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Cell-free Synthesis of Pea Seed Proteins

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ABSTRACT

Both polysomes and polysomal RNA, isolated from cotyledons of ripening pea (*Pisum sativum*) seeds and supplemented respectively with wheat germ S-100 and S-30 fractions, were used to program the cell-free synthesis of polypeptides. The relationship of these polypeptide products to seed storage proteins has been investigated. When fractionated on sucrose density gradients the translation products did not coincide with native storage proteins, nor were they exactly coincident with the subunits of storage proteins on dissociating gels. Treatment with antiserum prepared against storage proteins precipitated only a very small proportion of these products. Nonetheless, tryptic peptide mapping showed that a significant proportion (up to 65%) of the *in vitro* products from cell-free systems were related to the storage proteins. Alternative interpretations of these results are that either the translatable mRNAs for storage proteins make up a small proportion of the total template isolated from pea cotyledon polysomes, or that storage protein polypeptides are made in significant amounts *in vitro* but lack major antigenic determinants which *in vivo* may be acquired during chain completion or post-translational modification.

The maturing seed represents a stage of plant development during which large quantities of reserve materials, including characteristic proteins, are synthesized and stored. In the case of ripening pea seeds, and many other legumes, two major storage fractions have been characterized. These are the globulins legumin and vicilin, with mol wt of approximately 380,000 and 140,000, respectively (10). These two protein fractions constitute about 80% of the total protein of the mature pea seed (25). Earlier work has shown that their synthesis is initiated and terminated at well defined stages of seed development (23) and their occurrence is restricted to the cotyledons and axis of the seed (22). It follows that these tissues are committed to the large scale synthesis and deposition of these two protein fractions—a clear-cut case of biochemical differentiation involving the expression of otherwise latent genetic information. The developing pea seed is thus a potentially useful system in which to study the metabolic controls which regulate transcription and translation in plants.

Our immediate concern is to study the control of translation and toward this end we have sought to develop two cell-free systems capable of synthesizing pea seed storage proteins. One system involves protein synthesis by polysome preparations from cotyledons of ripening pea seeds and the other system utilizes pea seed polysomal RNA to program a mRNA-dependent, cell-free, protein-synthesizing system from wheat germ. Beevers and Poulson (4) have reported some of the quantitative features of *in vitro* amino acid incorporation by polysomes from developing pea cotyledons. A preliminary account of our results has been reported elsewhere (12).

MATERIALS AND METHODS

PLANT MATERIALS

Pisum sativum cv. Greenfeast was grown in artificially lit cabinets as described earlier (23). Flowers were tagged on 1st day of appearance and stage of seed development was subsequently measured from that date. Developing pods were collected and the immature cotyledons separated from the testa and embryonic axis and stored in liquid N_2 prior to use.

Epicotyls were harvested from peas germinated at 25 C for 8 days in the dark.

POLYRIBOSOME ISOLATION

Total polyribosomes were isolated using a modified buffer system first described by Breen *et al.* (7). The cotyledons were ground to a fine powder under liquid N_2 in a mortar. Buffer (50 mM tris-HCl [pH 8], 0.25 M sucrose, 0.4 M KCl, 20 mM Mg-acetate, 5 mM β-mercaptoethanol, 1% [w/v] Triton X-100, 1% [w/v] sodium deoxycholate) was added (3 ml/g tissue) and the mixture was allowed to thaw and stand on ice for 10 min. The extract was filtered through two layers of Miracloth and centrifuged at 10,000*g* for 10 min. The supernatant was layered over a cushion of 1.5 M sucrose in 50 mM tris-HCl (pH 8), 0.2 M KCl, 10 mM Mg-acetate, 5 mM β-mercaptoethanol, and centrifuged at 264,000*g* (av) for 4 hr in the 70 rotor of a Beckman L5-65B ultracentrifuge. The polysomes were resuspended in 50 mM tris-HCl (pH 7.8), 5 mM Mg-acetate, 2 mM β-mercaptoethanol, and 0.25 M sucrose, clarified by centrifuging at 10,000*g* for 10 min and either stored in small aliquots in liquid N_2 or used for the isolation of RNA.

PREPARATION OF POLYRIBOSOMAL RNA

Polysome preparations were made up to 50 mM tris-HCl, 0.1 M glycine (pH 8.5), 0.3 M NaCl, 5 mM EDTA, 2% SDS, and extracted with phenol followed by the addition of 1 volume of chloroform. The phenol phase was re-extracted with the tris-glycine buffer, the aqueous phases were pooled and extracted twice with phenol. Dissolved phenol was removed from the aqueous phase by ether extraction. Sodium acetate (pH 5.5) was added to a final concentration of 2% (w/v) followed by 2 volumes cold ethanol. After standing at −20 C overnight, the RNA was sedimented by centrifugation at 10,000*g* for 15 min and dissolved in water at a concentration of 1 mg/ml.

IN VITRO PROTEIN SYNTHESIS

Run-off system. A wheat germ S-100 fraction was prepared from an S-30 fraction (26) by centrifugation for 2 hr at 100,000*g*. The supernatant was stored in small aliquots in liquid N_2. Protein synthesis was carried out in a final volume of 50 μl containing 100 mM KCl, 20 mM HEPES-KOH (pH 7.9), 1 mM ATP, 0.1 mM GTP, 8 mM creatine phosphate, 3 mM Mg-acetate, 2 mM DTP, 40 μg polysomes, 10 μl wheat germ S-100

fraction, and 0.25 μCi of a mixture of 14 ^{14}C-amino-acids (54 mCi/m atom C) (Radiochemical Centre, Amersham). It was unnecessary to add further tRNA, unlabeled amino acids, or creatine phosphokinase since sufficient of each was present in the S-100. The reaction was allowed to proceed for 70 min at 25 C and was terminated by removal to an ice bath and the addition of unlabeled amino acids.

mRNA-dependent System. The wheat germ cell-free protein-synthesizing system (26) was prepared as described previously (11) except that the thawed S-30 was clarified by centrifuging for 10 min at 10,000*g* before use and the 2 mM Mg-acetate was replaced by 1 mM Mg-acetate and 0.8 mM spermidine. The dose response to each RNA preparation was determined and a subsaturating level of RNA was used subsequently, usually 5 to 10 μg/50 μl assay. The reaction was terminated after 90 min at 25 C by transfer to an ice bath and the addition of unlabeled amino acids.

ANALYSIS OF IN VITRO PRODUCTS

Total amino acid incorporation in both systems was assayed by treatment of aliquots of the reaction mixtures with 5% trichloroacetic acid at 80 C for 15 min and counting the precipitated material in a liquid scintillation spectrometer. The cell-free product (run-off system) was prepared for sucrose density gradient fractionation by removing the ribosomes at 40,000 rpm (40 rotor, Beckman model L ultracentrifuge) for 90 min. The supernatant was mixed with carrier storage protein isolated from protein bodies (see below) and layered onto 13-ml gradients of 10 to 30% (w/v) sucrose in 0.25 M NaCl, 100 mM tris-HCl, 10 mM ε-amino-*n*-caproic acid (Sigma) (a protease inhibitor; ref. 20), and centrifuged at 40,000 rpm in the SW 41 rotor (Beckman L5065B ultracentrifuge) for 28 hr at 5 C. The separation was monitored by pumping the sucrose upward through an ISCO fractionator and the protein was detected by absorption at 280 nm. Fractions (0.3 ml) were collected, 0.1 ml of each fraction was treated with 5% trichloroacetic acid at 80 C for 15 min, the insoluble residue was washed on glass fiber discs and counted by liquid scintillation.

Immunoprecipitation and analysis of the precipitated material on Na-dodecyl sulfate-polyacrylamide gels have been described previously (11). Details of preparation of specific antibodies in sheep and rabbits are given below. Further aliquots were fractionated by electrophoresis on SDS-PAG[1] and detected by fluorography of the dried gels as previously described (11).

For peptide mapping, two to four reaction mixtures were pooled and the ribosomes removed by centrifugation at 40,000 rpm for 90 min. The supernatant was precipitated with 5% trichloroacetic acid at 80 C in the presence of 5 mg unfractionated total storage protein isolated from protein bodies. The precipitated protein was washed once more with 5% trichloroacetic acid, then with ethanol-ether (1:1). The pellet was dried under N_2, oxidized with performic acid, digested with trypsin, and the peptides were separated on Whatman 3MM paper by chromatography followed by electrophoresis in the second dimension (2). The nonradioactive peptides were detected with 1% ninhydrin in acetone and the peptides of the *in vitro* translation products were detected by autoradiography with Kodak x-ray film.

ISOLATION OF STORAGE PROTEINS

This was carried out as will be described elsewhere (J. A. Thomson, H. Schroeder, and W. F. Dudman, in preparation) and in brief was as follows. Protein bodies were isolated from imbibed seeds, total salt-soluble protein was extracted from the protein bodies and designated as storage protein. Vicilin was prepared from this extract using zonal isoelectric precipitation (27). A crude legumin fraction was similarly obtained and was further purified by electrophoresis in agarose slabs.

ANTIBODIES AND SEROLOGY

Antibodies against legumin, vicilin, and unfractionated storage protein were produced in rabbits by sonicating 2 mg protein in 0.3 M NaCl, 0.02 M sodium phosphate (pH 7.4), and an equal volume of Freund's complete adjuvant. The mixture was injected subcutaneously at multiple sites and in both hind leg muscles. This was repeated twice at 14-day intervals; and 10 days after the third injection, blood was collected from an ear vein, allowed to clot, and the serum was collected after centrifugation. The serum was stored in the presence of 0.02% sodium azide at −20 C. Antibodies against native unfractionated storage protein were also produced in sheep by a procedure similar to the above except that 10 mg protein were used for each injection.

RESULTS

PEA POLYSOME RUN-OFF SYSTEM

This is an amino-acid-incorporating system consisting of polysomes from cotyledons of ripening seeds, radioactive amino acids, wheat germ S-100 fraction, and the necessary co-factors to allow completion *in vitro* of existing nascent polypeptide chains. Profiles of the pea cotyledon polysome preparations on sucrose density gradients showed a high proportion (80%) of the ribosomes in polysome form with a maximum at approximately the decamer region. Polysomes were destroyed by treatment with RNase (data not shown). Insensitivity to aurintricarboxylic acid (15% inhibition at 20 μM) indicated that this was essentially a noninitiating system (Table I). Optimum concentration of added Mg^{2+} was determined for each polysome preparation and for each wheat germ S-100 preparation. It varied between 0 and 3 mM added Mg^{2+}, and showed a sharp optimum for each preparation. KCl concentrations were also optimized for each combination and maximum activity was obtained in the range 70 to 120 mM KCl. Wheat germ S-100 was used routinely although S-100 from cotyledons of ripening pea seeds was also effective and the products of amino acid incorporation were the same as with the wheat germ preparation. However, pea S-100 fraction was only one-third to one-half as active as the wheat

Table I. Characteristics of protein synthesis in pea polysomal run-off system and pea polysomal RNA-directed wheat germ S-30 system

System	Treatment	Amino Acid Incorporation (% of complete assay)
Polysomal Run-off	Complete	100
	+ Aurintricarboxylic acid, 20μM	85
	+ Cycloheximide, 100 μg/ml	45
	+ D-threo-Chloramphenicol, 200μg/ml	81
	+ L-threo-Chloramphenicol, 200μg/ml	100
	+ D-threo-Chloramphenicol + cycloheximide	40
	+ Emetine, 0.1mM	21
	+ Emetine + D-threo-chloramphenicol	24
Polysomal RNA + Wheat germ S-30	Complete	100
	- Pea cotyledon polysomal RNA	2
	+ Aurintricarboxylic acid, 20μM	19
	+ Cycloheximide, 10μg/ml	5
	+ D-threo-Chloramphenicol, 200μg/ml	87

[1] Abbreviation: SDS-PAG: sodium dodecyl sulfate-polyacrylamide gels.

germ fraction at its optimum concentration. Pea polysomes and wheat germ S-30 and S-100 preparations retained full activity for at least 6 months when stored in liquid N_2.

Incorporation of radioactive amino acids by the polysome system was linear for approximately 45 min. Centrifugation of the incubated reaction mixture at 200,000*g* for 30 min to pellet most of the ribosomes left 70 to 90% of the incorporated amino acids in polypeptide chains that had been released into the supernatant. Occasionally batches of polysomes were prepared in which the amount of release was as low as 15 to 20%. However, when analyzed on SDS-PAG (see below), no difference could be detected between bound and released products. The run-off system was inhibited by cycloheximide, although at rather high concentrations (55% inhibition at 100 μg/ml; Table I). Emetine, another inhibitor of 80S ribosomes, reduced activity by 79% at 0.1 mM. D-*threo*-Chloramphenicol, at 200 μg/ml, inhibited by 19%, and L-*threo*-chloramphenicol was without effect. It is unlikely that the inhibition by D-*threo*-chloramphenicol was specific for 70S ribosomes because it was not additive in its effect with either cycloheximide or with emetine.

TRANSLATION OF PEA POLYSOMAL RNA (INITIATING SYSTEM)

In this system total polysomal RNA was isolated from cotyledons of ripening pea seeds and used to program protein synthesis in a wheat germ S-30 system. Sensitivity to aurintricarboxylic acid and a 50-fold response to RNA indicated that in this system chain elongation was largely preceded by chain initiation (Table I). Incorporation of amino acids into hot trichloroacetic acid-insoluble products was linear for 60 min at 25 C, and 80 to 90% of the product was released from the ribosomes into the supernatant. On SDS-PAG the small residues of ribosome-bound products were not distinguishable from those which were released.

The initiating system was highly sensitive to cycloheximide and only slightly affected by D-*threo*-chloramphenicol (Table I).

CHARACTERIZATION OF IN VITRO PRODUCTS

Sucrose Density Gradient Fractionation. Cell-free translation products of the run-off system were prepared using polysomes isolated from cotyledons 11 days after flowering (phase of rapid storage protein synthesis [23]) and were analyzed by sucrose density gradient fractionation in the presence of carrier storage proteins (Fig. 1). The pea storage proteins are resolved into two major peaks corresponding to legumin (mol wt 380,000) and vicilin (mol wt 140,000) (10) and a minor peak (mol wt 75,000). The major part of the trichloroacetic acid-insoluble radioactivity sedimented to a position on the gradient which did not correspond to any of the storage protein peaks. The average mol wt of the cell-free products was estimated to be 50,000 daltons by comparison with legumin and vicilin.

Immunoprecipitation. An extensive effort was made to obtain selective immunoprecipitation from the total *in vitro* reaction mixture using antiserum prepared against total storage protein. In the presence of carrier storage protein, legumin or vicilin, only low and variable levels of the radioactivity were precipitated under conditions where all of the carrier protein was recovered in the precipitate. Usually these accounted for only 2 to 4% of the incorporated radioactivity, although occasionally up to 15% of the counts were immunoprecipitated. The pattern of the immunoprecipitates on SDS-PAG was similar to that of the supernatants, but the polypeptides in the 50,000-dalton region were occasionally enriched.

SDS-PAG. Cell-free translation products of both the run-off and initiating systems were analyzed by electrophoresis on SDS-PAG followed by fluorography (Fig. 2, a and b). The run-off system gave rise to approximately 35 polypeptides and the

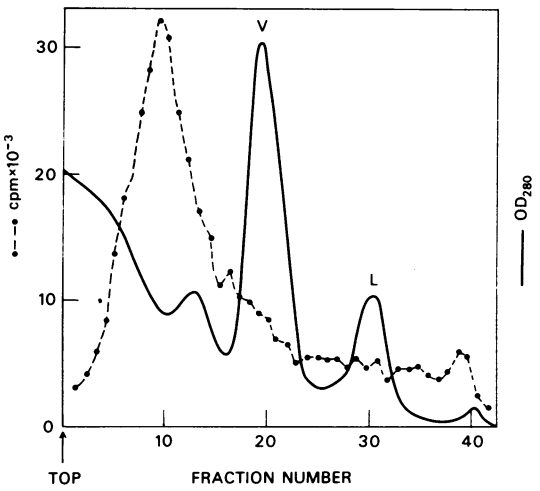

FIG. 1. Fractionation of storage proteins and cell-free translation products on a 10 to 30% (w/v) sucrose gradient. Four 50-μl reaction mixtures containing polysomes from cotyledons 11 days after flowering were incubated, then centrifuged to remove ribosomes, and the supernatant mixed with 2 mg of storage protein from mature seeds. The mixture was layered on a 13-ml sucrose gradient and centrifuged for 28 hr at 272,700*g*. ——: optical density at 280 nm; ---: trichloroacetic acid-insoluble radioactivity in a 0.1-ml aliquot of each 0.3-ml fraction; V: vicilin; L: legumin.

initiating system to about 30 discrete products. This spectrum of products was characteristic of each system and was reproducible between experiments and between preparations. The mol wt of the radioactive products ranged from greater than 80,000 to less than 10,000 daltons. Approximately 12 polypeptide products from the two systems showed equivalent mobilities on SDS gels but it is clear that the majority of the translation products of the two systems are of different size.

On SDS-PAG, the storage proteins of cv. Greenfeast are seen to be a complex mixture of subunits. Legumin consists of two major subunits of apparent mol wt about 38,500 and 20,000 with a number of trace components consistently present, and vicilin yields four major, five intermediate, and at least eight minor bands ranging in apparent mol wt from 80,000 to 10,000 daltons. In mature seeds both legumin and vicilin are localized in protein bodies, and these two purified fractions between them account for all of the polypeptide subunits which are present in the total salt extract of a protein body preparation (Fig. 2, c, d, and e).

Comparison of the subunits from pea seed storage proteins (revealed by staining with Coomassie blue [Fig. 2c]) with the *in vitro* products (revealed by fluorography) in adjacent tracks on an SDS-polyacrylamide slab gel (Fig. 2, a and b) showed near coincidence of radioactive bands with most of the subunits derived from legumin and vicilin but with considerable variation in relative amounts. Scrutiny of many such gels showed that in no case was coincidence precise. With such a large number of storage protein subunits, the probability of chance proximity is quite high. Other criteria were therefore sought to test whether the *in vitro* products were related to the storage proteins.

Peptide Mapping. The total radioactive products of amino acid incorporation by the initiating system were further analyzed by peptide mapping to determine whether their tryptic fingerprints were to any extent related to those of storage proteins from mature pea seeds. Comparison of radioactive tryptic peptides with those from a total storage protein extract in the same digest showed a marked degree of coincidence between radioactivity and ninhydrin-positive areas (Fig. 3, A, C, and E). In the example shown, in which polysomal RNA was prepared from cotyledons 11 days after flowering, 67% of the

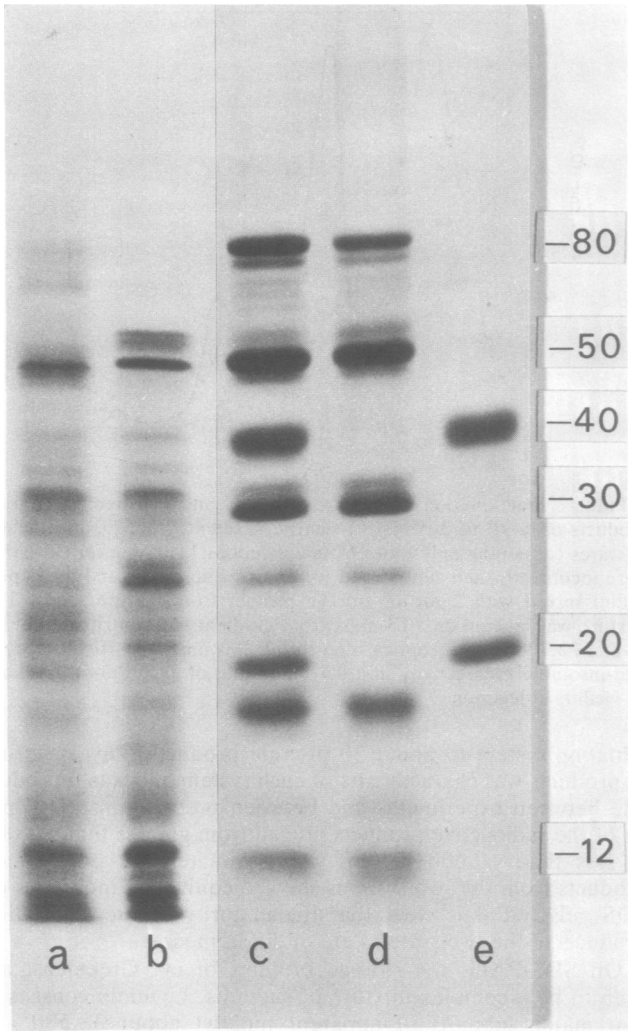

FIG. 2. Fractionation of legumin, vicilin, total extract of protein bodies from mature seeds, and the radioactive polypeptide products of amino acid incorporation formed by the run-off and initiating systems programmed by pea polysomal RNA (from 11-day cotyledons) on a single SDS-polyacrylamide slab gel. a and b show the radioactive polypeptides from the polysomal run-off and initiating systems, respectively, revealed by fluorography; c, d, and e show the polypeptides of total storage protein, vicilin, and legumin, respectively, revealed by Coomassie blue staining. The numbers refer to the apparent mol wt ($\times 10^{-3}$) of the major storage protein subunits.

radioactive peptides were associated with ninhydrin-positive, storage protein peptides. Of the total number of storage protein peptides resolved, only 46% were radioactive. When all resolved radioactive peptides were cut out of the peptide map and their radioactivity determined by scintillation counting, 66% of the total radioactivity was associated with storage protein peptides.

Tryptic fingerprints of the *in vitro* products of the run-off system using polysomes from 11-day cotyledons showed a similar relationship between radioactive peptides and those of mature storage protein (Fig. 3, A, B, and D). In this instance, 52% of the radioactive peptides were associated with storage protein peptides and 42% of the storage protein peptides had radioactive peptides coincident with them. Excision and counting of resolved radioactive areas showed that 55% of the incorporated radioactivity was coincident with storage protein peptides.

The autoradiographs of the translation products of the initiating and run-off systems were quite similar but contained some quantitative differences (*cf.* Fig. 3, B and C).

The large number (approximately 140) of peptides both from the radioactive *in vitro* products and the carrier storage proteins increases the possibility of chance coincidence between the two. To obtain an estimate of the level of chance coincidence in maps of similar complexity we prepared tryptic fingerprints of the following three combinations of unrelated proteins: (*a*) nonradioactive myosin together with ^{14}C-labeled *in vitro* products from the initiating system programmed with polysomal RNA from 11-day cotyledons; (*b*) nonradioactive BSA and ribulose 1,5-bisphosphate carboxylase (fraction I protein) together with the same ^{14}C-labeled *in vitro* products as above; and (*c*) nonradioactive storage protein together with ^{14}C-labeled products of a polysome run-off system prepared from pea epicotyl tissue 8 days after germination in the dark at 25 C. The proportion of radioactive peptides that were coincident with the unrelated nonradioactive peptides was 15%, 15%, and 20%, respectively, in the three combinations listed above. These can be compared with the values of 67% and 52%, respectively, for the *in vitro* products from the initiating and run-off systems from pea cotyledons with pea seed storage proteins. It would appear, therefore, that while some of this correspondence may be fortuitous there is a significant fraction of the *in vitro* products which are related to pea storage proteins as judged by the criterion of tryptic peptide mapping.

DISCUSSION

The subunit complexity of the pea seed storage proteins and the poor understanding of the relationship between the individual polypeptides have made the analysis of their relation to the cell-free translation products difficult. In order to maximize the possibility of detecting synthesis of any of the storage protein components we have chosen to study the total storage protein faction rather than either of its two major components, legumin and vicilin. Both legumin and vicilin are known to be complex mixtures of proteins (J. A. Thomson, H. Schroeder, and W. F. Dudman, in preparation), and furthermore Jackson *et al.* (13) have reported that vicilin and legumin contain common peptide sequences as determined by tryptic peptide analysis. Thus, it seemed reasonable to examine the products of *in vitro* protein synthesis in relation to the total storage protein fraction which we define as the salt-soluble proteins extracted from protein body preparations from mature pea seeds.

Four different criteria have been used to compare the *in vitro* translation products with the total storage protein fraction from mature seeds. Analysis of the cell-free translation products in the presence of carrier storage protein on sucrose density gradients showed no correspondence between the cell-free products and the holoproteins (Fig. 1). Although the formation of functional holoproteins by eucaryotic cell-free systems has only rarely been demonstrated it has been shown that the enzyme aldolase could be synthesized *in vitro* and that it was assembled into functional tetramers (17). The results of our sucrose gradient fractionation also show that subunit exchange does not occur between the *in vitro* products and the storage protein holoproteins.

The lack of coincidence between the *in vitro* translation products and the subunits of the total storage protein fraction on SDS-PAG (Fig. 2) also indicated that the equivalents of the storage protein subunits were not being synthesized in either of the two cell-free systems. Our immunoprecipitation studies strongly suggested that only a small fraction, if any, of the cell-free translation products carried the same antigenic determinants as the mature storage proteins which were used to prepare the antiserum.

Despite the lack of evidence for cell-free synthesis of storage proteins from these three analytical techniques, our fourth criterion, tryptic peptide fingerprinting, showed clearly that a significant proportion (up to 65%) of the peptides from the *in*

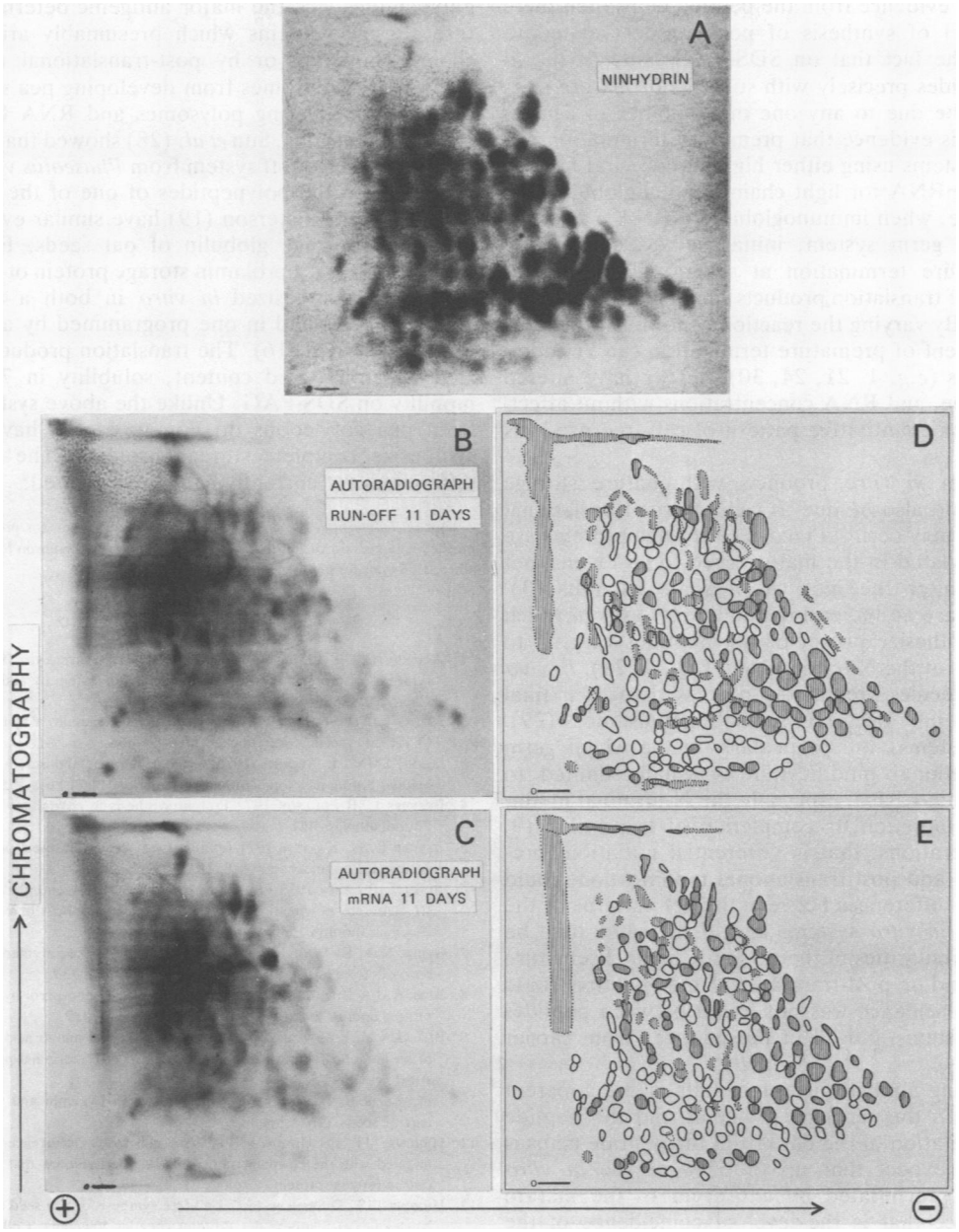

FIG. 3. Separation by paper chromatography and electrophoresis of the peptides obtained by tryptic digestion of the radioactive polypeptides synthesized by each of the two *in vitro* systems together with total storage protein fraction from mature seeds. A: typical pattern of peptides (revealed by ninhydrin) arising from the digestion of total storage protein; B and C: autoradiographs of peptides arising from cell-free translation products of polysomal run-off (B) and initiating (C) systems prepared from 11-day-old cotyledons; D and E: diagrammatic representations of ninhydrin-positive and radioactive peptides corresponding to B and C. Hatched areas are radioactive; solid outlines enclose ninhydrin-positive areas.

vitro products was coincident with storage protein peptides (Fig. 3). When the degree of chance coincidence was estimated by simultaneously mapping our labeled, *in vitro* translation products with a range of unrelated proteins, we found that coincidence was less than 20%.

This somewhat anomalous set of findings is open to a range of different interpretations depending upon the weight given to the data from the peptide maps. If one sets aside the evidence from the peptide maps, the data from the other three criteria indicate that storage proteins are not being made at an appreciable level in these cell-free systems. The *in vitro* products would therefore be essentially all nonstorage (metabolic) proteins. This interpretation carries several possible implications. One is that the mRNAs for storage proteins do not comprise a significant fraction of the total mRNA population of the cotyledon cells. This would be surprising in view of the fact that from as early as 11 days after flowering the total protein extracted from cotyledons and analyzed on SDS-PAG is dominated by the storage protein polypeptides pattern (unpublished observations). This interpretation would imply that nonstorage proteins must be turning over extremely rapidly.

A second possibility is that mRNAs for storage proteins are indeed major components of the total mRNA population but, for unknown reasons, the two *in vitro* systems selectively translate the mRNAs for nonstorage proteins. The fact that the polysomal run-off system and the initiating system programmed by total polysomal RNA should both reflect this selectivity is difficult to believe since a different mechanism would probably be involved in each system. For the run-off system the selectivity must occur at the level of chain elongation, and for the initiating system it is more likely to operate through control of the initiation step (18).

If one accepts the evidence from the peptide maps then there is a significant level of synthesis of polypeptides related to storage proteins. The fact that on SDS-PAG none of the *in vitro* products coincides precisely with subunits of mature storage proteins could be due to any one of a number of causes. For instance, there is evidence that premature termination can occur in cell-free systems using either high mol wt viral mRNA (6) or low mol wt mRNA for light chain immunoglobulin (9). In the latter instance, when immunoglobulin mRNA was translated in the wheat germ system, initiation was consistently correct but premature termination at several different sites generated a family of translation products smaller than authentic light chain protein. By varying the reaction conditions, the rates of initiation and extent of premature termination can be modified in some systems (*e.g.* 1, 21, 24, 30), but we have altered K^+, Mg^{2+}, polyamine, and RNA concentrations without affecting the qualitative or quantitative pattern of cell-free products (data not shown).

Noncoincidence of *in vitro* products with mature storage protein subunits could also be due to lack of post-translational modifications which may occur *in vivo*. Pea storage proteins are known to be glycosylated in the mature seed to an extent that could significantly alter the mol wt of some subunits (3). Furthermore, there are an increasing number of cases in which cell-free systems synthesize precursor polypeptides with up to 30 extra amino acids at the N-terminal end (*e.g.* 9, 29). *In vivo* these precursor molecules are rapidly processed to their final size but *in vitro* this only occurs in special circumstances (29). In most cell-free systems, and particularly in the wheat germ system, post-translational modification is usually limited to removal of single amino acids, especially the N-terminal methionine shortly after initiation or completion of translation (9, 14). Similar considerations, that is differential initiation, premature termination, and post-translational modification, could also account for the differences between the gel patterns of the products of the two *in vitro* systems and at the same time be consistent with the similarities of their peptide maps. Premature chain termination and/or post-translational modification could also explain why coincidence was not found between peptides from the *in vitro* systems and all of the peptides from carrier storage protein.

The most surprising anomaly in our results is the apparent contradiction between the serological studies and the peptide maps. If the interpretation of the data from the peptide maps is correct, one might expect that up to 65% of the *in vitro* products would be precipitated by antiserum to the mature storage protein, since that is the level of coincidence of the radioactive peptides from the *in vitro* products with those of mature storage proteins. There are many examples in which antisera prepared against completed, processed proteins are effective in precipitating incomplete, precursor, or unmodified versions of the same protein chain. It is possible that in the case of the storage proteins the major antigenic determinants may all arise during post-translational modification either by addition of carbohydrate side chains or removal of a number of peptide sequences from a precursor form. Berger and Kafatos (5) have shown that antiserum prepared against the proteolytic enzyme cocoonase gave only 30% cross-reaction with its zymogen whose mol wt is greater by 8,000 daltons. Even more striking is their finding that another proteolytic enzyme, namely trypsin, precipitated about 15% of the anti-cocoonase antibodies while the corresponding zymogen, trypsinogen, gave no precipitation at all. Trypsin and trypsinogen differ by only a hexapeptide (31). Clearly quite small changes in some proteins can have a major effect on their reactivity with antiserum.

On balance, we favor the view that polypeptides related to storage proteins are indeed being synthesized in both the *in vitro* systems from developing pea cotyledons but that these polypeptides lack the major antigenic determinants of the mature storage proteins which presumably arise *in vivo* during chain completion or by post-translational modification. Our results with polysomes from developing pea seeds contrast with those obtained using polysomes and RNA from a number of other seed systems. Sun *et al.* (28) showed that some translations products of a run-off system from *Phaseolus vulgaris* correspond in mol wt to the polypeptides of one of the storage globulins, and Luthe and Peterson (19) have similar evidence concerning the major storage globulin of oat seeds. Furthermore, both subunits of zein, a prolamin storage protein of maize endosperm, have been synthesized *in vitro* in both a polysomal run-off system (8, 15) and in one programmed by an mRNA fraction from this system (16). The translation products were characterized by amino acid content, solubility in 70% ethanol, and mobility on SDS-PAG. Unlike the above systems, preparations from pea cotyledons do not appear to have the capacity to synthesize complete storage proteins. The reasons for these differences are currently being investigated.

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LITERATURE CITED

1. Atkins JF, JB Lewis, CW Anderson, RF Gesteland 1975 Enhanced differential synthesis of proteins in a mammalian cell-free system by addition of polyamines. J Biol Chem 250: 5688-5695
2. Bailey CJ, D Boulter 1970 The structure of legumin, a storage protein of broad bean (*Vicia faba*) seed. Eur J Biochem 17: 460-466
3. Basha SMM, L Beevers 1976 Glycoprotein metabolism in the cotyledons of *Pisum sativum* during development and germination. Plant Physiol 57: 93-97
4. Beevers L, R Poulson 1972 Protein synthesis in cotyledons of *Pisum sativum* L. Plant Physiol 49: 476-481
5. Berger E, FC Kafatos 1971 Immunochemistry of an insect protease, cocoonase, and its zymogen. Immunochemistry 8: 391-403
6. Boime I, P Leder 1972 Protein synthesis directed by encephalomyocarditis virus mRNA. III. Discrete polypeptides translated from a monocistronic messenger *in vitro*. Arch Biochem Biophys 153: 706-713
7. Breen MD, EI Whitehead, DG Kenefick 1971 Requirements for extraction of polyribosomes from barley tissue. Plant Physiol 49: 733-739
8. Burr B, FA Burr 1976 Zein synthesis in maize endosperm by polyribosomes attached to protein bodies. Proc Nat Acad Sci USA 73: 515-519
9. Burstein Y, F Kantor, I Schechter 1976 Partial amino acid sequence of the precursor of an immunoglobulin light chain containing NH_2-terminal pyroglutamic acid. Proc Nat Acad Sci USA 73: 2604-2608
10. Derbyshire E, DJ Wright, D Boulter 1976 Legumin and vicilin, storage proteins of legume seeds. Phytochemistry 4: 3-24
11. Higgins TJV, PB Goodwin, PR Whitfeld 1976 Occurrence of short particles in beans infected with the cowpea strain of TMV. II. Evidence that short particles contain the cistron for coat protein. Virology 71: 486-497
12. Higgins TJV, D Spencer 1977 Cell-free synthesis of pea seed storage proteins. *In* Acides Nucleiques et Synthese des Proteins chez les Vegetaux. Colloq Int Cent Natl Rech Sci 261: 413-418
13. Jackson P, D Boulter, DA Thurman 1969 A comparison of some properties of vicilin and legumin isolated from seeds of *Pisum sativum*, *Vicia faba* and *Cicer arietinum*. New Phytol 68: 25-33
14. Jackson R, T Hunter 1970 The role of methionine in the initiation of haemoglobin synthesis. Nature 227: 672-676
15. Larkins BA, A Dalby 1975 *In vitro* synthesis of zein-like protein by maize polysomes. Biochem Biophys Res Commun 66: 1048-1054
16. Larkins BA, RA Jones, CY Tsai 1976 Isolation and *in vitro* translation of zein messenger ribonucleic acid. Biochemistry 15: 5506-5511
17. Lebherz HG, D Doyle 1976 Synthesis of functional aldolase tetramers in a heterologous cell-free system. J Biol Chem 251: 4355-4358
18. Lodish HF 1976 Translational control of protein synthesis. Annu Rev Biochem 45: 39-72
19. Luthe DS, DM Peterson 1977 Cell-free synthesis of globulin by developing oat (*Avena sativa* L.) seeds. Plant Physiol 59: 836-841
20. MacGregor CH 1975 Solubilization of *Escherichia coli* nitrate reductase by a membrane-bound protease. J Bacteriol 121: 1102-1110
21. Matthews MB, M Osborn 1974 The rate of polypeptide chain elongation in a cell-free system from Krebs II ascites cells. Biochim Biophys Acta 340: 147-152
22. Millerd A 1975 Biochemistry of legume seed proteins. Annu Rev Plant Physiol 26: 53-72
23. Millerd A, D Spencer 1974 Changes in RNA-synthesizing activity and template activity in nuclei from cotyledons of developing pea seeds. Aust J Plant Physiol 1: 331-341
24. Palmiter RD 1974 Differential rates of initiation on conalbumin and ovalbumin messenger ribonucleic acid in reticulocyte lysates. J Biol Chem 249: 6779-6787
25. Raacke ID 1957 Protein synthesis in ripening pea seeds. I. Analyses of whole seeds. Biochem J 66: 101-110

26. 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 Nat Acad Sci USA 70: 2330-2334
27. Scholz G, J Richter, R Manteuffel 1974 Studies on seed globulins from legumes. I. Separation and purification of legumin and vicilin from *Vicia faba* L. by zone precipitation. Biochim Physiol Pflanzen 166: 163-172
28. Sun SM, BU Buchbinder, TC Hall 1975 Cell-free synthesis of the major storage protein of the bean, *Phaseolus vulgaris* L. Plant Physiol 56: 780-785
29. Szczesna E, I Boime 1976 mRNA-dependent synthesis of authentic precursors to human placental lactogen: conversion to its mature hormone form in ascites cell-free extracts. Proc Nat Acad Sci USA 73: 1179-1183
30. Tse TPH, JM Taylor 1977 Translation of albumin messenger RNA in a cell-free protein-synthesizing system derived from wheat germ. J Biol Chem 252: 1272-1278
31. Walsh KA, DL Kauffman, KSV Sampath Kumar, H Neurath 1964 On the structure and function of bovine trypsinogen and trypsin. Proc Nat Acad Sci USA 51: 301-308