence comparison for preferential mutagenesis as follows. A sequence named TC2 element, named TC1, localized at position −119.

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**Fig. 2. Localization of an Iron-dependent Regulatory Sequence by site-directed mutagenesis analysis of the −124 ZmFer1 promoter region.** A, partial nucleotide sequence of the −124-bp DNA fragment of ZmFer1 promoter. The putative regulatory sequences are boxed and their names indicated. Stars indicate the mutated bases in the various constructs used for transient expression (see B). B, effect of promoter mutations on iron-activated expression of the ZmFer1 gene in a transient assay. White boxes represent the target sequences for mutation. Black boxes represent mutated sequences. BMS cells were transformed and treated as described in Fig. 1 except that the plasmid used for luciferase activity standardization was pRD109, which constitutively expresses the GUS reporter gene.

**A**

| TC1  | GC  | TC2  |
|------|-----|------|
| −124 | GAGGCCTCCA | CATCAGAATAT |
| −69  | CTCGCCAGTC | TCTCCCGGCG |

**B**

| Construct | Actin introns | Luciferase | 3SS polyA |
|-----------|--------------|------------|-----------|
| p124Luc   | −124 bp      |            |           |
| pmTC1     |              |            |           |
| pmGC      |              |            |           |
| pmTC2     |              |            |           |
| pmTC12    |              |            |           |
| pmSHS     |              |            |           |
| pmGb      |              |            |           |
| pmCAATb   |              |            |           |

| Normalized luciferase activity = luc activity/GUS activity (Relative light units) |
|-----------------------------------------------|
| No treatment | 5 mM iron citrate |

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bp (Fig. 2A). TC1 sequence mutation alone did not affect the iron-induced regulation of the reporter gene (Fig. 2B, pmTC1). However, a double mutation of TC1 and TC2 resulted in enhanced basal expression of Luc and absence of induction by iron (Fig. 2B, pmTC12). These data suggests that, in the absence of a functional TC2 element, the TC1 sequence could partially substitute for TC2, whereas this sequence does not play a major role in the wild type ZmFer1 promoter. Sequences flanking the TC2 sequence were also analyzed to define more precisely this putative cis-acting element. The region upstream of TC2 was of particular interest, because mutations in the sequence called GC (Fig. 2A) lead to a pattern very similar to the TC2 mutant (Fig. 2B, pmGC). This result extends the regulatory region to both TC2 and GC sequences. This DNA fragment of 16 bp, which appears to be essential for the ZmFer1 ferritin gene regulation by iron, was named Iron-Dependent Regulatory Sequence (IDRS).

The ZmFer1 promoter region contains a CAAT box sequence in position –23 (Fig. 2A). This element has been described as a general cis-acting element, but can also be involved in specific responses. Indeed, ferritin H gene transcriptional control by heme in Friend Leukemia cells is mediated by a CCAAT element (28), and this sequence plays also a role in the transactivation of genes in response to antioxidant treatment in a human colorectal cancer cell line (29). Mutation of the CAAT box sequence present in the ZmFer1 promoter did not affect the expression pattern of the fusion gene in low or high iron conditions (Fig. 2). The Gbox element (CACGTG) (Fig. 2A) is a common regulatory sequence in plant gene promoters involved in many environmental stress responses (30). Mutagenesis of the ZmFer1 promoter Gbox revealed this sequence not to be required for the iron-dependent regulation of the ZmFer1 maize ferritin gene. Transcriptional regulation of soybean ferritin gene has been clearly demonstrated by nuclear run-on experiments (9). The corresponding gene has been cloned (31), and we have compared the promoter sequences of this soybean ferritin gene and ZmFer1. Interestingly, a conserved region (Fig. 2A, SHS), distinct from the IDRS element, was found in the proximal region of these two promoters. However, mutational analysis of this sequence in a transient expression assay revealed that this sequence is not involved in ZmFer1 gene regulation by iron in maize cells (Fig. 2B, pmSIS).

By a combined approach of promoter deletion and site-directed mutagenesis, we have, therefore, been able to localize an essential region for ZmFer1 regulation by iron, which we have named IDRS.

The IDRS Element Is Not Involved in the Antioxidant or Okadaic Acid Inhibition of ZmFer1 Ferritin Gene Expression—We have previously shown, by Northern blot experiments and transient expression in maize cells, that the iron-induced expression of ZmFer1 gene is inhibited by NAC or OA (13). In the presence of NAC, the pSL5 ZmFer1-GUS fusion expression was strongly reduced in both untreated and iron-treated cells, but an increase of GUS activity was still induced by iron. Such results suggested that antioxidant agents could affect the level of expression of this gene, rather than the iron-dependent activation of ZmFer1 gene. Therefore, it was interesting to determine whether these effects are mediated by the IDRS element. To address that question, p124Luc, pmTC12, and pmGC (see Fig. 2A) construct expression was analyzed by transient expression in the presence of NAC or OA (data not shown). These experiments revealed that OA and NAC inhibitions of ZmFer1 gene expression are not mediated by the IDRS, which appears to be specific to the iron response. Regulatory elements, distinct from the IDRS, are therefore required to mediate the antioxidant effect at the promoter level.

Characterization of the Iron-dependent Regulatory Sequence—To further characterize the IDRS element, gain-of-function experiments were performed to establish whether this sequence is sufficient to confer iron-dependent regulation to a minimal promoter. A construct containing a −80-bp 35S CAMV promoter sequence fused to rice actin intron sequences, and the luciferase reporter gene was prepared. The IDRS element was cloned upstream of this construct, conserving the distance between this regulatory sequence and the TATA box as in ZmFer1 gene. The IDRS fragment corresponds to the ZmFer1 promoter region from −88 to −62 bp and was cloned in 1, 2, or 3 copies. For each construct, a significant level of expression was measured, but no iron-dependent regulation was observed (data not shown). Increasing the fragment length to −124 to −62 of the ZmFer1 promoter led to the same result. These data suggest that some additional elements are required to establish the iron regulation or that a specific chromatin context is essential and not reproduced in the fusion gene.

To determine if the IDRS element could effectively bind nuclear transcription factors, gel shift experiments were performed. For this purpose, a double-stranded deoxyoligonucleotide corresponding to the IDRS was used (Fig. 3B). This probe was incubated in the presence of nuclear extracts prepared from maize cells treated or not by iron (Fig. 3A). In both low or high iron conditions, only one complex was observed (Fig. 3A). The signal was more abundant after iron treatment, but a signal was also clearly detectable when iron level was low (Fig. 3A, lane 2). Addition of 1 μM iron citrate in the protein extract from iron-depleted cells, or β-mercaptoethanol as a reducer, or diaminod as an oxidant in both extracts, had no effect on the observed complex (data not shown). To address the specificity of this complex, the reaction was performed in the presence of a range of molar excess of cold probe. A dose-dependent decrease of the binding was detected (Fig. 3A, lanes 4–7). In contrast, in the presence of a 100-fold molar excess of nonspecific DNA, which did not contain IDRS, no effect on DNA binding was observed. Therefore, a nuclear protein, or protein
complex, specifically binds the IDRS element in vitro. Site-directed mutagenesis of the ZmFer1 promoter enabled us to establish that base changes within IDRS affect the chimeric ZmFer1-Luc gene regulation in vivo. To investigate if such mutations alter the binding of nuclear proteins to the IDRS in vitro, a competition experiment was performed in the presence of different double-stranded oligonucleotides containing three base substitutions compared with the WT sequence (Fig. 3B). When the binding reaction was performed in the presence of oligonucleotides M1 to M3, a partial inhibition of binding was detected, but the complex was still clearly observed (Fig. 3A, lanes 9–11). Oligonucleotides M4 and M5 resulted in less inhibition of binding of nuclear protein(s) to the labeled IDRS (Fig. 3B), which affect the binding of nuclear proteins to the IDRS. These experiments confirm that the IDRS is specifically bound by nuclear protein(s) consistent with its in vivo ability to regulate reporter gene expression in response to iron.

The IDRS Element Is Involved in the Iron-regulated Expression of the AtFer1 Ferritin Gene—Maize is a graminaceous plant and shares with this group some specificities in iron metabolism. A phytosiderophore-mediated uptake of iron by the roots has been described in this plant group, whereas it does not exist in non-graminaceous monocotyledonous or dicotyledonous plants (32). This raises the question of whether the IDRS element could also play a role in iron-regulated expression in non-graminaceous plants. To address this question, we have investigated the regulation of AtFer1 ferritin gene from A. thaliana, a dicotyledonous plant. It has been previously shown, using AtFer1 cDNA as a probe in Northern experiments, that this gene is regulated by an ABA-independent pathway, and iron-induced accumulation of AtFer1 mRNA is inhibited in the presence of NAC (17). These features match with ZmFer1 gene regulation and suggest that AtFer1 would be the A. thaliana ferritin gene orthologous to ZmFer1. We have cloned the AtFer1 ferritin gene encoding the previously identified AtFer1 cDNA (GenBank accession no AF229850). This gene contains an exon/intron structure similar to known plant ferritin genes. A sequence comparison between the proximal region of AtFer1 and ZmFer1 promoters is presented in Fig. 4A. This alignment reveals three major blocks of identity. One corresponds to the G-box sequence, which is not involved in the iron-dependent regulation of ZmFer1 gene (see above). A second region of similarity was revealed close to the putative TATA box of both promoters (Fig. 4A, block II). The third block corresponds to 14 bp with high similarity between the two promoters and corresponds to the IDRS element. This region matches exactly with the ZmFer1 IDRS base substitutions analyzed by gel shift (Fig. 3B), which affect the binding of nuclear proteins to the IDRS (Fig. 3A). To determine whether this sequence corresponds to a functional IDRS in the AtFer1 gene, a transient expression assay was set up using particle bombardment-mediated transformation of Arabidopsis cell suspension cultures. AtFer1 promoter deletions were fused to the GUS reporter gene and introduced into cell cultures. The pIDRS-WT construct contains 100 bp of the AtFer1 promoter and the putative IDRS element. A 6-fold increase in GUS activity was measured in the transient expression assay in response to a 500 μM iron citrate treatment (Fig. 4). When the AtFer1 promoter sequence was reduced from −100 to −61 bp, deleting the IDRS, an increase of GUS activity in low iron conditions was detected, and the response to iron treatment was eliminated. This result is similar to the data obtained by deleting the ZmFer1 IDRS (Fig. 1). Using the pIDRS-WT construct, site-directed mutagenesis of the AtFer1 IDRS was performed. In the pmIDRS-1 construct, the 3′ region of the IDRS was mutagenized, corresponding to the ZmFer1 TC2 mutations (Fig. 2) and M4–M5 in the gel shift assay. The complete IDRS was mutated in the pmIDRS-2 construct. Transient expression analysis of these plasmids revealed a pattern similar to what is observed by the IDRS deletion. Site-directed mutagenesis of block II had no significant effect on the iron-dependent regulation of the construct (data not shown). Therefore, the IDRS is essential for the iron-dependent regulation of both AtFer1 and ZmFer1 ferritin genes, in transient assays.

Because stable transformation of A. thaliana plants is a routine procedure, in contrast to maize transformation, we decided to verify whether the IDRS was essential for the iron-mediated derepression of AtFer1 gene expression in transgenic A. thaliana plants. The approach consisted of transforming A.
Iron-dependent Regulation of Plant Ferritin Genes

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**DISCUSSION**

Using promoter deletion and site-directed mutagenesis analysis, we have identified a *cis*-regulatory element, IDRS, essential for *ZmFer1* maize ferritin gene iron regulation. This element does not match with known regulatory sequences identified in plant promoters like the Metal Regulatory Element for example, nor with such elements from yeast or animals (33). IDRS is required for iron-dependent regulation of both *ZmFer1* and *AtFer1* genes, illustrating its broad functionality in the plant kingdom. It is important to notice that the IDRS is not only essential in transient assays using cell suspension cultures but also in stable multicellular transformants such as transgenic *A. thaliana* plants (Fig. 5). Gain-of-function experiments failed to demonstrate that IDRS is sufficient to confer iron-dependent regulation, suggesting that other elements are necessary. However, promoter sequence analysis of *ZmFer1* and *AtFer1* ferritin genes coupled to transient expression analysis showed that IDRS was the only conserved sequence essential for iron regulation. Furthermore, the first evidence of iron transcriptional regulation of a plant ferritin gene was obtained with soybean (9). The corresponding gene was cloned (31) and contains an IDRS element within its promoter at position -121 to -108. Very recently, it has been reported that the expression of a GUS reporter gene fused downstream of this soybean promoter was repressed under low iron conditions, in agreement with our findings (34). The soybean promoter sequence suggested to be involved in this transcriptional repression is different from the IDRS sequence. It is, however, important to notice that this soybean regulatory region is not conserved in *AtFer1* and *ZmFer1* promoter sequences. Also, the result by Wei and Theil (34) does not exclude a role of the soybean IDRS in iron control of ferritin gene expression in this plant species.

Our results suggest that the IDRS element is involved in ferritin gene repression at low iron concentrations. This could require a specific chromatin conformation to allow IDRS bound protein(s) to interact with the transcription initiation complex or to limit the TATA box access for example. Then, if the IDRS fusion to the 35S CAMV minimal promoter fails to reproduce such a correct conformation, the IDRS activity could not be revealed.

In plant and animal cells, a common regulatory scheme of ferritin genes by iron is emerging. Low iron conditions repress ferritin synthesis, at the translational level in animals (2), and at the transcriptional level in plants. In animals, a *cis*-element called Iron Responsive Element (IRE), localized in the 5'-untranslated repeat of ferritin mRNA, binds Iron Regulatory Proteins (IRPs). When iron level is low, IRPs occupy IRE and prevent the initiation of mRNA translation (2). Two types of IRPs, IRP1 and -2, have been characterized. IRP1 enables integration on the IRE of at least two different signals: the iron and the redox status of the cell. IRP1 binding is stimulated by iron deficiency or by H2O2 treatment. Iron could have a direct effect on the protein, whereas the H2O2 signal would require a transduction pathway (35). We have also described such an integration of iron and redox signals for *ZmFer1* maize ferritin gene regulation (13). In this case, iron treatment promotes ferritin synthesis, whereas antioxidant agents like NAC or glutathione inhibit ferritin synthesis. However, in contrast to animal cells for which such responses are targeted to the same molecule, IRP1, the redox signal in plants is not mediated by the IDRS, which is specific to the iron response. These results suggest that other(s) regulatory sequence(s) in *ZmFer1* mediate(s) the antioxidant response. This integration of redox and iron signals within the ferritin promoter could allow adjustment of the ferritin level and protection of the cell according to the free iron concentration and the redox status of the cell.

IDRS implication in the transcriptional regulation of ferritin genes by iron led us to search for this element in other gene promoter sequences. Because iron uptake and storage need to be coordinated, some iron-dependent regulatory mechanisms are required to modulate gene expression implicated in these processes. In animal cells, the IRE sequence regulating ferritin mRNA translation is also localized in the 3'-untranslated repeat of the transferrin receptor mRNA (2). In low iron conditions, the binding of IRPs on the IRE stabilizes the transferrin receptor transcript and allows the synthesis of the corresponding protein. Therefore, the same regulatory sequence enables the adjustment of iron storage and iron uptake capacity of the...
cell. Searching for IDRS in plant sequence data bases using BLAST and FASTA programs (36, 37), we have found no sequences similar to IDRS in the promoter regions of genes that could be involved in plant iron uptake, like fro2 (6), irt1 (7), or the Nramp gene family (38, 39). Iron could also regulate genes implicated in the protection against oxidative stress such as superoxide dismutases or catalases. Again, we did not find the IDRS in these genes. The A. thaliana genome is now almost completely sequenced, and we have found some sequences similar to IDRS. However, they were not localized in promoter regions, or were present in unidentified sequences.

Gel shift experiments show that IDRS is able to bind nuclear protein(s) in vitro, and these data correlate with in vivo functional assays. The mechanistic significance of the apparent increase of binding to the IDRS under iron overload remains to be worked out. The molecular characterization of the protein(s) interacting with the IDRS will allow the elucidation of the mechanisms involved in the repression of ferritin synthesis during low iron conditions, and the derepression by iron, of ZmFer1 and AtFer1 ferritin genes.

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Characterization of an Iron-dependent Regulatory Sequence Involved in the Transcriptional Control of \textit{AtFer1} and \textit{ZmFer1} Plant Ferritin Genes by Iron

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