Plant Physiol. (1985) 77, 747–752
0032-0889/85/77/0747/06/$01.00/0

Biosynthesis and Intracellular Transport of 11S Globulin in Developing Pumpkin Cotyledons[1]

Received for publication July 25, 1984 and in revised form November 27, 1984

IKUKO HARA-NISHIMURA*[2], MIKIO NISHIMURA, AND TAKASHI AKAZAWA
Research Institute for Biochemical Regulation, School of Agriculture, Nagoya University, Chikusa, Nagoya 464, Japan

ABSTRACT

In vitro **studies to explore the biosynthesis of 11S globulin developing cotyledons of pumpkin (*Cucurbita* sp.) demonstrated that 11S globulin is synthesized on membrane-bound polysomes. M_r of the translation products (preproglobulin) synthesized by the poly(A)$^+$-RNA isolated from developing cotyledons were determined to be 64,000 and 59,000, which are larger than those of the mature globulin subunit (62,000 and 57,000). Preproglobulin is then cotranslationally processed by cleavage of the signal peptide to produce proglobulin. *In vivo* pulse-chase experiments showed the sequential transformation of the single-chain proglobulin to mature globulin subunit (disulfide-linked doublet polypeptides) indicating posttranslational modification of the proglobulin.**

Subcellular fractionation of the pulse-chased intact cotyledons showed that the [^{35}S]methionine label is detectable in proglobulin in rough endoplasmic reticulum shortly after the pulse label. With time, the labeled proteins move into other cellular fractions: proglobulin in the density = 1.24 grams per cubic centimeter fractions after 30 minutes and mature globulin subunit associated with protein bodies after 1 to 2 hours. The distribution of proglobulin in sucrose density gradients did not correspond with those of catalase (microbody marker) or fumarase (mitochondria marker). An accumulation of proglobulin occurred in the density = 1.24 grams per cubic centimeter fractions, whereas the mature globulin was scarcely detectable in this fraction. In contrast, proglobulin was not detected by immunochemical blotting analysis in the protein bodies prepared under the mild conditions from cotyledon protoplasts. The results suggest that the d = 1.24 grams per cubic centimeter fractions are engaged in the translocation of proglobulin into the protein bodies.

11S globulin proteins are present in seeds of both mono- and dicotyledonous plants as a major reserve protein. The only reserve protein of pumpkin (*Cucurbita* sp.) seed is 11S globulin which comprises more than 85% of the total protein of dry seed. Most of the insoluble 11S globulin molecules are limited-hydrolyzed during the early stage of germination by the globulin-specific proteinase to produce the soluble molecular species (11). Thus, the pumpkin cotyledon can be used to explore the mechanism of biosynthesis as well as mobilization of seed protein. Similar studies with seeds of leguminous species are complicated by the presence of multiple reserve proteins.

The pumpkin 11S globulin subunit consists of two polypeptide chains linked together by a disulfide bond (9, 10). The N-terminal amino acid sequences and C-terminal residues have been previously reported (22). There is a high degree of structural resemblance to other 11S globulins such as glycinin (20) and legumin (5).

The globulin molecules synthesized during the step of seed development, about 30 to 40 d after anthesis, are stored as crystalloids in the spherical protein bodies (5–7 μm) localized in cotyledons of dry seeds (8). The mechanisms involved in the biosynthesis and intracellular transport of seed proteins have received a great deal of attention in recent years (12). *In vitro* translation systems have shown that a single polypeptide precursor form is synthesized on the RER (12). Higgins *et al.* (13) have conducted similar studies of the biosynthesis and processing of pea lectin, which is also localized in the protein bodies. However, the mechanism involved in processing the single chain precursor polypeptide to the mature form of 11S globulin (consisting of disulfide-linked doublet polypeptide chains) is unknown. In attempting to answer this unsolved question, we have conducted experiments using the pumpkin 11S globulin as a model system. The specific objective of this study was to identify the intracellular transport of the precursor form of the protein molecules synthesized on the RER.

MATERIALS AND METHODS

Plant Materials. Seeds of pumpkin (*Cucurbita* sp. cv Kurokawa Amakuri Nankin) were grown during the summer season of 1983 on the university farm and experiments were carried out using cotyledons (120–160 mg/cotyledon pair) of developing plants 35 to 40 d after fertilization.

Isolation of Poly(A)$^+$-RNA. Frozen cotyledons (10 g) were ground in liquid N_2 to a fine powder, to which was added 50 ml of 50 mM Tricine-KOH (pH 9.5) containing 150 mM NaCl, 20 mM EDTA, and 2% SDS. An equal volume of phenol-chloroform (1:1, v/v) was added to the suspension, and RNA was extracted into the aqueous phase by vigorous stirring. After centrifugation, the aqueous phase was treated once again with 30 ml of phenol-chloroform (1:1). The subsequent procedures were basically the same as those described by Miyata *et al.* (19).

Isolation of Polysomes. Developing cotyledons (27 g) or etiolated cotyledons (3-d) (30 g) were pulverized to a fine powder in liquid N_2. To the powder was added 70 ml of 0.2 M Tris-HCl (pH 8.5) containing 60 mM KCl, 50 mM $MgCl_2$, 0.1 mM EDTA, 0.2 M sucrose, 5 mM DTT, and yeast RNA (3 mg/ml). Subsequent procedures were essentially as described by Larkins *et al.* (15), except that at the final step both free and membrane-bound polysomes were pelleted by centrifugation at 230,000 *g* for 3 h in a Beckman R45Ti rotor. The pellets were resuspended in a small volume of 20 mM Hepes-KOH (pH 7.5) containing 120 mM KCl, 2.5 mM $MgCl_2$, 0.25 M sucrose, and 1 mM DTT.

In Vitro **Protein Synthesis.** Poly(A)$^+$-RNA or isolated polysomes were used in an *in vitro* translation system containing

[1] This is paper no. 9 in a series "Pumpkin (*Cucurbita* sp.) Seed Globulin". Paper no. 8 is Ref. 11.

[2] Recipient of a postdoctoral fellowship from the Japan Society for the Promotion of Science (JSPS), 1983.

wheat germ extracts as described by Roberts and Paterson (27) or Larkins *et al.* (15), respectively. The reaction mixture (50 μl) for translation of poly(A)$^+$-RNA contained [^{35}S]methionine (50 μCi) and 2.5 μg of poly(A)$^+$-RNA. The reaction mixture (72 μl) for either free or membrane-bound polysomes contained [^{35}S]methionine (30 μCi) and 1.5 A_{260} units of polysomes. Subsequent procedures were exactly those described by Yamaguchi *et al.* (30). Characterization of the globulin precursor synthesized in this system was performed using specific anti-11S globulin IgG and the immunoprecipitates were subjected to SDS gel electrophoresis and fluorography.

Preparation of Protein Bodies. Protoplasts were prepared as described by Nishimura and Beevers (21). Sliced tissues from seven developing cotyledon halves were incubated in 5 ml of an enzyme solution (2% Cellulase 'Onozuka' R-10 and 0.1% Pectolyase dissolved in 0.7 M mannitol). After 4 to 5 h, the solution was removed, and 5 ml of 0.7 M mannitol was added to the slices and the flask was briefly swirled by hand. The mannitol solution was collected by filtration through a Nylon bolting cloth (35 mesh) and the filtrate was layered on 2 ml of 40% (w/v) sucrose. After slow speed centrifugation at 200*g* for 5 min, the pellet was collected and used as the protein body fraction. The isolated protein bodies were inspected by an Olympus BHS phase-contrast microscope and found to be free from contamination by other cellular components (Fig. 4). The extracts from the protein body fraction and the whole cotyledon tissue were then subjected to immunochemical (western) blotting analysis.

Sucrose Density Gradient Centrifugation. Developing cotyledons (5 g) were homogenized in a Petri dish placed in an ice bath by chopping with a stainless steel razor blade for 5 min using 3 ml of chilled medium containing 150 mM Tricine-KOH (pH 7.5), 1 mM EDTA, and 13% (w/v) sucrose. The homogenates were passed through four layers of cheesecloth and the filtrate was directly layered onto a sucrose gradient consisting of a 1-ml cushion of 60% (w/w) sucrose, 11 ml of sucrose solution increasing linearly from 30 to 60% sucrose, and 2 ml of 20% sucrose in a 17-ml tube. The gradient solution was then centrifuged at 21,000 rpm for 3 h in a Beckman Spinco model L8-70 ultracentrifuge using an SW 27-1 rotor at 4°C. After centrifugation, fractions (0.4 ml) were collected by an ISCO Density Gradient Fractionator (model 640) and used for the assays of each respective marker enzyme, the sucrose density, and the protein content, which was analyzed with a Bio-Rad Protein Assay Kit using bovine γ-globulin as a standard. The localization of globulin or proglobulin was determined using SDS gel electrophoresis or immunochemical (western) blotting analysis, respectively.

Pulse-Chase Labeling. Five μl of [^{35}S]methionine (58 μCi) was administered to the inner surface of the isolated cotyledon on moistened filter paper in a Petri dish and incubation was performed at 25°C. One to six cotyledons were used per treatment. After 15 or 30 min of [^{35}S]methionine uptake, the tissue was briefly rinsed in 10 mM unlabeled methionine solution, followed by further incubation for 0.5, 1, 2, or 3 h of chase period. At the indicated times, cotyledons were homogenized with 2% SDS solution containing 10 mM Tris-HCl (pH 6.8), 5% 2-mercaptoethanol, 10% glycerol, and 0.001% bromophenol blue in a mortar. After centrifugation, 1 to 3 μl of the supernatant solution was subjected to SDS gel electrophoresis and fluorography.

For subcellular separation of the labeled cotyledons, the procedures of pulse-chase labeling and sucrose density gradient centrifugation were exactly the same as those described above.

Immunochemical (Western) Blotting. Electrophoretic blotting was performed basically as described by Vaessen *et al.* (29) and Yamaguchi *et al.* (30).

Marker Enzyme Assays. All enzyme assays were carried out at room temperature (25°C). Assay methods of catalase (17), NADPH-Cyt *c* reductase (16), fumarase (25), and alcohol dehydrogenase (26) followed those described in the literature.

Estimation of S Values. Sucrose density gradient centrifugation was performed according to the method of Martin and Ames (18). The internal marker enzymes, *i.e.* bovine liver catalase (0.5 k units) and yeast alcohol dehydrogenase (100 μg), were added to 200 μl of the extract from the developing cotyledons (0.06 g). The sample solution was layered onto 4.5 ml of the linear sucrose gradient (8–24% [w/v]) containing (a) 0.1 M Tris-HCl (pH 7.5) and 2 M NaCl or (b) 0.1 M Tris-HCl (pH 7.5), 1% Tween, and 0.25 M NaCl. Centrifugation was carried out at 40,000 rpm for 48 h (a) or 24 h (b) in a Beckman Spinco model L8-70 ultracentrifuge using an SW 65Ti rotor at 4°C. After centrifugation, fractions (0.1 ml) were collected by an ISCO Density Gradient Fractionator (model 640), and aliquots were assayed for catalase and alcohol dehydrogenase and subjected to immunochemical (western) blotting analysis.

RESULTS

Precursor Forms of 11S Globulin. As a first step to study the *in vitro* synthesis of pumpkin 11S globulin, we prepared both free and membrane-bound polysomes from cotyledons of either developing seeds or etiolated seedlings, and examined the nature of the translation products directed by these preparations. From the data shown in Table I, given on the basis of the same quantity of RNA, the maximal incorporation of [^{35}S]methionine into the immunoprecipitable polypeptides was observed with membrane-bound polysomes obtained from the developing cotyledons. The results indicated that the 11S globulin is synthesized on the RER, and that the globulin mRNA is not present in the cotyledons of germinating seedlings.

The fluorographic analysis of Figure 1 clearly shows that M_r of the *in vitro* translation products directed by poly(A)$^+$-RNA are 64,000 and 59,000, which are approximately 2,000 D larger than those of the constituent subunits (α and β) of the mature globulin molecules. Consequently, the protein appears to be synthesized as a larger precursor form (preproglobulin).

The time-sequential formation of *in vitro* translation products on membrane-bound polysomes has been analyzed employing the 'read-out' system. The initial products detected are M_r 62,000 and 57,000 polypeptides, followed by the formation of preproglobulin molecules having a larger molecular size (data not shown). These results indicate that cotranslational cleavage of the signal peptide of preproglobulin occurs on the RER to produce proglobulin molecules.

The mature form of pumpkin 11S globulin contains two major subunits (α: M_r 62,000 and β: M_r 57,000) (Fig. 1, lane 2), each containing two polypeptide chains linked by a disulfide bond (9, 10). 2-Mercaptoethanol treatment produces smaller cleaved products (γ: M_r 34,000–36,000 and δ: M_r 22,000–26,000) (lane 3). However, this treatment does not alter the electrophoretic mobilities of the *in vitro* translation products (preproglobulin) (lane 1), indicating that both γ and δ chains are synthesized as a single polypeptide chain.

Table I. *In Vitro Translation of 11S Globulin Directed by Free and Membrane-Bound Polysomes from Either Developing or Etiolated Cotyledons*

Polysomes	Incorporation of [^{35}S]Methionine[a]			
	Developing cotyledons		Etiolated cotyledons	
	dpm	%	*dpm*	%
Membrane bound	114,400	100	4,680	4.1
Free	6,570	5.7	1,190	1.0

[a] Incorporation of [^{35}S]methionine into immunoprecipitable polypeptides was measured on the basis of the same quantity of RNA.

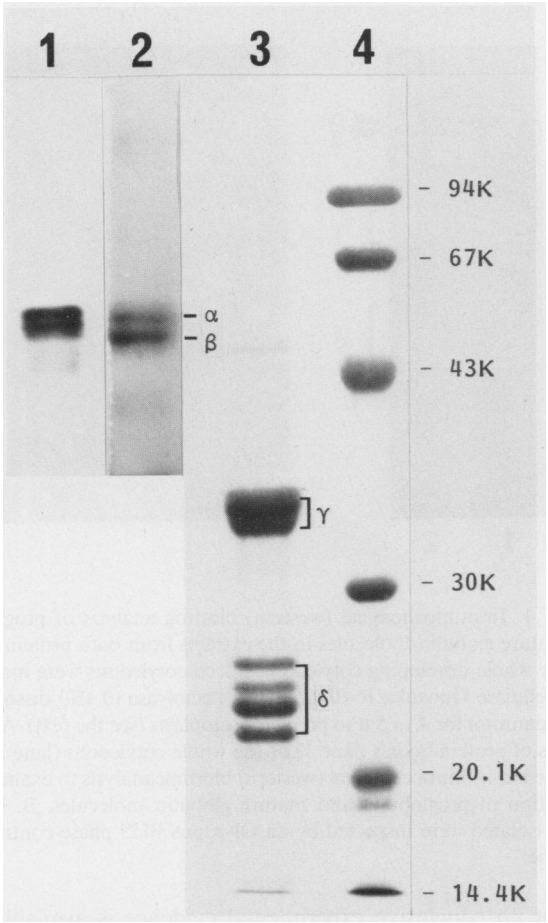

FIG. 1. *In vitro* synthesis of 11S globulin directed by poly(A)$^+$-RNA. Lane 1, Fluorogram of immunoprecipitate of 11S globulin directed by poly(A)$^+$-RNA. Lane 2 and 3, SDS gel electrophoretic pattern of mature globulin with (lane 3) or without (lane 2) 2-mercaptoethanol. α and β are represented to subunits of proglobulin or unreduced mature globulin, and γ and δ are represented to constituent polypeptide chains of reduced mature globulin. Lane 4, M_r marker proteins: phosphorylase b (94,000), BSA (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), and α-lactoalbumin (14,400).

In vivo pulse-chase labeling of the globulin molecules revealed that radioactivity was initially detected in proglobulin (M_r 62,000 and 57,000), and after 30 to 60 min in both γ and δ chains of the mature form (Fig. 2). A radioactive band of M_r 51,000 was detectable after 1 h. Although this component was precipitated by anti-11S globulin IgG, it is unlikely to be derived from the proteolytic hydrolysis of proglobulin, based on peptide mapping analysis (unpublished observation).

Overall, these results show that the single proglobulin polypeptide is posttranslationally processed to the doublet globulin subunit containing both γ and δ chains.

Intracellular Transport of Globulin. The crucial questions remaining to be answered relate to identifying the sites of posttranslational processing of proglobulin molecules and assembly to form 11S globulin molecule. To answer the first question, we have subjected homogenates of cotyledons to subcellular fractionation on linear sucrose density gradients. As shown in Figure 3, proglobulin was detected in both ER (fraction number 7) and density (d) = 1.24 g/cm^3 (51.7% [w/w] sucrose) fraction (fraction number 22), whereas most of the mature globulin molecules were contained in the bottom fraction, which is likely to be protein bodies and/or suborganellar inclusions, *i.e.* crystalloids (Fig. 3C).

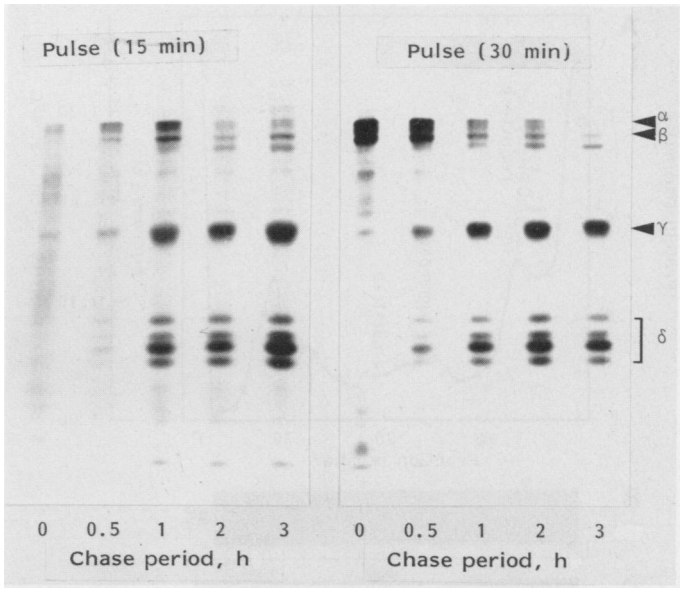

FIG. 2. Labeling patterns of polypeptides during pulse-chase periods. Cotyledons were exposed to [^{35}S]methionine for 15 min (left) or 30 min (right) and subsequently transferred to medium containing unlabeled methionine. Cotyledons were homogenized at the selected chase periods as indicated, and an aliquot of the supernatant fraction obtained after centrifugation was subjected to SDS gel electrophoresis and fluorography as described in the text.

What is the structural entity of the d = 1.24 g/cm^3 fractions? From the measurements of several marker enzyme activities, they are clearly denser than ER, mitochondria, or microbodies (glyoxysomes). To assure that the fractions were not derived from the degradation of protein bodies, protoplasts were prepared from the developing cotyledons and used to isolate the intact protein bodies. Immunochemical methods did not identify proglobulin in the protein bodies (Fig. 4), thus showing that d = 1.24 g/cm^3 fractions are not derived from these protein bodies. It is evident that at least three organelles, *i.e.* ER, d = 1.24 g/cm^3 fractions and protein bodies, are involved in the biosynthesis and intracellular transport of the globulin molecules.

The pulse-chase [^{35}S]methionine labeled cotyledons were subjected to subcellular fractionation experiments. Results presented in Figure 5 show that immediately after the pulse (0 h), radioactivity was detected in proglobulin localized in the ER and not in preproglobulin. After 30 min chase, radioactivity had spread into the proglobulin present in two different organelles, ER and d = 1.24 g/cm^3 fractions. During the subsequent 1 to 2 h chase periods, the γ and δ chains of globulin molecules in the protein body fraction became prominently labeled. For the purpose of further exploring the mechanism of the molecular assemblage of the globulin subunit, the S value of the proglobulin or globulin molecules was determined to be 8S or 12S, respectively.

DISCUSSION

On the basis of these data, we propose that preproglobulin synthesized on the RER is cotranslationally processed to make up trimeric proglobulin, which is then transported to the d = 1.24 g/cm^3 fractions. Subsequent posttranslational processing results in the formation of hexameric molecules of the mature 11S globulin which are deposited in the protein bodies. The biosynthetic mechanism of the pumpkin 11S globulin can be diagrammatically summarized as in Figure 6. The M_r of the *in vitro* synthesized singlet polypeptide (preproglobulin) was shown to be approximately 2000 D larger than that of the doublet globulin subunit composed of disulfide-linked polypeptide chains

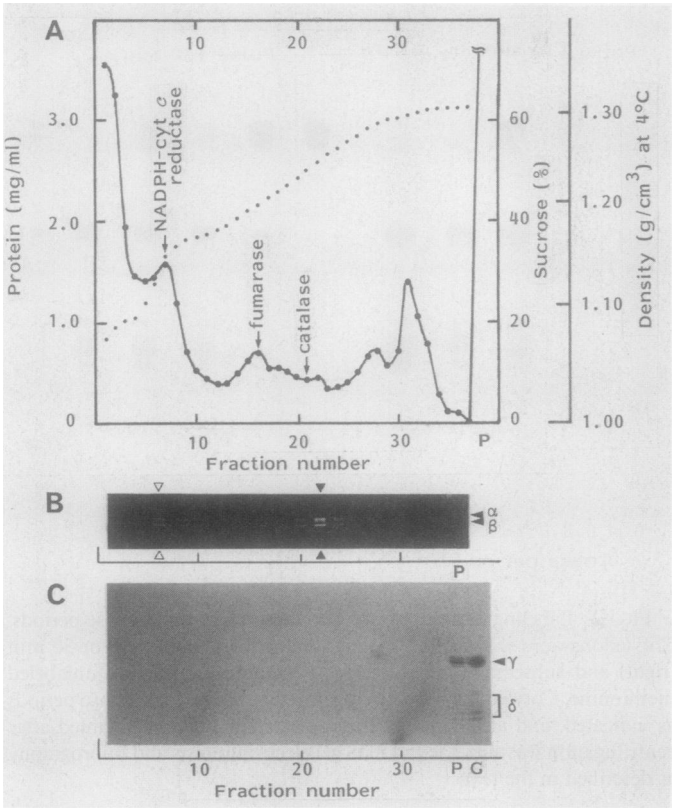

FIG. 3. Subcellular fractionation of developing cotyledons and localizations of proglobulin and mature globulin. Developing cotyledons (5 g) were homogenized by chopping with a razor blade for 5 min. After briefly squeezing the homogenate, an aliquot of the filtrate was subjected to sucrose density gradient centrifugation at 21,000 rpm for 3 h using an SW 27-1 rotor of a Beckman Spinco ultracentrifuge at 4°C (A). Aliquots of each fraction were subjected to immunochemical (western) blotting analysis for proglobulin (B) and SDS gel electrophoresis for mature globulin (C). Locations of the marker enzyme activities, NADPH-Cyt *c* reductase (ER), catalase (microbodies), and fumarase (mitochondria) are indicated by arrows. Protein content was determined by a Bio-Rad Protein Assay Kit using γ-globulin as a standard. (——), Protein content; (. . . .), concentration of sucrose; P, precipitate; G, mature 11S globulin.

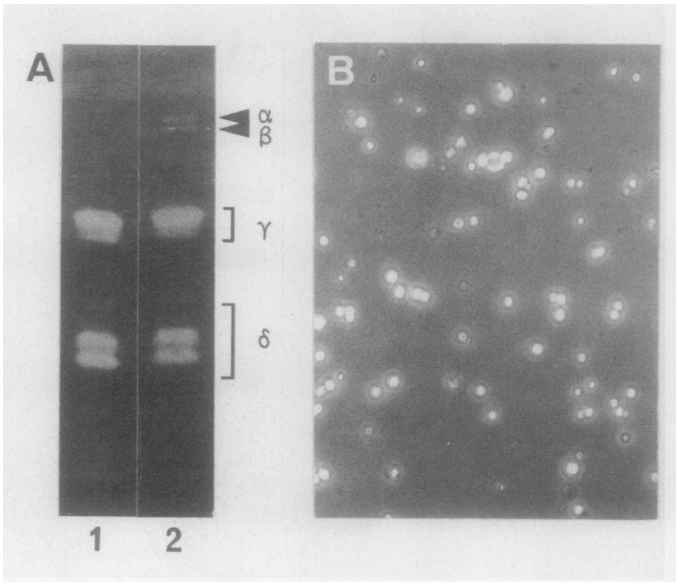

FIG. 4. Immunochemical (western) blotting analysis of proglobulin and mature globulin molecules in the extracts from both protein bodies and the whole developing cotyledons. Sliced cotyledons were incubated with Cellulase Onozuka R-10 (2%) and Pectolyase (0.1%) dissolved in 0.7 M mannitol for 4 to 5 h to prepare protoplasts (see the text). A, Total extracts of protein bodies (lane 1) or the whole cotyledons (lane 2) were subjected to immunochemical (western) blotting analysis to examine the association of proglobulin and mature globulin molecules. B, Protein bodies isolated were inspected by an Olympus BHS phase-contrast microscope.

(Fig. 1), and the signal peptide was cotranslationally cleaved. In contrast, Bassüner *et al.* (1) have recently reported that the M_r of the *in vitro* translation product of the *Vicia faba* legumin (11S globulin) has a size similar to that of the mature molecule.

Employing subcellular fractionation experiments of the pulse-chased cotyledons, we have found that the proglobulin molecules, produced after cotranslational processing on the RER, are transported to the d = 1.24 g/cm³ organelles (Fig. 5). Indeed, a marked accumulation of proglobulin in the d = 1.24 g/cm³ organelles is clearly demonstrable (Fig. 3). Similar results have been reported for the intracellular transport of phytohemagglutinin in French bean (*Phaseolus vulgaris* L.) (2). It was found that phytohemagglutinin is transiently bound to the dense vesicles (d = 1.22 g/cm³) derived from the Golgi membranes. Electron micrographs have demonstrated the structure of vesicles as electron dense small particles (0.1–0.4 μm). The presence of the electron dense small vesicles has also been reported in developing rice endosperm tissues (23). There is thus a good possibility that the d = 1.24 g/cm³ fractions presently observed are structurally related to the dense vesicles identified by other workers.

It is generally accepted that in animal cells both secretory and lysosomal proteins are transported via the Golgi complex (7), where the posttranslational modifications occur. However, at present no conclusive experimental evidence is available supporting this mechanism operating in the biosynthetic pathway of nonglycosylated type 11S globulin molecules (12). If the d = 1.24 g/cm³ fractions are analogous to the Chrispeels' dense vesicles (d = 1.22 g/cm³), we must take into account the possible role of the Golgi complex during the intracellular transport of the pumpkin 11S globulin molecules.

Other crucial questions remaining to be answered are the mechanisms of assemblage to form the mature form of 11S globulin, as well as its posttranslational processing. The quaternary structure of proglobulin and the mature globulin appears to correspond with the precursor and mature molecule of legumin in developing pea cotyledons (3). However, in contrast to our observations concerning the transient accumulation of the trimeric proglobulin in the d = 1.24 g/cm³ fractions (Figs. 3 and 5) and its scarce presence in the protein bodies (Fig. 4), Chrispeels *et al.* (3) proposed that the legumin precursor (trimer) is directly transported to the protein bodies from ER. They also reported that the legumin precursor is processed in the protein bodies to produce the mature form of legumin. However, they only examined ER and protein bodies, and it is conceivable from such experimental protocols that the protein bodies obtained are not homogeneous but contain the denser membranous components, the latter being probably engaged in the accumulation and/or the posttranslational processing of the legumin precursor.

Subcellular fractionation of the pulse-chased cotyledons clearly demonstrated an accumulation of proglobulin in the d = 1.24 g/cm³ vesicles (Fig. 5). On the contrary, protein bodies are only associated with the mature globulin species, with little proglobulin molecules being accumulated (Figs. 3–5). All these findings substantiate the view that the two organelles are distinctly different in their protein compositions. In corroboration with the results of other investigators, it is reasonable to postulate that the posttranslational processing proceeds after the d = 1.24 g/cm³

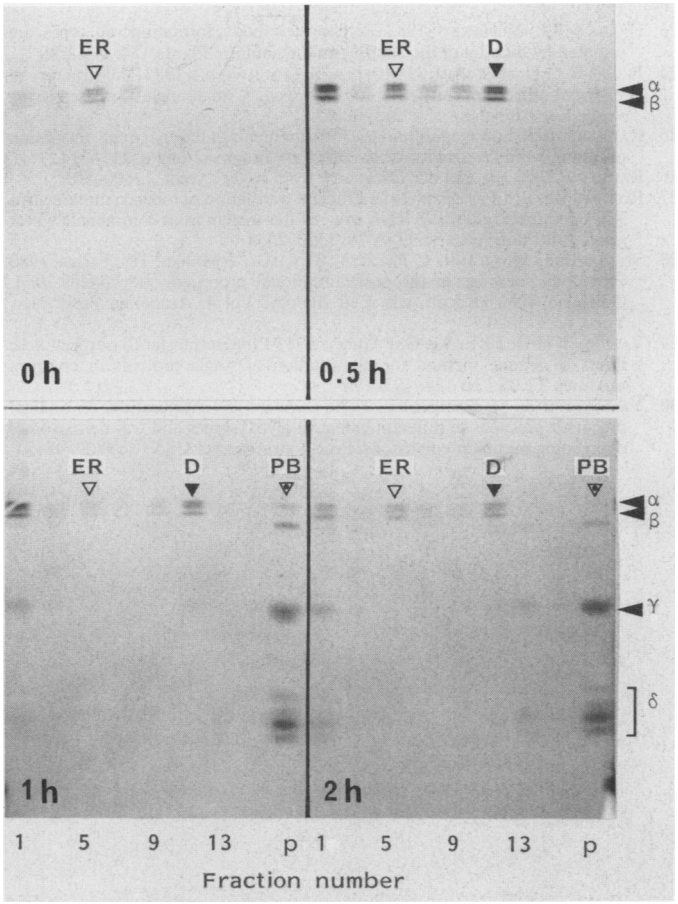

FIG. 5. Subcellular fractionation of pulse-chase labeled cotyledons by sucrose density gradient centrifugation. After a prior pulse with [^{35}S]-methionine for 15 min, cotyledons were subsequently transferred to a chase medium containing unlabeled methionine for the selected chase periods as indicated (0, 0.5, 1, and 2 h), and then immediately homogenized by chopping with a razor blade for 5 min. After filtration of the homogenate, an aliquot was subjected to sucrose density gradient centrifugation at 21,000 rpm for 3 h using an SW 27-1 rotor of a Beckman Spinco ultracentrifuge at 4°C. After fractionation, an aliquot of each fraction was subjected to SDS gel electrophoresis and fluorography. D, d = 1.24 g/cm^3 fractions; PB, protein bodies; P, precipitate.

vesicles, but we have not succeeded in identifying organelle(s) containing both substrate (proglobulin) and product (globulin). One possibility is that the processing enzyme(s) involved is present in protein bodies and the proglobulin in the protein bodies is cleaved to form mature globulin during isolation or separation of these organelles.

It is pointed out that the postulated mechanism illustrated in Figure 6 is basically similar to the currently accepted mechanism(s) of both biosynthesis and transport of some animal secretory proteins and hormones (28). It has been demonstrated that the adjacent basic amino acid residues have a distinct role as the recognition sites during the step of the posttranslational processing of these proteins. From a base sequence analysis of cDNA coding pea legumin precursor, Croy *et al.* (4) suggested that there is an Arg-Arg sequence at five residues upstream from the N-terminal amino acid of the basic chain of legumin (corresponding to the δ chain of pumpkin globulin), which is conceivably the site of processing. Therefore, it is reasonable to postulate that the mechanism of the processing of 11S globulin is basically analogous to that of animal hormones such as insulin. Knowledge concerning the localization of the proinsulin-converting enzyme

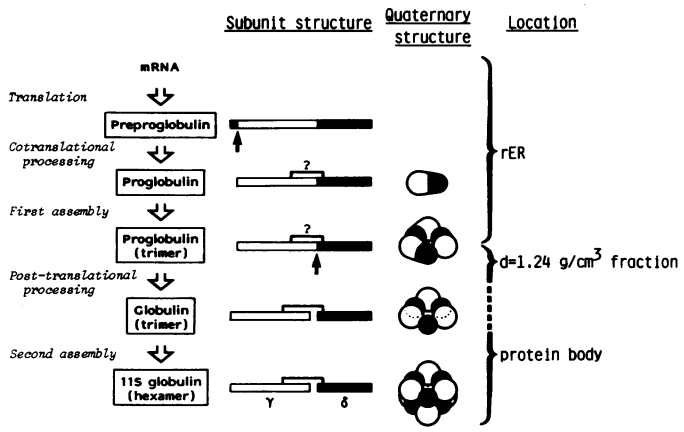

FIG. 6. Hypothetical mechanism of biosynthesis and intracellular transport of 11S globulin molecule in developing pumpkin cotyledons. Black arrows indicate the cleavage of signal peptide in preproglobulin and posttranslational processing of proglobulin, respectively. Site of the introduction of disulfide bridge is not known. Quaternary structure is based on the date of x-ray analysis of sunflower and rape seed 11S globulins (24) and Brazil nut 11S globulin (14); rER, RER.

activities in the secretory granules (28) will help us in the full characterization of d = 1.24 g/cm^3 fractions in future experiments. Docherty *et al.* (6) have recently reported that a unique acid thiol protease (M_r 31,500) may play a role in the processing of proinsulin. Further studies on the processing enzyme(s) as well as structural comparison between proglobulin and globulin will give us a clue to understanding the general mechanism of the posttranslational processing of seed reserve proteins.

LITERATURE CITED

1. BASSÜNER R, R MANTEUFFEL, K MÜNTZ, M PÜCHEL, P SCHMIDT, E WEBER 1983 Analysis of in vivo and in vitro globulin formation during cotyledon development of field beans (*Vicia faba* L. var. *minor*). Biochem Physiol Pflanzen 178: 665–684
2. CHRISPEELS MJ 1983 The Golgi apparatus mediates the transport of phytohemagglutinin to the protein bodies in bean cotyledons. Planta 158: 140–151
3. CHRISPEELS MJ, TJV HIGGINS, D SPENCER 1982 Assembly of the storage protein oligomers in the endoplasmic reticulum and processing of the polypeptides in the protein bodies of developing pea cotyledons. J Cell Biol 93: 306–311
4. CROY RRD, GW LYCETT, JA GATEHOUSE, JN YARWOOD, D BOULTER 1982 Cloning and analysis of cDNAs encoding plant storage protein precursors. Nature 295: 76–79
5. DERBYSHIRE E, DJ WRIGHT, D BOULTER 1976 Legumin and vicilin, storage proteins of legume seeds. Phytochemistry 15: 3–24
6. DOCHERTY K, RJ CARROLL, DF STEINER 1982 Conversion of proinsulin to insulin: Involvement of a 31,500 molecular weight thiol protease. Proc Natl Acad Sci USA 79: 4613–4617
7. FARQUHAR MG, GE PALADE 1981 The Golgi apparatus (complex)—(1954–1981)—from artifact to center stage. J Cell Biol 91: 77s–103s
8. HARA I, H MATSUBARA 1980 Pumpkin (*Cucurbita* sp.) seed globulin VII. Immunofluorescent study on protein bodies in ungerminated and germinating cotyledon cells. Plant Cell Physiol 21: 247–254
9. HARA I, M OHMIYA, H MATSUBARA 1978 Pumpkin (*Cucurbita* sp.) seed globulin III. Comparison of subunit structures among seed globulins of various *Cucurbita* species and characterization of peptide components. Plant Cell Physiol 19: 237–243
10. HARA I, K WADA, S WAKABAYASHI, H MATSUBARA 1976 Pumpkin (*Cucurbita* sp.) seed globulin I. Purification, characterization and subunit structure. Plant Cell Physiol 17: 799–814
11. HARA-NISHIMURA I, M NISHIMURA, H MATSUBARA, T AKAZAWA 1982 Suborganellar localization of proteinase catalyzing the limited hydrolysis of pumpkin globulin. Plant Physiol 70: 699–703
12. HIGGINS TJV 1984 Synthesis and regulation of the major proteins in seeds. Annu Rev Plant Physiol 35: 191–221
13. HIGGINS TJV, MJ CHRISPEELS, PM CHANDLER, D SPENCER 1983 Intracellular sites of synthesis and processing of lectin in developing pea cotyledons. J Biol Chem 258: 9550–9552
14. KAMIYA N, K SAKABE, N SAKABE, K SASAKI, M SAKAKIBARA, H NOGUCHI 1983 Structural properties of Brazil nut 11S globulin, excelsin. Agric Biol Chem 47: 2091–2098

15. LARKINS BA, CE BRACKER, CY TSAI 1976 Storage protein synthesis in maize. Isolation of zein-synthesizing polyribosomes. Plant Physiol 57: 740–745
16. LORD JM, T KAGAWA, TS MOORE, H BEEVERS 1973 Endoplasmic reticulum as the site of lecithin formation in castor bean endosperm. J Cell Biol 57: 659–667
17. LÜCK H 1965 Catalase. *In* HU Bergmeyer, ed, Methods of Enzymatic Analysis. Academic Press, New York, pp 885–894
18. MARTIN RG, BN AMES 1961 A method for determining the sedimentation behavior of enzymes: application to protein mixtures. J Biol Chem 236: 1372–1379
19. MIYATA S, K OKAMOTO, A WATANABE, T AKAZAWA 1981 Enzymic mechanism of starch breakdown in germinating rice seeds. 10. *In vivo* and *in vitro* synthesis of α-amylase in rice seed scutellum. Plant Physiol 68: 1314–1318
20. MOREIRA MA, MA HERMODSON, BA LARKINS, NC NIELSEN 1979 Partial characterization of the acidic and basic polypeptides of glycinin. J Biol Chem 254: 9921–9926
21. NISHIMURA M, H BEEVERS 1978 Hydrolases in vacuoles from castor bean endosperm. Plant Physiol 62: 44–48
22. OHMIYA M, I HARA, H MATSUBARA 1980 Pumpkin (*Cucurbita* sp.) seed globulin IV. Terminal sequences of the acidic and basic peptide chains and identification of pyrroglutamyl peptide chain. Plant Cell Physiol 21: 157–167
23. OPARKA KJ, N HARRIS 1982 Rice protein-body formation: all types are initiated by dilation of the endoplasmic reticulum. Planta 154: 184–188
24. PLIETZ P, G DAMASCHUM, JJ MÜLLER, K-D SCHWENKE 1983 The structure of 11-S globulins from sunflower and rape seed. A small-angle X-ray scattering study. Eur J Biochem 130: 315–320
25. RACKER E 1950 Spectrophotometric measurements of the enzymatic formation of fumaric and *cis*-aconitic acids. Biochim. Biophys. Acta 4: 211–214
26. RACKER E 1955 Alcohol dehydrogenase from baker's yeast. 1: 500–503
27. ROBERTS BE, BM PATERSON 1973 Efficient translation of tobacco mosaic virus RNA and rabbit globin 9S RNA in a cell-free system from commercial wheat germ. Proc Natl Acad Sci USA 70: 2330–2334
28. STEINER DF, PS QUINN, C PATZELT, SJ CHAN, J MARSH, HS TAGER 1980 Proteolytic cleavage in the posttranslational processing of proteins. *In* L Goldstein, DM Prescott, eds, Cell Biology, Vol 4. Academic Press, New York, pp 175–202
29. VAESSEN RTMJ, J KREIKE, GSP GROOT 1981 Protein transfer to nitrocellulose filters. A simple method for quantitation of single proteins in complex mixtures. FEBS Lett 124: 193–196
30. YAMAGUCHI J, M NISHIMURA, T AKAZAWA 1984 Maturation of catalase precursor proceeds to different extent in glyoxysomes and leaf peroxisomes of greening pumpkin cotyledons. Proc Natl Acad Sci USA 81: 4809–4813