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EXOGENOUS METHIONINE DEPRESSES LEVEL OF mRNA FOR A SOYBEAN STORAGE PROTEIN

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In vitro translation experiments indicate that absence of the β-subunit of 7S storage protein in soybean (*Glycine max* L. Merr. cv. Provar) cotyledons cultured on methionine-supplemented medium is due to lack of functional mRNA for that polypeptide. Relative amounts of functional mRNA for the 7S α'- and α-subunits were unaffected by methionine in the cotyledon culture medium. Measurements of β-subunit accumulation in cotyledons transferred from basal medium to methionine-supplemented medium show that methionine inhibits continued accumulation of the β-subunit after synthesis of the β-subunit has begun, and that methionine does not promote degradation of existing β-subunit.

Soybean storage proteins and the genes which code for them constitute a model system for studies on eucaryotic gene regulation, because these storage proteins rapidly accumulate to high levels during a discrete period in seed development. *In vitro* culture of soybean cotyledons (1) provides a means to study the physiology and molecular biology of the developing cotyledons under defined conditions. The 7S and 11S storage protein subunits from soybean cotyledons cultured on basal medium are indistinguishable on SDS-polyacrylamide gels from subunits synthesized by seeds which have developed on intact plants (2). In seeds on intact plants, the 7S β-subunit appears 5-10 days later than do the 7S α'- and α-subunits (3, 4, 5). When cotyledons at *ca.* 12 days after flowering (20-50 mg) are placed into basal culture medium, the α'- and α-subunits are always prominent within 24 hours. The β-subunit appears only after 2-3 days of growth in culture (2). Our laboratory has reported that adding methionine (4 mM) to the culture medium results in increased growth, increased

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aminoacylation of methionyl tRNA, and increased methionine content of total protein (6). In addition, exogenous methionine suppresses accumulation of the 7S β-subunit in cultured cotyledons (2). This study was conducted to determine whether exogenous methionine prevents formation of functional mRNA for the β-subunit in cultured soybean cotyledons.

MATERIALS AND METHODS

Cotyledon culture. Cotyledons from immature soybean seeds (weighing 40-50 mg each) taken from greenhouse-grown plants (Glycine max L. Merr. cv. Provar) were cultured as previously described (1), except that 1-liter Erlenmeyer flasks, with 200 ml of medium and 50 cotyledons per flask were used. Cotyledons were grown on basal medium [(-) met] (1), and on basal medium plus 4 mM L-methionine [(+) met]. After 6 days in culture, cotyledons were frozen in liquid nitrogen and stored at -80°C.

Isolation of mRNA. Poly(A)+ mRNA was isolated from cultured cotyledons by the method of Barton et al. (7). This poly(A)+ mRNA was dissolved in 10 mM sodium phosphate buffer, pH 7.0, and loaded onto a column (1.5 cm x 8 cm) of hydroxylapatite (LKB, Rockville, Md.)[1]. The column was washed with 120 ml of 10 mM phosphate buffer to remove polysaccharides. The RNA was then eluted with 400 mM sodium phosphate, pH 7.0. The RNA was recovered in a 12 ml volume and desalted on a column (1.5 cm x 16 cm) of Sephadex G-25 (8).

In vitro translation. Wheat germ extract was prepared according to the procedures of Bruening et al. (9). Translation "reaction mix" was purchased from BRL, Gaithersburg, Md. Final concentrations of potassium acetate and magnesium acetate were 125 mM and 2 mM, respectively. Each reaction volume (30 µl) contained 2.7 µCi of [^{3}H]leucine. Translations were carried out for 60 min. at 20°. Analysis of in vitro translation reaction products by polyacrylamide gel electrophoresis and autoradiography was carried out as previously described (10).

Cotyledon transfer experiments. Cultures were set up with 10 cotyledons (in 40 ml of medium) per 125 ml flask. Cotyledons were harvested or transferred to fresh medium on the fourth day. All cotyledons were in basal medium for the first four days, and transfers were to basal medium or to basal medium plus 4 mM methionine. Those cotyledons transferred on the fourth day were harvested on the eighth day. Harvested cotyledons were stored at -20°C until used. Cotyledons from each culture flask were pooled and homogenized. Proteins were extracted into 30 mM Tris-HCl, pH 8.0, 10 mM mercaptoethanol, at room temp. Polyacrylamide gel electrophoresis of proteins was as described by Maizel (11). Protein bands were stained with Coomassie Blue, and the stained polypeptides were assayed on an Ortec model 4310 scanning densitometer. Since there could be slight differences in staining and de-staining of gels processed on different occasions, raw data from each experiment were handled separately and expressed on a relative basis. Arbitrarily, the amount of β-subunit present at day 4 was designated as 1.0 unit.

RESULTS AND DISCUSSION

Products of in Vitro Translation Reactions. Full-length 7S subunit precursors were obtained from in vitro translations of poly(A)+ mRNA

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1 Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

isolated from methionine-fed cotyledons and with poly(A)+ mRNA isolated from cotyledons grown on basal medium (Fig. 1). Due to post-translational modifications that take place in vivo, the α'-, α-, and β-subunit precursors synthesized in the wheat germ translation system do not have the same molecular weights as the mature α'-, α-, and β-subunits (12). Translations of mRNA from cotyledons cultured in the presence of 4 mM L-methionine yielded no detectable amount of β-subunit precursor (12). However, in translations of mRNA isolated from cotyledons cultured on basal medium, similar amounts of β-subunit precursor and α-subunit precursor were synthesized. The ratio of α'-subunit precursor to α-subunit precursor produced in translations of mRNA from control cotyledons and mRNA from methionine-fed cotyledons was approximately the same. This indicates that lack of β-subunit precursor production in reactions with mRNA from methionine-fed cotyledons was not an artifact of mRNA isolation or translation reaction conditions.

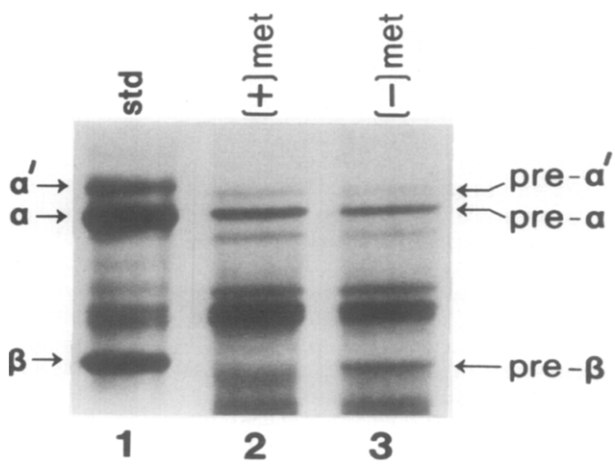

Fig. 1. Autoradiogram of Products of in Vitro Translation of mRNA from Soybean Cotyledons Cultured on Basal Medium or on Methionine-Supplemented Medium. The basal medium (lane 3) contained 1.5 mM sulfate as the sole sulfur source. Methionine-supplemented medium (lane 2) differed from basal medium only in that it contained L-methionine (4 mM). Standards for the autoradiogram (lane 1) were proteins extracted from cotyledons which were cultured on basal medium plus [^{3}H]leucine. Due to darker background on the lower part of the autoradiogram, the α'-subunit precursor is only faintly visible on photographs printed to clearly show the β-subunit precursor.

Cotyledon Transfer Experiments. We reasoned that if soybean storage protein messenger RNA's have half-lives on the order of several hours to a few days (13, J.T. Madison, unpublished results), and if exogenous methionine turns off production of functional mRNA for the β-subunit, there should be a reduction in the rate of accumulation of the β-subunit during the 4-day period following transfer of cotyledons from basal medium to methionine-supplemented medium. Such a reduction was observed (Table 1). Accumulation of the α' and α-subunits was not affected by transfer to a methionine-containing medium (data not shown). We have shown that exogenous methionine prevents the initiation of the β-subunit synthesis (2). The reduction in accumulation of the β-subunit following a transfer from basal medium to methionine-supplemented medium now shows that exogenous methionine can inhibit accumulation of the β-subunit even after synthesis has begun. The presence of 1.6 units of the β-subunit in cotyledons grown for 4 days on basal medium and then for 4 days on methionine-supplemented medium (Table 1) shows that exogenous methionine does not selectively promote degradation of existing β-subunit.

The results presented here indicate that high levels of intracellular methionine (or perhaps a metabolite of methionine) greatly depress the level of functional mRNA coding for the soybean 7S storage protein β-subunit. These experiments do not distinguish between prevention of transcription and prevention of mRNA processing. A third possibility, which seems less

Table 1. Relative Amount of β-subunit in Cotyledons Cultured Under Different Transfer Regimens Involving Basal Medium and Basal Medium Supplemented with Methionine. "B" designates basal medium; "M" designates basal medium supplemented with 4 mM methionine; "H" designates harvest; arrows represent 4 days in culture.

Regimen	Densitometer Peak Area/Cotyledon Expt 1	Expt 2	Mean	Relative Amount of β Subunit
B → H	2.8	3.4	3.1	1.0
B → B → H	8.5	8.9	8.7	2.8
B → M → H	4.8	5.2	5.0	1.6

likely, is that mRNA for the β-subunit is formed but rapidly and selectively degraded. Chandler et al. (14) have reported that reduced legumin accumulation in developing pea seeds in response to sulfur deficiency is primarily a consequence of reduced levels of legumin mRNA. The specific inhibition by methionine of β-subunit production in cultured soybean cotyledons provides a model system in which to carry out studies on regulation of a eucaryotic gene.

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