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Ferritin gene transcription is regulated by iron in soybean cell cultures

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ABSTRACT **Iron-regulated ferritin synthesis in animals is dominated by translational control of stored mRNA; iron-induced transcription of ferritin genes, when it occurs, changes the subunit composition of ferritin mRNA and protein and is coupled to translational control. Ferritins in plants and animals have evolved from a common progenitor, based on the similarity of protein sequence; however, sequence divergence occurs in the C termini; structure prediction suggests that plant ferritin has the E-helix, which, in horse ferritin, forms a large channel at the tetrameric interface. In contemporary plants, a transit peptide is encoded by ferritin mRNA to target the protein to plastids. Iron-regulated synthesis of ferritin in plants and animals appears to be very different since the 50- to 60-fold increases of ferritin protein, previously observed to be induced by iron in cultured soybean cells, is accompanied by an equivalent accumulation of hybridizable ferritin mRNA and by increased transcription of ferritin genes. Ferritin mRNA from iron-induced cells and the constitutive ferritin mRNA from soybean hypocotyls are identical. The iron-induced protein is translocated normally to plastids. Differences in animal ferritin structure coincide with the various iron storage functions (reserve for iron proteins and detoxification). In contrast, the constancy of structure of soybean ferritin, iron-induced and constitutive, coupled with the potential for vacuolar storage of excess iron in plants suggest that rapid synthesis of ferritin from a stored ferritin mRNA may not be needed in plants for detoxification of iron.**

Ferritin synthesis and accumulation is regulated by iron, the metal stored inside the protein coat as hydrated ferric oxide (1–3). Ferritin is widely distributed among eukaryotes and prokaryotes; the similar structure of ferritins in plants and animals suggests a common evolutionary origin. In contemporary plant ferritins, a transit peptide targets the protein to plastids (4). Ferritin stores iron for protein synthesis and for detoxification of iron excess; vacuoles may also detoxify iron in plants (5, 6).

Regulation of ferritin gene expression by iron was first observed decades ago in animals (7, 8) and plants (9); the mechanism in animals is mainly posttranscriptional, involving ferritin mRNA storage and translational competition (10–12). Detection of ferritin mRNA-specific trans-acting factors (13), isolation and cloning of regulator protein(s) (14–16), and identification of a conserved regulatory sequence (17–21) with a distinctive structure (22) have made ferritin mRNA a model for translational control (23, 24). Iron-induced transcription and/or accumulation of ferritin mRNA also occur in animals. The effects are subunit-specific and are coupled to increases in translation (25–28) and, apparently, to detoxification of the excess iron.

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In plants, regulation of the ferritin gene by iron has not been studied as thoroughly. Earlier measurements of translatable ferritin mRNAs suggested that in contrast to animals, iron regulation may affect mRNA transcription rather than translation in bean leaves (29) and cultured soybean cells (30). Measurement of ferritin mRNA by translation in cell-free extracts could have been complicated by ferritin-mRNA-specific translational effects (13). To determine if the regulation of ferritin expression by iron is different in plants and animals, we have investigated the synthesis and sequence of ferritin mRNA[§] in iron-induced cultured soybean cells and the fate of the ferritin produced. The results show that iron induced a 45-fold increase in the transcription rate of soybean ferritin mRNA and a corresponding increase in the accumulation of ferritin protein. The sequence of induced ferritin mRNA was identical to its hypocotyl counterpart and the transport of the newly synthesized ferritin to plastids appeared to be normal. Thus the domination of translational events in iron-regulated ferritin gene expression (24) appears to be specific to animals. In plants, by contrast, iron has a dramatic effect directly on transcription of ferritin mRNA.

MATERIALS AND METHODS

Materials. Isolation of a partial ferritin cDNA (SoF35) from a soybean hypocotyl cDNA library has been described elsewhere (4). The glyceraldehyde-3-phosphate dehydrogenase C probe (GapC) from pea (31) was a gift from R. Cerff (Technical University, Braunschweig, F.R.G.). Soybean cell cultures (*Glycine max* cv. Mandarin line Sbe4) were grown as described (32). Ferritin synthesis was induced by 500 μM iron(III) citrate as described (30).

cDNA Cloning, Sequencing, and Prediction of Protein Secondary Structure. A cDNA library, constructed using poly(A)$^+$ RNA from iron-induced soybean cell suspensions (30), *Eco*RI–*Not* I adaptors (Pharmacia), and *Eco*RI arms of λNM1149 (33, 34), and the cDNA library from hypocotyls (4) were screened with ^{32}P-labeled SoF35, a fragment of hypocotyl ferritin cDNA (4). After phage purification (35) and subcloning into pEMBL18 or pUC18, the complete sequence was obtained from both strands by the dideoxynucleotide method (36). Prediction of protein secondary structure (according to the Chou–Fasman and Garnier–Arguthorp–Robson methods) used the programs of the University of Wisconsin Genetics Computer Group.

RNA Isolation and Characterization. Total RNAs were extracted from frozen cells by using guanidine hydrochloride (37), analyzed on Northern blots with the cDNA clone SoF35 as a probe (4) or on dot blots on nitrocellulose [hybridized for 48 h at 42°C in 50% (vol/vol) formamide/5× SSPE (1× SSPE

Abbreviation: IRE, iron regulatory element.

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§The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M64337 from cultured soybean cells and M58336 from soybean hypocotyl).

= 0.18 M NaCl/10 mM sodium phosphate, pH 7.4/1 mM EDTA) and washed with 50% formamide/5× SSPE at 42°C], and quantitated using a Beckman scintillation counter; amounts of RNA were 10 and 5 μg, respectively.

***In Vitro* Transcription in Isolated Nuclei.** Crude nuclei (10 g of cells) were prepared as described (38) and could be stored at −70°C for 2 months in a Triton-free buffer containing 30% (vol/vol) glycerol (39) at 4 mg of proteins per ml. Protein was measured according to Bradford (40). Transcription reactions (250 μg of protein) were incubated 10 min at 30°C, and purified transcripts (41) were hybridized (1×10^7 cpm/ml) to denatured plasmids (5 μg) on nitrocellulose. Quantitation of autoradiograms used a Uniscan II spectrophotometer at 595 nm (Labsystems, Les Ullis, France).

Immunolocalization of Ferritin by Electron Microscopy. Preparation of soybean cell samples for electron microscopy using 200-mesh nickel grids (42) (Touzard et Matignon, Paris) and protein A-gold to localize ferritin has been described (43). Grids were analyzed at a magnification of ×20,000.

RESULTS

Properties of Iron-Induced Ferritin mRNA in Cultured Soybean Cells. Specific members of the ferritin subunit family can be induced by excess iron in animals (25–27). To determine whether iron induces the same or different ferritin mRNA in plants, the sequence of a ferritin cDNA clone from a library constructed with poly(A)$^+$ RNA from iron-induced soybean cells was compared to that from hypocotyl cDNA. No ferritin mRNA could be detected in iron-depleted cells. The longest hypocotyl cDNA (986 base pairs) ended at the guanine of the initiator AUG whereas the longest cDNA clone from iron-induced soybean cells (pSoFIC1) was 1066 base pairs and extended 89 nucleotides into the 5′ untranslated region. Three regions of soybean ferritin mRNA were particularly scrutinized, the plant-specific transit peptide, an N-terminal extension peptide present in iron-loaded mature ferritin of plants (4, 44), and the 30% of the C terminus that was absent in the soybean hypocotyl partial cDNA clone previously described (4).

Comparison of the complete nucleotide sequences of the inserts from pSoFH2 and from pSoFIC1 revealed an identity of 100%, showing that iron did not induce a variant ferritin mRNA. Only the nucleotide sequence of pSoFIC1, the longer insert, is presented in Fig. 1*A*. Both the transit peptide and the extension peptide are conserved in the iron-induced mRNA. [Note that the two N-terminal residues, glutamic acid and phenylalanine, in the transit peptide of the hypocotyl ferritin (4), actually the *Eco*RI cloning site of the vector, are omitted from the amino acid sequence.] The C terminus of the coding region and the complete 3′ untranslated region of hypocotyl ferritin mRNA is also faithfully represented in the iron-induced mRNA (Fig. 1*A*). No evidence was obtained for the conserved translational regulatory sequence of animal ferritin mRNA, the iron regulatory element (IRE) (17–24), in the soybean ferritin cDNA sequences, but such a sequence could reside in the 5′ untranslated region yet to be characterized.

The amino acid sequence predicted for soybean ferritin shows that conservation of sequences in plant and animal ferritins includes much of the C-terminal end of the molecule (Fig. 1*B*); the bundle of four α-helices in animal ferritins (2) should also form in soybean ferritins. However, residues that could form the fifth or E-helix (residues 160–169) in animal ferritins are absent in soybean ferritin (Fig. 1*B*). A channel at the fourfold axis of horse spleen ferritin is formed by the E-helix (2) and, by sequence analogy, in all vertebrate ferritins (1–3). The functional significance, if any, of this difference between plant and animal ferritins is not clear. An insertion of four amino acids (Asn-Ala-Pro-Ser) occurs be-

A

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CACAAATCTTAGCCGCCATTGATTTTTCCTCTTCCATTTCTCTTACTTTCTCAGCCTTT 59
                                  M   A   L   A   P   S   K
TTCGATTACCCATTTGGTTATTTCGCCCTA  ATG GCT CTT GCT CCA TCC AAA 110
 V   S   T   F   S   G   F   S   P   K   P   S   V   G   G
GTT TCC ACC TTT TCT GGT TTT TCT CCC AAA CCC AGT GTT GGG GGT 155
 A   Q   K   N   P   T   C   S   V   S   L   S   F   L   N
GCT CAG AAA AAC CCA ACT TGC TCT GTT TCT CTG AGC TTT TTG AAT 200
 E   K   L   G   S   R   N   L   R   V   C   A  ▼S   T   V
GAG AAA CTT GGA AGC AGA AAC CTT AGG GTT TGT GCC TCA ACG GTG 245
 P   L   T   G   V   I   F   E   P   F   E   E   V   K   K
CCT CTC ACT GGG GTG ATT TTT GAA CCG TTT GAG GAG GTT AAG AAG 290
 S   E   L   A   V   P   T   A   P   Q   V   S   L   A   R
AGC GAG CTT GCT GTT CCA ACT GCT CCC CAA GTC TCG TTG GCT CGT 335
 Q   N   Y   A   D   E   C   E   S   A   I   N   E   Q   I
CAG AAC TAC GCT GAT GAG TGT GAA TCT GCG ATT AAC GAG CAG ATA 380
 N   V   E   Y   N   A   S   Y   V   Y   H   S   L   F   A
AAT GTG GAA TAC AAT GCT TCC TAC GTG TAC CAC TCC TTG TTT GCA 425
 Y   F   D   R   D   N   V   A   L   K   G   F   A   K   F
TAC TTT GAC AGG GAC AAC GTG GCT CTC AAG GGA TTT GCC AAG TTC 470
 F   K   E   S   S   E   E   E   R   E   H   A   E   K   L
TTC AAG GAA TCT AGT GAG GAA GAA AGA GAG CAC GCT GAA AAG CTC 515
 M   K   Y   Q   N   T   R   G   G   R   V   V   L   H   P
ATG AAA TAT CAG AAC ACT CGC GGT GGA AGA GTT GTA CTT CAC CCC 560
 I   K   N   A   P   S   E   F   E   H   V   E   K   G   D
ATC AAG AAT GCC CCC TCA GAA TTT GAG CAT GTG GAA AAG GGG GAT 605
 A   L   Y   A   M   E   L   A   L   S   L   E   K   L   V
GCA TTG TAT GCA ATG GAA TTA GCT TTG TCT TTG GAG AAG TTA GTG 650
 N   E   K   L   L   N   V   H   S   V   A   D   R   N   N
AAT GAG AAA CTT CTG AAT GTG CAC AGT GTG GCA GAT CGC AAC AAT 695
 D   P   Q   M   A   D   F   I   E   S   E   F   L   S   E
GAC CCT CAA ATG GCC GAC TTC ATT GAA AGC GAG TTT TTG TCT GAA 740
 Q   V   E   S   I   K   K   I   S   E   Y   V   A   Q   L
CAG GTT GAA TCA ATT AAG AAA ATT TCA GAG TAT GTG GCT CAG TTG 785
 R   R   V   G   K   G   H   G   V   W   H   F   D   Q   R
AGA AGG GTT GGA AAG GGT CAC GGT GTT TGG CAC TTT GAT CAA AGA 830
 L   L   D
CTT CTT GAT TAG GAAGATGCTGCTTAATCTTGAATAGCCTTATTATTAGTCTTCA 885
TTTACATTTGGTCTTTTCTGAAATTCTGGGTTGTTTTCTAATTTAGTAGTATTTAAATG 944
TTTTTCCTATGTACTGAGATTTAGGAACTCGGAGTAATGTAATTGCTAGTAGCACATGT 1003
AAAAGGCTGCAAGCCTATTATGCAATAACATTTGATATCTGCATTTC(A)16         1066
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B

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                10        20        30        40        50        60
HoS-L SSQIRQNYSTEVEAAVNRLVNLYLRASYTYLSLGFYFDRDDVALEGVCHFFRELAEEKRE
HuL-L ----------D------S------Q----------------------S------------
HuL-H T--V----HQDS---I--QI--E-Y---V---MSX---------KNFAKY-LHQSH-E--
ChR-H P--V----HQDC---I--QI--E-Y---V---MSY---------KNFAKY-LHQSH-E--
TaR-L E--V---FHQDC--GL--T---KFHS--V---MAS--N------SNFAK----RS--EK-
TaR-M V--V----HSDC------ML--E-Y-----S-MYAF--------HN-AE--K-HSH-E--
TaR-H D--V---FHRDC---I--M--ME-Y------MA-------I--HN-AK--K-QSH-E--
SoIC- V-LA----AD-C-S-I-EQI-VE-N---V-H--FA-----N---K-FAK--K-SS--E--

                70        80        90        100       110       120
HoS-L GAERLLKMQNQRGGRALFQDLQKPSQDEWGTTLDAMKAAIVLEKSLNQALLDLHALGSAQ
HuL-L -Y-----------------IK--AE----K-P------MA---K-----------R
HuL-H H--K-M-L-------IFL--IK--DC-D-ESG-N--EC-LH---NV--S--E--K-ATDK
ChR-H H--K-M-L-------IFL--IK--DR-D-ENG-T--EC-LH---NV--S--E--K-ATDK
TaR-L H--K-IEY-------VFL-SVE--ER-D-ANG-E-LQT-LK-Q--V---------VAADK
TaR-M H--KFM-Y--K----VVL--IK--ER----N--E--Q--LQ---TV--------K-ATDK
TaR-H H--K-M-D--K----IVL--VK--ER----N--E--Q--LQ---TV--------KV--DK
SoIC- H--K-M-Y--T----VVLHPIKEFEHV-K-DA-Y--EL-LS---LV-EK--NV-SVADRN

                130       140       150       160       170
HoS-L ADPHLCDFLESHFLDEEVKLIKKMGDHLTNIQRLVGSQAGLGEYLFERLTLKHD
HuL-L T---------T-----------------LH--G-PE---------------
HuL-H N-------I-T-Y-N-Q--A--EL---V--LRKMGAPES--A----DKH--GDSDNES
ChR-H N-------I-T-Y---Q--A--QL---V--LRKMGAPKY-MA----DKH--GESDS
TaR-L S---MT-----PY-S-S-ET---L---I-SLKK-WS-HP-MA----NKH--G
TaR-M V----------EY-E-Q--D--RI--FI--LK--GLPEN-M-----DKHSV-ESS
TaR-H V---------TEY-E-Q--S--QL--YI--LK--GLP-N-M-----FKH-MGESS
SoIC- N--QMA--I--E--S-Q-ES---ISEYVAQLR-VGKGHGVWHFDQRLLD
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FIG. 1. Primary structure of soybean ferritin mRNA. (*A*) Nucleotide sequence of pSoFIC1, a ferritin cDNA clone isolated from an iron-treated soybean cell cDNA library. The sequence from clone pSoFH2 isolated from a soybean hypocotyl cDNA library is 100% identical; both sequences were obtained from each strand of both inserts. The two N-terminal residues of the transit peptide (glutamic acid and phenylalanine) shown in ref. 4 can now be omitted since they belong to the *Eco*RI cloning site of the vector. The vertical arrow indicates the site of cleavage of the transit peptide in the ferritin precursor; underlined amino acids correspond to the N-terminal sequence of ferritin purified from iron-induced soybean cells (5). No sequence analogous to the IRE of animal ferritin was observed. (*B*) Comparison of the predicted amino acid sequence of soybean ferritin with selected sequences from animal ferritins. Residue 1 is the first amino acid in the ferritin from horse spleen, for which the three-dimensional structure has been described (2), and the transit and extension peptides (4) are omitted from the N terminus. An insertion (Asn-Ala-Pro-Ser) occurs in the soybean sequence between residues 82 and 83 of horse spleen ferritin. HoS-L, horse spleen L subunit (45); HuL-L and -H, human liver L and H subunits, respectively (46); ChR-H, chicken reticulocyte H subunit (47); TaR-L, -H, and -M, tadpole reticulocyte L, H, and H′ subunits (25); SoIC (see *A*). Among mammalian sequences, identity between H and L subunit classes is ≈65% but within H or L subunit classes it is 85–90%. Note the variation of sequence in the C-terminal end of the soybean subunit compared to the animal subunits, particularly in the region of the fifth or E-helix (residues 160–169).

tween residues 82 and 83 (Fig. 1) at a splice junction in animal ferritin genes (2).

Effect of Iron on Ferritin mRNA Accumulation in Cultured Soybean Cells. Iron increased the amount of translatable ferritin mRNA in bean leaves and in soybean cell cultures by ≈40-fold (29, 30). To quantify the effect of iron on induction of ferritin mRNA and protein, total cellular RNAs of iron-treated soybean cells were analyzed by electrophoresis and by hybridization. Only one size class of ferritin mRNA was observed, corresponding to ≈1400 nucleotides (Fig. 2*A*), as was observed in normal soybean hypocotyl (4). The absence of detectable ferritin mRNA in iron-depleted soybean cells suggests that the mRNA concentration was lower than in mature leaves where an equivalent amount of RNA gave a readily detectable ferritin mRNA signal (4).

Quantitation of the iron-induced increase in hybridizable ferritin mRNA was obtained by dot-blot analysis of total RNA, using glyceraldehyde-3-phosphate dehydrogenase as an internal control. The results show that ferritin mRNA increased as much as 52-fold during incubation of the soybean cells with iron (Fig. 2 *B* and *C*), which corresponds well to the increase in the amount of ferritin protein induced in the cultured cells (Table 1). Iron increased ferritin mRNA only transiently (maximum after 15 h) in analogy to animal cells such as cultured HeLa cells (27) and adult liver (26).

Effect of Iron on Ferritin mRNA Synthesis in Cultured Soybean Cells. Iron-induced accumulation of ferritin mRNA (Fig. 2) could result from either a change in ferritin mRNA stability or synthesis. To determine if iron stimulation of transcription accounted for the 52-fold increase in accumulation of ferritin mRNA observed (Fig. 2), synthesis of ferritin mRNA was measured in nuclei isolated from the iron-treated soybean cells, compared to nuclei from iron-deprived cells. Iron had no effect on glyceraldehyde-3-phosphate dehydrogenase gene transcription (Fig. 3). In contrast, ferritin gene transcripts could only be detected in the iron-treated cells. Based on the lowest concentration of ferritin mRNA that could have been detected, the synthesis of ferritin mRNA was induced up to 45-fold by iron, accounting for most, if not all, of the iron-induced accumulation of ferritin mRNA observed (Fig. 2) and the increase in ferritin protein (Table 1).

Intracellular Transport of Iron-Induced Ferritin. Normally, ferritin in plants is found in plastids (51) after transport and processing of a precursor (29, 30, 52). To determine if the response of soybean cells to excess iron corresponds to the normal intracellular physiology of ferritin, the fate of iron-induced ferritin was monitored by immunoelectron microscopy. No ferritin was detectable in iron-deficient cells (Fig. 4*A*). However, after 24 h of culture with iron citrate, gold-decorated ferritin could be seen in amyloplastids (Fig. 4*B*). The amount of ferritin in plastids was even higher after 48 h, even though ferritin mRNA had declined to 28% of the maximum (Fig. 2*C*). The presence of ferritin outside the plastids after 48 h (Fig. 4*C*) indicates either ferritin that is yet to be incorporated into plastids or leakage from the amyloplasts. In contrast to the iron-rich ferritin observed earlier by electron microscopy studies (9, 51), the use of an antibody (Fig. 4) permits the detection of iron-rich and iron-poor ferritins and ferritin precursors.

Table 1. Effect of iron on ferritin mRNA and protein in cultured soybean cells

Ferritin mRNA, protein, or iron	Value 0 μM	Value 500 μM	Relative increase
mRNA synthesis,* OD_{595}	0.007	0.315	45
mRNA (12–15 h)†			
Hybridization, cpm × 10^{-3}	0.12	6.4	52
Translation, OD_{595}	<0.02	0.089	>40
Protein (72 h),‡ pmol/mg of cells	<0.005	0.30	>60
Iron (72 h),‡ nmol/mg of cells	ND	0.54	—
Intracellular iron (72 h)‡, nmol/mg of cells	0.4	10.5	26

Soybean cells were cultured in the presence of 500 μM iron citrate or its absence. Results are the average of two or three experiments. The time selected for the data on mRNA and protein illustrates the maximum effect of iron. Iron-induced transcription and accumulation of translatable ferritin mRNA preceded accumulation of ferritin protein (ref. 30 and this work). ND, not done.

*Quantitation was achieved by scanning autoradiograms of hybrids obtained between nuclear run-on transcripts and various DNA probes immobilized on nitrocellulose filters (Fig. 3). Absorbance of the pUC negative control (OD_{595} = 0.02) was deduced from the assays.

†Data were obtained by scanning (at 595 nm) the autoradiograms of the *in vitro* translation experiments reported in ref. 30. Hybridization data were from Fig. 2*C*.

‡Data were taken from ref. 5. Note that, in contrast to animals (1–3), the iron content of iron-induced ferritin in cultured soybean cells was comparable to that in constitutive conditions [e.g., in pea (48, 49), lentil (48), or soybean (50)].

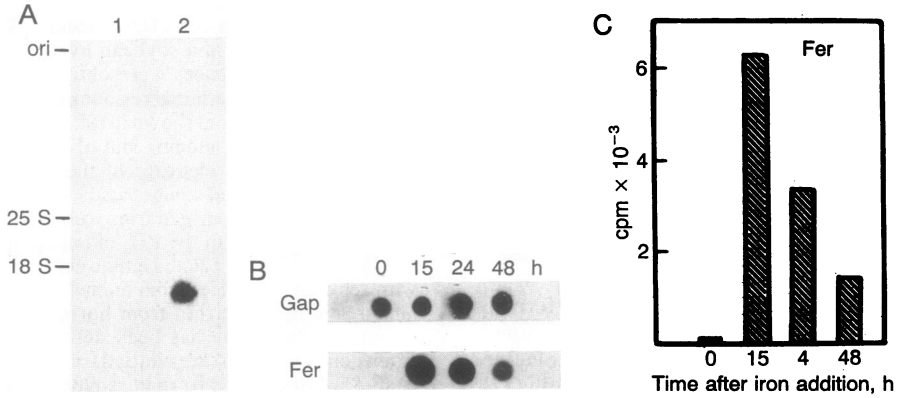

FIG. 2. Effect of iron on ferritin mRNA concentration in soybean cultured cells. (*A*) Iron stimulates ferritin mRNA accumulation. Total RNA was isolated from cells grown with or without 500 μM iron citrate for 15 h. RNAs (10 μg) were analyzed on a Northern blot using the ^{32}P-labeled ferritin cDNA insert of pSoF35. Lanes: 1, RNA from iron-deprived cells; 2, RNA from iron-induced cells. Positions of the 25S and 18S rRNAs are indicated. Two independent experiments have been performed giving the same result. Note the absence of detectable ferritin mRNA before treatment with iron. (*B*) Kinetics of ferritin mRNA accumulation after iron addition. Total cellular RNA was isolated from cells 0, 15, 24 and 48 h after addition of 500 μM iron citrate. RNA (5 or 10 μg) were analyzed using labeled GapC (Gap) and ferritin (Fer) cDNAs as probes. These results represent two experiments with two RNA concentrations each. (*C*) Quantification of ferritin mRNA accumulation. Radioactivity was quantitated by excising the RNA and measuring radioactivity in a Beckman scintillation counter. Experimental variation is between 5 and 10%.

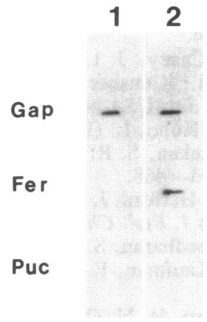

FIG. 3. Iron stimulates the rate of ferritin gene transcription. *In vitro* nuclear transcription run-on assays with nuclei purified from cells grown without (lane 1) or with (lane 2) 500 μM iron citrate for 15 h. pSoF35 (Fer; 5 μg; linearized with *Pst* I), pPS46A3 (glyceraldehyde-3-phosphate dehydrogenase, Gap; 5 μg), and pUC18 (Puc; 5 μg; linearized with *Eco*RI) were immobilized on nitrocellulose filters. Radioactive run-on nuclear transcripts (2×10^7 cpm) were hybridized to the various probes. Hybrids were quantitated by scanning the autoradiograms at 595 nm with an Uniscan II spectrophotometer. The results are representative of two experiments.

DISCUSSION

Ferritin from plants and animals share similar morphologies and primary sequence suggesting a common evolutionary precursor (4). Nevertheless, the apparent absence of the C-terminal E-helix in soybean ferritin (Fig. 1*B*) does not affect iron core formation. Ferritin gene regulation also has common features in plants and animals, since excess iron leads to the accumulation of ferritin protein to store iron.

In animals, the ferritin multigene family is expressed cell specifically with ferritin mRNA varying in amounts and composition. Translational control of ferritin mRNA leads to changes in ferritin synthesis that is dependent on intracellular iron concentration. Iron uptake in animal cells is regulated coordinately with iron storage, through a common regulator protein (53) and structural motif (the IRE) in ferritin and transferrin receptor mRNAs (23, 24). In certain cell types, changes in ferritin gene transcription accompany changes in translation (25–27), but whether the IRE sequence in the DNA is involved is not known. Ferritin genes are also regulated transcriptionally during development and by monokines, growth factors, and hormones (1, 3, 28).

If the common evolutionary features of ferritin genes in plant and animal cells extended from protein structure to genetic regulation by iron, the amount of ferritin mRNA in soybean cells would not be significantly changed by iron; large amounts of ferritin mRNA would be found stored in iron-depleted plant cells. Such is not the case: no ferritin mRNA was detected by hybridization (Fig. 2) or translation (30) in iron-depleted cultured soybean cells; similar results were obtained (29) when translatable ferritin mRNA in leaves from iron-deficient and iron-loaded bean plants was measured.

In plants, 50- to 60-fold increases in ferritin protein induced by iron were accompanied by equivalent increases in the transcription and accumulation of hybridizable ferritin mRNA (Table 1). By contrast, 40- to 50-fold increases in ferritin protein synthesis induced by iron in animals could be achieved with no change in the amount or type of translatable or hybridizable ferritin mRNA (11, 25, 26).

Induction of transcription of ferritin mRNA by iron in animal cells is accompanied by changes in the protein structure (25–29) and, often, in the iron content (25–27). In contrast, in the plant cell both the ferritin mRNA and protein induced in iron-treated soybean cells appear to be unchanged from the normal or constitutive type (Fig. 1*A* and refs. 5 and 30). Moreover, the physiology of the iron-induced ferritin is unchanged, based on iron content, subunit size (5, 30), and intracellular transport (Fig. 4). Iron simply induces the plant

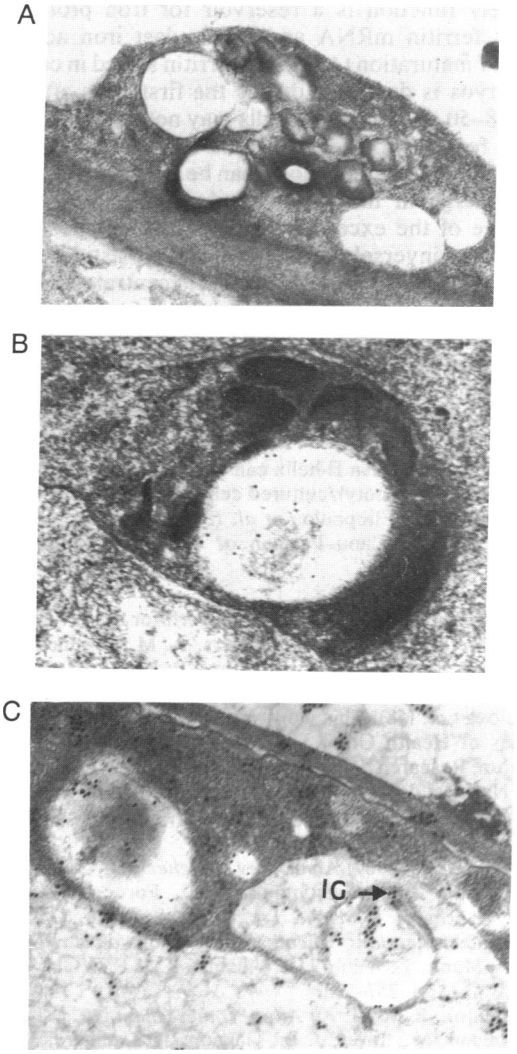

FIG. 4. Intracellular distribution of iron-induced ferritin in cultured soybean cells. Soybean cells untreated (*A*) or treated with 500 μM iron citrate for 24 h (*B*) and 48 h (*C*) were used for immunoelectron microscopy with rabbit polyclonal antibodies to pea seed ferritin (49) and goat anti-rabbit IgG coupled with 15-nm gold particles. Gold decoration of ferritin is observed only after cells have been treated with iron for 24 or 48 h and is mainly found in amyloplastids. IG, gold label. (×16,000.)

cells to synthesize more constitutive ferritin mRNA and protein. At the present time, evidence for the absence of translational regulation of ferritin in plants includes translation rates related to concentrations of hybridizable animal ferritin mRNA in wheat germ extract (13) and absence of IRE recognition proteins in plant cells (54). However, the heterogeneity of ferritin genomic sequences in pea, soybean, and maize (M.R., Y. Kimata, and E.C.T., unpublished data) emphasizes that understanding of ferritin gene regulation in plants may not yet be complete.

An explanation of the difference in iron regulation of ferritin synthesis in plants and animals may relate to differences in ferritin function. In animals, ferritin is mainly a cytoplasmic protein although incorporation into lysosomes appears to occur by autophagy. Iron stored in ferritin in animal cells is used for at least three purposes, (*i*) a reservoir for iron proteins within the cell, (*ii*) an iron reserve for other cell types, and (*iii*) a detoxification site for toxic concentrations of iron that coincide with variations in ferritin expression and protein structure (25, 26). Although the function of iron stored in ferritin in plants has not been fully explored, the

most likely function is a reservoir for iron proteins. For example, ferritin mRNA and chloroplast iron accumulate during leaf maturation (4, 55) and ferritin stored in cotyledons and embryos is degraded during the first days after germination (48–50, 56, 57). Plant cells may not need to synthesize alternate ferritins for detoxification of iron because, in contrast to animals, plant vacuoles can be used. In fact, vacuoles are very large in the iron-treated soybean cells (5). The percentage of the excess intracellular iron stored in plants appears to be inversely related to the low plastid number (5). In addition, in yeast excess iron is concentrated in vacuoles (6). The use of vacuoles to safely store potentially toxic excess iron would eliminate the necessity of rapidly synthesizing ferritin and storing ferritin mRNA required in animals. Whether any plant cells depend on iron-dependent translational control of ferritin synthesis remains to be determined.

Note Added in Proof. An E-helix can be predicted at the C terminus of the soybean hypocotyl/cultured cell ferritin by using the combination program of Eliopoulos *et al.* (58) but not by using default parameters of the Chou–Fasman or Garnier–Arguthorp–Robson programs.

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