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VARIABLE AMOUNTS OF TRANSLATABLE FERRITIN mRNA IN BEAN LEAVES WITH VARIOUS IRON CONTENTS

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SUMMARY: RNA was isolated from bean leaves with various iron and ferritin contents. The amount of translatable ferritin mRNA in these RNAs was determined by wheat-germ cell-free translation and immunoprecipitation with anti-bean seed ferritin serum. The levels of ferritin mRNA present in total RNA of the leaves are reflected by the *in vivo* levels of ferritin in the corresponding leaf tissues. This implies that the iron-dependent regulation of phytoferritin synthesis, in contrast with the regulation of animal ferritin synthesis, occurs at the level of transcription.

The iron storage protein ferritin is widely distributed in nature and is known to play a prominent role in the metabolism of iron in eukaryotic cells (see recent reviews (1,2,3)). Ferritin protects the organism against an overdose of iron in the cell and its synthesis is induced by iron administration. It has been suggested that ferritin synthesis in mammals is regulated at the translational level (4). Zähringer *et al.*(5) found that a post-ribosomal fraction of normal rat liver contained ferritin mRNA (see also (6,7)). The amount of these free ferritin messengers was strongly reduced in liver after iron administration, while the amount of polysomal ferritin mRNA was concomitantly increased. Yet, the total amount of ferritin messengers was not changed.

The iron content of leaf tissue is easy to manipulate, and can range from 0.9 to 22 nmol iron/mg dry weight. The ferritin protein content of bean leaves is correlated with

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their iron content; iron-deficient leaves do not contain ferritin, while iron-loaded leaves show large deposits of highly-filled ferritin (8,9). Thus, as in the mammalian system, the synthesis of phytoferritin is subject to regulation. Recently, we showed that ferritin in plants is coded for by nuclear DNA and is synthesized *in vitro* in a precursor form. The precursor is taken up by intact chloroplasts and is subsequently processed to its mature size (10).

The elucidation of the biosynthetic pathway of phytoferritin and its chloroplast localization prompted us to investigate the regulation of ferritin synthesis in plants with respect to what is known about the synthesis in the mammalian systems. In the present study we report that there is a close correlation between ferritin messenger content and ferritin protein content of leaf tissues with varying iron levels. For the study of ferritin messenger content, mRNA prepared from iron-deficient, iron-sufficient (normal) and iron-loaded leaves were tested for their capacity to synthesize the precursor form of ferritin. The mRNA samples were translated in a wheat-germ cell-free protein-synthesizing system and synthesized precursors for ferritin were precipitated with an antiserum against bean seed ferritin. The immunoprecipitates were analyzed by SDS-gel electrophoresis followed by fluorography. The amount of ferritin precursor synthesized was estimated by scanning the developed film.

MATERIALS AND METHODS

Plant material: *Phaseolus vulgaris* L. cv. Prélude were grown as described by van der Mark *et al.*(9). The glass pots for the culture of iron-deficient plants were cleaned before use with 10% HNO_3, 10% H_2SO_4 and rinsed with hot tap water and distilled water. Iron-loaded leaves were obtained by feeding iron-deficient plants with ferric-sodium-ethylene-diamine-tetraacetic acid (FeNaEDTA) as described by van der Mark *et al.*(9). The first trifoliate leaves were used for all the experiments. The preparation of leaf homogenates,

ferritin protein and total protein determinations were performed as described earlier (11). Immunoelectrophoresis was optimized in order to improve the determinations of small quantities of antigen. For this purpose a known amount of purified bean seed ferritin was added to samples of the leaf homogenates. RNA from leaves was prepared and used for translation as described by van der Mark et al.(10). For the proteinase treatment of RNA, samples (± 3 mg/ml) were incubated prior to the translation experiment with proteinase K (1µg/ml) for 30 min at 4°C. After this treatment 2 mM phenylmethanesulphonyl fluoride (PMSF) was added, and the preparation was used in the protein-synthesizing system. In the control experiments proteinase and PMSF were added together. Immunoprecipitation with anti-bean seed ferritin serum, SDS-gel electrophoresis and fluorography were performed as described by van der Mark et al.(10). The relative incorporation of [^{35}S] Met into the precursor of ferritin was assessed by scanning the developed film in a Laser Scanning Densitometer (LKB instruments).

RESULTS AND DISCUSSION

Table I shows the amount of ferritin as the percentage of total protein in the first trifoliate leaves of plants grown under different iron regimes. Iron-deficient leaves do not contain detectable amounts of ferritin, whereas the ferritin content of iron-loaded leaves is about four times higher than that in leaves of plants grown continuously on medium with a normal iron content. In an earlier study we reported in more detail on the relation between ferritin content and iron fluxes in the trifoliate leaf (9). In order to investigate the regulation of the ferritin synthesis in plants, RNA was extracted from normal, iron-deficient and iron-loaded leaves and used in the wheat-germ cell-free protein-synthesizing system. The relative abundance of ferritin-mRNA was estimated by determining the relative amount of

Table I. Ferritin protein levels in normal, iron-deficient and iron-loaded leaves as measured with rocket immunoelectrophoresis.

Leaf tissue	Ferritin (% total protein)	Ratio
A. Normal	0.21 ± 0.04	1
B. Iron-deficient	<0.02	<0.1
C. Iron-loaded	0.80 ± 0.15	3.9

ferritin translation product. For that purpose, [^{35}S] Met labelled translation products were indirectly immunoprecipitated with an antiserum against bean seed ferritin and with Staphylococcus aureus cells. The immunoprecipitates were analyzed by SDS-gel electrophoresis and fluorography. Variable amounts of labelled products from in vitro translation, using RNA from iron-loaded leaves, were used to check the reliability of the method used to quantify the amount of ferritin precursor synthesized. Figure 1 shows a proportionality between the amount of translation product and the relative density of the precursor ferritin band in the fluorogram.

Zähringer et al.(5) proposed that the translation of post-ribosomal ferritin messengers is blocked by adhering ferritin subunits. It is known that ribonucleoprotein (RNP) particles can sustain the phenol/cresol step of the RNA isolation procedure. We therefore subjected the RNA preparations to a proteinase treatment in order to ensure the removal of any proteinaceous inhibitor of translation still present in the

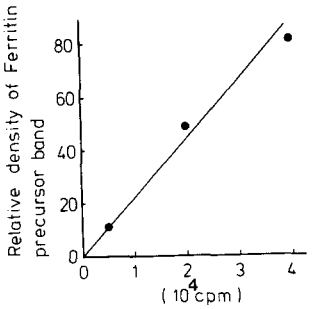
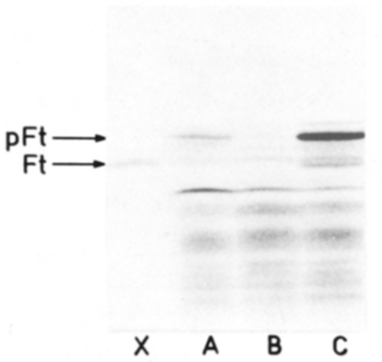


Fig.1. Density of ferritin precursor bands by SDS-gel electrophoresis as a function of the amount of products from in vitro protein synthesis. RNA from iron-loaded leaves was translated in a wheat-germ system. Variable amounts of trichloroacetic acid precipitable products were used for immunoprecipitation with an antiserum against bean seed ferritin. The immunoprecipitates were analyzed by SDS-gel electrophoresis and fluorography. The density of the precursor ferritin band was measured by scanning the fluorogram.

RNA solution. This extra proteinase treatment of the RNA species used for in vitro translation resulted in an increase of 25-30% of the trichloroacetic acid precipitable products. However, with respect to the amount of ferritin precursor as a fraction of total precipitable products, no significant difference could be observed after immunoprecipitation, gel electrophoresis and fluorography (results not shown).

The three RNA species extracted from leaves with different iron status were translated in the wheat-germ system and equal amounts of trichloroacetic acid precipitable counts were used for immunoprecipitation. Figure 2 shows the fluorogram resulting from such an experiment. According to the measured density of the precursor ferritin bands, RNA from iron-deficient plants gave less than 15% and RNA from iron-loaded



Density of precursor Ferritin band as related to A: 100 15 370

Fig.2. Analysis of the products of in vitro protein synthesis with RNAs from leaves with different iron content. RNAs from leaves with different iron levels were translated in a wheat-germ system. Equal amounts of trichloroacetic acid precipitable products were used for immunoprecipitation with anti-bean seed ferritin serum. The immunoprecipitates were analyzed by SDS-gel electrophoresis and fluorography. Fluorogram: A, normal leaves; B, iron-deficient leaves; C, iron-loaded leaves. Lane x shows the migration of [^{125}I] labelled pure bean seed ferritin (Ft), pFt indicates the migration of the precursor form of ferritin.

leaves about 370% of the amount of ferritin precursor translated on RNA from iron-sufficient leaves.

These data were obtained with RNA preparations which were free from any proteinaceous inhibitor of translation. Hence we may assume that the ratio of ferritin precursor to the total protein synthesized, reflects the relative amounts of ferritin mRNA in the RNA preparation used. This ratio, 0.15:1:3.7, is very well comparable to the ratio of contributions of ferritin protein to total protein present in the leaves, namely $<0.1:1:3.9$ for iron-deficient, normal and iron-loaded leaves respectively (Table I). This suggests that *in vivo*, under the conditions tested, all ferritin messengers are translated and that the presence of a non-polysomal pool for ferritin mRNA is not likely. Our results indicate that the appearance of ferritin protein in leaves with different iron levels is correlated with the levels of corresponding mRNA species, suggesting that their *in vivo* level is regulated primarily by transcriptional control. In tadpole reticulocytes, with a 40-fold induction of ferritin synthesis in intact cells, there appeared to be no difference in the percentage of translatable ferritin mRNA in iron-induced or non-induced cells, using the wheat-germ translation system (12). Thus, as in mammalian cells, in amphibians the ferritin synthesis is thought to be regulated in a post-transcriptional way. In higher plants however, the regulation is more likely to occur at a transcriptional level.

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