PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Biosynthesis, Processing and Transport of Storage Proteins and Lectins in Cotyledons of Developing Legume Seeds

M. J. Chrispeels

Phil. Trans. R. Soc. Lond. B 1984 **304**, 309-322
doi: 10.1098/rstb.1984.0026

Phil. Trans. R. Soc. Lond. B **304**, 309–322 (1984)
Printed in Great Britain

Biosynthesis, processing and transport of storage proteins and lectins in cotyledons of developing legume seeds

By M. J. Chrispeels

Department of Biology C-106, *University of California, San Diego, La Jolla, California* 92093, *U.S.A.*

The storage proteins and lectins that accumulate in the protein bodies of developing legume cotyledons undergo a number of processing steps along the transport pathway from their site of synthesis to their site of deposition. The polypeptides are synthesized on polysomes attached to the endoplasmic reticulum. Synthesis of the polypeptides is always accompanied by the co-translational removal of a signal peptide. Those proteins that are glycoproteins in their mature form are co-translationally glycosylated with high-mannose oligosaccharide side chains. Co-translational sequestration into the lumen of the endoplasmic reticulum is followed by the formation of oligomers. Transport of these oligomers to the Golgi complex may occur via tubular connections between the endoplasmic reticulum and the Golgi. In the Golgi complex some of the high-mannose side chains are modified by the removal of five to six mannosyl residues, and the addition of fucosyl and terminal *N*-acetylglucosaminyl residues. This phenomenon has so far been observed only for phytohaemagglutinin, the lectin of *Phaseolus vulgaris*. From the Golgi complex the storage proteins and lectins are transported to the protein bodies. This transport is mediated by small electron-dense vesicles. In the protein bodies two types of processing occur: proteolytic processing resulting in the formation of smaller polypeptides, and glycolytic processing resulting in the removal of the terminal *N*-acetylglucosaminyl residues from the modified carbohydrate side chains. All storage proteins and lectins undergo some of these processing steps, and specific examples are discussed in this paper.

Introduction

Seeds of leguminous plants synthesize and accumulate large amounts of storage proteins as well as lectins and hydrolytic enzymes in the course of their development. In mature seeds the storage proteins are present in protein bodies, membrane-bounded organelles measuring 1–10 μm in diameter, in the storage parenchyma cells of the cotyledons (Varner & Schidlovsky 1963; Graham & Gunning 1970). Recent immunocytochemical evidence indicates that the lectins, which occur so abundantly in the seeds of leguminous plants, are also contained in the protein bodies (Horisberger & Volanthen 1980; Van Driessche *et al.* 1981; Manen & Pusztai 1982). Analysis of isolated protein bodies shows that they contain not only storage proteins and lectins, but also numerous hydrolytic enzymes with an acid pH optimum, such as phosphatase, α-mannosidase and ribonuclease (Van der Wilden *et al.* 1980). The same hydrolytic enzymes are also found in the central vacuoles of parenchyma cells. Studies on the origin of protein bodies show that they arise from the central vacuoles of storage parenchyma cells after the deposition of storage protein has started (Öpik 1968; Bain & Mercer 1966; Harris & Boulter 1976; Neumann & Weber 1978; Yoo & Chrispeels 1980; Craig *et al.* 1980). Thus, protein bodies are in effect small vacuoles that have differentiated for storing protein during seed maturation and allowing the controlled hydrolysis of this protein during seedling growth.

Molecular structure of storage proteins and lectins

Legumin-like storage proteins such as legumin from pea (*Pisum sativum*) or broadbean (*Vicia faba*) and glycinin from soybean (*Glycine max*) have a molecular mass ratio (M_r) of 300000–360000 and a sedimentation constant of 11–12 *S*, and consist of six subunits each with M_r 60000–65000. Each subunit contains two polypeptides with M_r 38000–42000 and M_r 17000–20000 held together by a single disulphide bond (Derbyshire *et al.* 1976). Experiments with radioactive precursors indicate that these proteins do not have covalently attached sugar residues (Badenoch-Jones *et al.* 1981).

The vicilin-like proteins such as pea or broadbean vicilin, soybean β-conglycinin or bean (*Phaseolus vulgaris*) phaseolin have a somewhat more complex structure. They have sedimentation constants in the 6–7 *S* range and abundant subunits with M_r between about 75000 and 50000. These polypeptides form trimers with M_r ranging from about 140000 to about 225000. Vicilin-like proteins may also have polypeptides with an M_r smaller than 50000. In the pea these polypeptides have an M_r between 12000 and 30000, while in mung bean (*Vigna radiata*) they have an M_r of 25000. These smaller polypeptides occur in the same 6–7 *S* oligomers as the larger polypeptides and are thought to be fragmentation products of the larger ones (Ericson & Chrispeels 1976). The presence of these smaller polypeptides is not the result of polypeptide breakdown during the extraction and purification of oligomers, because their relative abundance is genotype-specific and a heritable characteristic (Thomson *et al.* 1980). Vicilin-like storage proteins have 4–6% covalently attached carbohydrate as residues of mannose (Man) and *N*-acetylglucosamine (GlcNAc). These sugar residues form branched oligosaccharide chains consisting of two GlcNAc residues and eight to nine Man residues, which are linked to an asparagine (Asn) residue of the polypeptide chain. There are an average of two such oligosaccharide side chains per polypeptide (M_r 50000), but many of the smaller polypeptides are not glycosylated. The structure of these side chains has not been determined (but see Davies & Delmer 1981), but it is assumed to be similar to the structure of the oligosaccharide moiety of soybean agglutinin (see below) and to the structure of the high-mannose side chains present in glycoproteins of animal cells.

The molecular structure of the lectins of leguminous seeds also shows many similarities. Lectins are generally tetramers with M_r between 100000 and 140000 and with polypeptides with M_r between 25000 and 35000 (reviewed in Goldstein & Hayes 1978). The four subunits in each oligomer may be identical or there may be small differences in M_r and also in biological properties. Phytohaemagglutinin (PHA) is a classical example of such heterogeneity. It is composed of E subunits that exhibit erythrocyte binding and are responsible for haemagglutination, and L subunits that are potent lymphocyte mitogens. In the protein bodies these subunits occur in arrangements E_4, L_1E_3, L_2E_2, L_3E_1 and L_4 representing a series of isolectins (Leavitt *et al.* 1977). The E and L subunits may have the same M_r, or slightly different M_r as is the case in the cultivar Greensleeves in use in our laboratory (Bollini & Chrispeels 1978). Lectins such as those of pea and lentil (*Lens culinaris*) have an $\alpha_2\beta_2$ structure where α and β have quite different M_r (α, M_r 6000; β, M_r 17000).

Some lectins such as pea lectin and concanavalin A have no covalently attached carbohydrate, but most seed lectins are glycoproteins. Soybean agglutinin has Asn-linked oligosaccharide side chains with a $GlcNAc_2Man_9$ composition. The structure of the side chains has been determined with nuclear magnetic resonance spectroscopy and is shown in figure 1 (Dorland *et al.* 1981).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

A number of storage proteins (vicilin, phaseolin, conglycinin) have Asn-linked oligosaccharide side chains with similar compositions, and it may be assumed that their structures are probably similar or identical to that of soybean agglutinin.

Many seed lectins contain sugars other than GlcNAc and Man, especially fucose (Fuc), xylose (Xyl) and galactose (Gal). For example, the oligosaccharide side chain of lima bean (*Phaseolus*

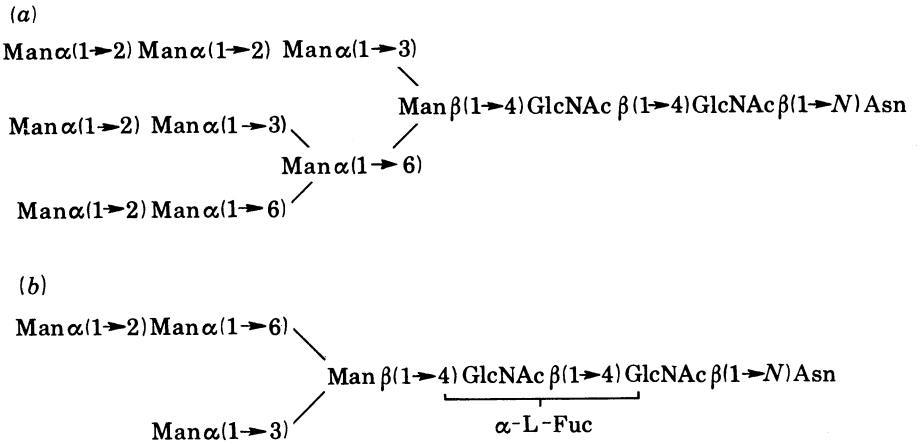

FIGURE 1. (*a*) Structure of high-mannose oligosaccharide of soybean agglutinin (Dorland *et al.* 1981). (*b*) Structure of the complex oligosaccharide of lima bean lectin (Misaki & Goldstein 1977).

lunatus) agglutinin has the composition $GlcNAc_2Man_4Fuc_1$ (Misaki & Goldstein 1977), whereas the lectin of *Erythrina cristagalli* has a carbohydrate composition of $GlcNAc_2Man_{3.5}Fuc_1Xyl_1$ (Iglesias *et al.* 1982). *Wistaria floribunda* has an Asn-linked carbohydrate moiety with the composition $GlcNAc_2Man_2Gal_5$ (Kurokawa *et al.* 1976). There is no agreement in the literature with regard to the carbohydrate composition of PHA; some have found it to contain only GlcNAc and Man (Leavitt *et al.* 1977; Rsänen *et al.* 1973) whereas others have reported the presence of Fuc, Xyl and Gal (Mialonier *et al.* 1973; Allen *et al.* 1969). The analyses of Jaffé *et al.* (1974) indicate that the sugar composition of PHA may be cultivar-specific. Work from my own laboratory (A. Vitale, T. G. Warner and M. J. Chrispeels, unpublished) shows that PHA from the cultivar Greensleeves has two different oligosaccharide side chains: one is a high-mannose type with the composition $GlcNAc_2Man_9$, while the other contains the sugars GlcNAc, Man, Fuc and Xyl, in the molar ratios 2:3.9:0.6:0.5 respectively. The presence of sugars other than GlcNAc and Man in the Asn-linked oligosaccharides of animal glycoproteins indicates that these glycoproteins have complex, rather than high-mannose, side chains. While there is as yet little information about glycoproteins with complex side chains in plant cells, it may be assumed that the lectins that contain Xyl, Fuc and Gal probably have such complex side chains.

Like storage proteins, lectins may also contain fragmented subunits. Both concanavalin A and soybean agglutinin have been shown to contain fragmented subunits (Abe *et al.* 1971; Wang *et al.* 1971; Lotan *et al.* 1975). Another property that the various storage proteins and the lectins have in common is that the subunits referred to above are often families of closely related polypeptides. The number of individuals in each family is difficult to determine and varies with the resolving power of the technique used to separate them. Thus, the term 'polypeptides with M_r about 50000' usually refers to a family of closely related polypeptides (Bollini & Vitale

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

1981; Brown *et al.* 1980). It appears therefore that the polypeptides of storage proteins and lectins are encoded by small gene families (Goldberg *et al.* 1981). For example in the case of the α and α′ subunits of β-conglycinin it was found by sequence analysis of cloned cDNA that each subunit was encoded by a small family of very closely related genes and that these two families differed in approximately 6% of their nucleotides (Schuler *et al.* 1982).

The rough endoplasmic reticulum

Electron micrographs of storage parenchyma cells of developing legume cotyledons show that these cells contain an extensive rough endoplasmic reticulum (e.r.) consisting of numerous cisternae and vesicles (e.g. Bain & Mercer 1966; Öpik 1968). Development of *P. vulgaris* cotyledons is accompanied by an increase in the amount of rough e.r. per cell and in the proportion of the cytoplasmic volume occupied by the rough e.r. (Briarty 1973). Determinations of the phospholipid content of isolated e.r. and of the activity of the e.r. marker enzyme NADH cytochrome *c* reductase also show that cotyledon development is accompanied by a dramatic increase in the total amount of e.r. in the cotyledons (Bollini & Chrispeels 1979). When thick sections of cotyledon tissue impregnated with zinc iodide and osmium tetroxide are viewed with a high-voltage electron microscope the e.r. appears as a continuous system of double-membrane sheets interconnected by tubules. The vesicles seen in conventional thin-section appear to be artefacts of sectioning, and probably, do not exist *in situ* (Harris 1979). Such thick sections also show that there are connecting tubules between the e.r. and the Golgi apparatus (Juniper *et al.* 1982; Harris & Oparka 1983).

Bailey *et al.* (1970) postulated on the basis of radioautographic experiments that the rough e.r. of the storage parenchyma cells is the main site of storage protein synthesis. This postulate was later confirmed by experiments in which polysomes or RNA obtained from the rough e.r. were used for *in vitro* protein synthesis. Experiments from a number of laboratories (Sun *et al.* 1975; Beachy *et al.* 1978; Püchel *et al.* 1979; Bollini & Chrispeels 1979; Higgins & Spencer 1981) showed that polysomes that synthesize storage proteins are largely or exclusively associated with membranous organelles. The degree to which the membrane-bound polysomes were found to be the exclusive site of storage protein synthesis depended on the methods used to separate free and membrane-bound polysomes. In each case the products of *in vitro* synthesis were identified as storage protein polypeptides by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (p.a.g.e.), immunoaffinity chromatography, or both. However, the polypeptides that were recognized by antibodies were usually not of the same size as the polypeptides synthesized *in vivo*. This indicates that they are processed *in vivo* to reach their mature size.

Experiments in which rough e.r. (rather than polysomes or RNA obtained from rough e.r.) was used for *in vitro* protein synthesis (chain completion) show that the storage protein polypeptides formed were similar in size to the *in vivo* synthesized polypeptides; this indicates that some of the processing steps reside in the e.r. (Bollini *et al.* 1983). The polypeptides that are formed in this way are protected from proteolytic digestion (Hurkman & Beevers 1982); this indicates that they have been sequestered within the microsomal vesicles. Higgins & Spencer (1981) were able to reproduce the processing steps *in vitro* by using a reconstituted system that consisted of polysomal RNA obtained from the rough e.r. of developing pea cotyledons, and microsomes stripped of their polysomes. The addition of the microsomal membranes during the translation of the polysomal RNA resulted in the formation of

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

polypeptides of pea storage proteins that had the correct size and were sequestered within the vesicles. The processing steps thought to be associated with the e.r. involve the co-translational removal of a signal sequence and addition of Asn-linked high-mannose oligosaccharides.

Signal sequences on storage proteins and lectins

Storage proteins and lectins are sequestered in membrane-bounded organelles, and therefore must cross a membrane as an obligatory step in their sequestration. Evidence that this sequestration occurs first in the e.r. comes from both immunocytochemical and biochemical experiments. Baumgartner *et al.* (1980) used affinity-purified antibodies against phaseolin to localize phaseolin at the ultrastructural level in the storage parenchyma cells of *P. vulgaris*. They found that phaseolin was located in protein bodies, in numerous vesicles with an electron dense content and in the cisternae of the e.r. When such cotyledons were labelled with [^{3}H]amino acids newly synthesized phaseolin and PHA were found to be associated with the rough e.r. isolated on isopycnic sucrose gradients (Bollini *et al.* 1982; Chrispeels & Bollini 1982). Similar observations have been made with pea cotyledons (Chrispeels *et al.* 1982*a*, *b*). These results, together with the results indicating that storage proteins are synthesized on e.r.-bound polysomes, suggests that vectorial discharge of the polypeptides leading to sequestration occurs within the e.r.

The vectorial discharge of polypeptides into the lumen of the e.r. is the basic sequestration event in the transport of secretory proteins in animal cells (for review see Sabatini *et al.* 1982). The mechanism for vectorial discharge through the membrane involves the *N*-terminal amino acid portion of the newly synthesized polypeptide. The *N*-terminal portion of the nascent polypeptide that emerges from the ribosome is the signal that determined polysome–membrane interaction and in some way assists in co-translational translocation of the nascent polypeptide across the e.r. membrane. Thus, the pre-secretory proteins contain an extra segment termed the signal peptide at their *N*-terminal ends. Many signal peptides have been sequenced, and although their composition is variable they all contain a high proportion of hydrophobic amino acids. A peptidase located on the luminal side of the e.r. membrane removes the signal peptide when the nascent polypeptide is 70–90 amino acids long.

The presence of a signal sequence on a polypeptide may be inferred from an analysis by SDS p.a.g.e. of polypeptides synthesized *in vitro* with extracted polysomal RNA as template, synthesized *in vitro* by isolated polysomes (chain completion), or synthesized *in vivo*. If the polypeptides made *in vitro* by isolated mRNA are slightly larger than the polypeptides made by polysomes or than the polypeptides made *in vivo* one can tentatively conclude that the difference in size may be because of the presence of a signal sequence. This type of analysis was used to demonstrate that the A polypeptide of castor bean (*Ricinus communis*) agglutinin (Roberts & Lord 1981) and the M_r 23000 precursor of pea lectin (Higgins *et al.* 1983*b*) are made as pre-proteins with a transient signal sequence. Proteins that are co-translationally glycosylated may give confusing results because chain-completion assays show that both glycosylated and unglycosylated polypeptides are present on polysomes as nascent chains. Furthermore, removal of the signal sequence and attachment of the carbohydrate may result in a glycopolypeptide that is the same size or even larger than the initial translation products. To find the exact sizes of the glycosylated and unglycosylated polypeptides synthesized under different conditions one needs to introduce several other methods. Concanavalin A–sepharose

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

can be used to separate glycosylated from unglycosylated polypeptides. *In vivo* labelling can also be carried out after pretreatment of the cotyledons with tunicamycin, a drug that inhibits the co-translational glycosylation of nascent chains by preventing the synthesis of lipid-linked oligosaccharides. In the presence of tunicamycin the cells synthesize unglycosylated polypeptides that are nevertheless normally sequestered and transported (Badenoch-Jones *et al.* 1981). By using these various techniques it was shown that the α and α′ subunits of β-conglycinin (Sengupta *et al.* 1981) and the subunits of phaseolin of M_r about 50000 (Bollini *et al.* 1983) are made as pre-proteins with a transient signal sequence (figure 2).

A different approach to the demonstration of the presence of a signal sequence was taken by Ereken-Tumer *et al.* (1982) who examined glycinin polypeptides made *in vitro* by isolated polysomal RNA, and made *in vivo* by *Xenopus* oocytes. The found that the oocytes processed the polypeptides by the removal of a small peptide. Microsequence analysis of the radioactive products showed that this small peptide was present at the *N*-terminal end of the acidic polypeptide and was hydrophobic in nature.

Evidence for the existence of signal sequences is now being obtained by sequence analysis of cloned cDNAs that contain the entire coding sequence for the pre-proteins. The identity of the signal sequence is inferred from the presence of a hydrophobic amino acid sequence at the *N*-terminal end of the polypeptide, between the start codon for methionine and the *N*-terminal end of the mature polypeptide as determined by amino acid sequence analysis of the purified protein. The amino acid sequence of the signal peptides of PHA (Hoffman *et al.* 1982) and pea lectin (Higgins *et al.* 1983*a*) have been determined in this way.

Co-translational glycosylation

Many storage proteins and lectins are glycoproteins with high-mannose or complex type Asn-linked side chains (see above). In animal cells as well as in plant cells high-mannose oligosaccharide side chains are assembled on lipid (dolichol pyrophosphate) carriers and transferred *en bloc* to the polypeptide chains (for a review see Hubbard & Ivatt 1981 and Elbein 1981). The side chains are initially synthesized as $Glc_3Man_9GlcNAc_2$ groups attached to dolichol pyrophosphate (Hori *et al.* 1982; Staneloni *et al.* 1980; Lehle 1981). The glycosyltransferases involved in the synthesis of these oligosaccharide side chains and in their transfer to the nascent polypeptide chains are associated with the endoplasmic reticulum (Nagahashi & Beevers 1978; Dürr *et al.* 1979; Lehle *et al.* 1978; Mellor *et al.* 1980). That the transfer of the oligosaccharide side chains to the polypeptide is a co-translational process can be shown by carrying out *in vitro* translations (chain completion) with polysomes detached from the membranes of the e.r. The polysomes can be detached by dissolving the membranes with detergent and this removes the dolichol pyrophosphate-linked oligosaccharides as well as the transferase involved in their synthesis and transfer. Completion of the nascent chains with an *in vitro* protein synthesizing system nevertheless results in the formation of partially and fully glycosylated polypeptides besides the unglycosylated ones. We isolated the products of run-off synthesis obtained with polysomes from developing *P. vulgaris* cotyledons and analysed them by SDS p.a.g.e. after affinity chromatography on concanavalin A-sepharose (Bollini *et al.* 1983). Phaseolin, isolated by immunoaffinity chromatography from the mixture of *in vitro* synthesized (completed) polypeptides was represented by unglycosylated and partially glycosylated (one instead of two oligosaccharides per polypeptide) chains. Chain completion with polysomes did not result in

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

the formation of fully glycosylated polypeptides (two oligosaccharide chains per polypeptide). Fully glycosylated chains were only formed if rough e.r. was used in a chain completion system (figure 2). The polysome preparation that synthesized phaseolin also synthesized PHA. Phytohaemagglutinin was represented by unglycosylated, partially glycosylated and fully glycosylated (two oligosaccharides per polypeptide) chains (A. Vitale, M. J. Chrispeels, unpublished observations). The reason for this difference between phaseolin and PHA is unknown, but the experiments clearly show that polypeptides in different stages of glycosylation are present as nascent chains on the polysomes.

Role of the Golgi complex in transport and post-translational modification

There has been considerable speculation in the literature about the role of the Golgi complex in the transport of proteins from the e.r. to the protein bodies. This speculation is based primarily on the presence in Golgi-associated vesicles of electron dense material which is similar in appearance to the proteinaceous material in the protein bodies (Harris & Boulter 1976; Dieckert & Dieckert 1976; Neumann & Weber 1978; Harris 1979). Whether this electron-dense material in the Golgi vesicles is indeed storage protein can only be shown by immunocytochemical experiments, and the published evidence (Baumgartner *et al.* 1980) is inconclusive. Recent immunocytochemical experiments by E. Herman and L. M. Shannon (personal communication) in which antibodies against concanavalin A and against the seed lectin of *Bauhinia purpurea* were used, show quite unequivocally that these lectins are located in the protein bodies, the e.r. and the Golgi complex of the storage parenchyma cells of developing seeds.

A role for the Golgi complex in the modification of protein body proteins can be postulated on the basis of the sugar compositions of the carbohydrate moieties of lectins. Most lectins are glycoproteins, and many lectins contain sugars other than GlcNAc and Man, especially Fuc, Xyl and Gal (see above). The presence of sugars other than GlcNAc and Man in the Asn-linked oligosaccharide side chains of animal glycoproteins indicates that these side chains are of the complex type. By extrapolation one could postulate that many lectins also contain complex oligosaccharide side chains. In animal cells, complex side chains are formed by post-translational modification of high-mannose side chains. Such modifications involve the removal of Man and the addition of the peripheral sugars occur in the Golgi complex (for review see Hubbard & Ivatt 1981). If this is also the case in plant cells, then lectins with complex side chains would have to pass through the Golgi apparatus on their way to the protein bodies. Our laboratory has recently obtained the first evidence that this is indeed the case for phytohaemagglutinin (Chrispeels 1983*a*, *b*).

When developing cotyledons of *P. vulgaris* are labelled with [^{3}H]fucose, the precursor becomes rapidly incorporated into PHA, and PHA is the major fucosylated glycoprotein synthesized in the cotyledons (Chrispeels 1983*a*). Analysis of the organelles on sucrose gradients shows that [^{3}H]fucose-PHA is present in two classes of organelles: class I particles with an average density of 1.13 g cm^{-3} and class II particles with an average density of 1.22 g cm^{-3}. These particles do not band in the same region of the sucrose gradient as the e.r. marker enzyme NADH-cytochrome *c* reductase, and their density does not change when $MgCl_2$ or EDTA are in the isolation media (Chrispeels 1983*b*). Such results indicate that fucosylated PHA is not present in the e.r. but is associated with other organelles. Further analysis indicates that the class I

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

particles sediment with the Golgi-marker enzyme inosine diphosphatase in both rate zonal and isopycnic gradients; the class II particles on the other hand are small (0.05–0.2 μm) electron dense vesicles which look like very small protein bodies. Pulse chase experiments show that [^{3}H]fucose-PHA moves from the class I (Golgi) particles to the class II particles (dense vesicles) (Chrispeels 1983*b*).

Further evidence for a role of the Golgi apparatus in the transport of storage proteins comes from experiments in which the monovalent ionophore monensin was used to disrupt Golgi function. Evidence available from animal cells indicates that monensin interferes with the secretion and the late post-translational modifications of secreted proteins by interfering with late Golgi functions. Pre-treatment of cotyledons with monensin did not inhibit the biosynthesis of either phaseolin or PHA, but in a pulse chase experiment monensin prevented their chase-out from the membranous organelles. The block appeared to be between the Golgi apparatus and the dense vesicles. Phaseolin has high-mannose oligosaccharide side chains. (Davies & Delmer 1981) that are co-translationally attached to the polypeptides (Bollini *et al.* 1983) and it does not need to be processed in the Golgi complex. Yet it passes through the Golgi on its way to the protein bodies. I would predict from these results that all protein body proteins, whether they are glycosylated or not, and whether they have high-mannose or complex oligosaccharide side chains, probably pass through the Golgi complex on their way to the protein bodies.

Transition between the e.r. and the Golgi complex, and between the Golgi complex and the protein bodies

Recent detailed ultrastructural observations on thick sections of zinc iodide and osmium tetroxide impregnated cotyledon tissue show that there are numerous connections between the tubular form of the e.r. and the cisternae of the Golgi complex (Juniper *et al.* 1982; Harris & Oparka 1983). The tubular form of the e.r. is continuous with the cisternal form that is engaged in the synthesis of protein body proteins. These connections appear to be quite numerous in cell types that either secrete proteins or transport proteins to protein bodies, but are absent from cell types where the main function of the Golgi complex is the biosynthesis and secretion of polysaccharides. Thus it may be unnecessary to postulate a vesicular transport between the e.r. and the Golgi complex.

Transport from the Golgi complex to the protein bodies would appear to be mediated by the class II particles (dense vesicles) described in the previous section. Such dense vesicles are frequently seen in electron micrographs in the vicinity of the Golgi complex. That they contain protein body proteins is shown by the analysis of their polypeptide content by SDS p.a.g.e., (Chrispeels 1983*b*) and by immunocytochemical experiments (Baumgartner *et al.* 1981; E. Herman and L. M. Shannon, unpublished results).

Post-translational modifications in the protein bodies

Proteolytic processing

While attempting to show that polysomes and polysomal RNA isolated from developing pea cotyledons can be used for the *in vitro* synthesis of storage proteins, Higgins & Spencer (1977) observed major discrepancies in the size and relative abundance of the polypeptides synthesized *in vitro*, and those present in the protein bodies of mature seeds. Tryptic digests nevertheless

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

confirmed that most of the protein synthesized *in vitro* was indeed vicilin and legumin. These discrepancies were later resolved when it was shown that legumin is synthesized as precursors with M_r about 60000–65000, which are later converted to mature legumin with subunits of M_r about 40000 and 19000 (Croy *et al.* 1980; Spencer & Higgins 1980). Similar results have been obtained with glycinin (Tumer *et al.* 1981). These experiments led me to a detailed examination of such processing events using *in vivo* pulse labelling of developing pea cotyledons to discover the subcellular site of this processing step.

When pea cotyledons are labelled *in vivo* with ^{3}H-amino acids the most abundant storage protein polypeptides synthesized are the M_r 60000–65000 precursors of legumin, and the M_r 70000, 75000 and 50000 polypeptides of vicilin. These polypeptides are associated with the rough e.r. and in a pulse chase experiment they chase out of the e.r. with a $t_{\frac{1}{2}}$ of 90 min (Chrispeels *et al.* 1982*a*). Other legumin polypeptides (M_r about 40000 and 19000) and vicilin polypeptides (M_r 30000, 24000, 18000, 14000, 13000 and 12000), which are normally found in protein bodies, are not present as translation products in the e.r. (Chrispeels *et al.* 1982*b*). Pulse chase experiments show that these smaller polypeptides are formed in the protein bodies from the larger molecular mass precursors, but that the rate at which the polypeptides are processed is not the same for all proteins. The legumin precursors are completely processed within 2 h after they have been synthesized, while the processing of the vicilin precursors takes much longer (up to 24 h). Pea lectin is also present in the e.r. as a precursor with M_r 23000. This precursor chases out of the e.r. quite slowly ($t_{\frac{1}{2}}$ around 5 h) and is processed in the protein bodies to mature lectin, with polypeptides with a M_r 17000 and 6000. In the case of legumin and lectin the limiting step in processing seems to be the rate at which newly synthesized protein chases out of the e.r. and into the protein bodies. In the case of vicilin, which chases out of the e.r. as fast as legumin, the limiting step may be the amount of enzyme, the accessibility of the processing site to the proteinase, or the existence of several sequential processing steps for the same precursor.

An interesting aspect of this proteolytic processing involves the extent to which a particular set of precursors is processed. In the case of legumin, glycinin and pea lectin, all the high M_r precursors that arrive in the protein bodies are processed. In the case of the pea vicilin precursors only some of the polypeptides with M_r of about 75000 and 50000 are processed. This incomplete processing can be explained in two ways. Processing may be very slow and it is possible that the enzyme levels are too low, or the processing sites too inaccessible for processing to come to completion. The alternative explanation is that some of the members in the M_r 75000 and M_r 50000 class are completely processed, while other members are not processed at all. Whether a particular polypeptide is processed would then depend on its amino acid sequence rather than on the level of enzyme, or the accessibility of the processing site. Similar processing steps have been documented for a few other protein body proteins, but the site of processing has not been determined. For example, the B chain of *Ricinus communis* agglutinin which has an M_r 37000 is synthesized as a precursor of M_r 59000; this becomes glycosylated (M_r 66000–69000) and is later processed to its mature form (Roberts & Lord 1981). Earlier I drew attention to the fact that many protein body proteins have fragmented polypeptides. I would predict that all such polypeptides are the result of proteolytic processing in the protein bodies. The function, if any, of this proteolytic processing remains as yet unknown. It is interesting to note, while bearing in mind that protein bodies are small protein-filled vacuoles (lysosomes), that similar proteolytic processing steps have been shown to occur in a number

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

of acid hydrolases found in the lysosomes of animal cells. Cathepsin D, β-hexosaminidase and α-glucosidase in fibroblasts (Hasilik & Neufeld 1980), β-galactosidase in rat macrophages (Skudlarek & Swank 1979) and carboxypeptidase Y in yeast vacuoles (Hasilik & Tanner 1978) all undergo proteolytic processing after they arrive in their respective lysosomal compartments.

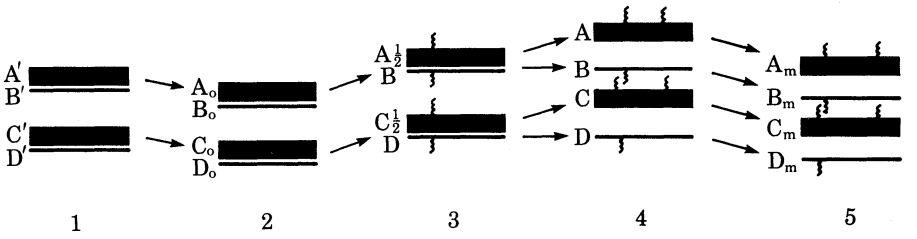

FIGURE 2. Schematic drawing of the different processing steps of the polypeptides of phaseolin. The scheme was derived by analysing the mobility of the polypeptides by SDS p.a.g.e., and comparing the polypeptides synthesized *in vitro* using different systems (RNA, polysomes, rough e.r.) or synthesized *in vivo* and present in the e.r. or in the protein bodies. Note that polypeptides that are drawn closely together such as A′ and B′ or A_o and B_o are not visible as distinct bands on the gels. A and B are only resolved on the gels after one of the polypeptides (A) has received an additional oligosaccharide side chain. The processing steps are as follows. (1) Polypeptides A′, B′, C′ and D′ synthesized *in vitro* with polysomal RNA as template; signal peptides present. (2) Unglycosylated polypeptides A_o, B_o, C_o, and D_o synthesized *in vitro* by polysome run-off; signal peptides absent. (3) Glycosylated polypeptides $A\frac{1}{2}$, B, $C\frac{1}{2}$ and D synthesized *in vitro* by polysome run-off; signal peptides absent; one oligosaccharide side chain present. (4) Glycosylated polypeptides A, B, C, and D synthesized *in vitro* by run-off of rough e.r., or synthesized *in vivo* and present in the e.r.: a second oligosaccharide present on A and C. (5) Mature glycosylated polypeptides A_m, B_m, C_m and D_m present in protein bodies after *in vivo* labelling and chase; nature of the processing step not entirely clear. Reproduced from the *Journal of Cell Biology* (1983), **96**, 999–1007 by copyright permission of The Rockefeller University Press.

Processing of the oligosaccharide side chains

Comparison of the mobilities on denaturing gels of the glycosylated polypeptides show that the polypeptides of phaseolin (Bollini *et al.* 1982) and of phytohaemagglutinin (Chrispeels & Bollini 1982) in the protein bodies have a slightly smaller M_r than those in the e.r. Pulse chase experiments with *P. vulgaris* cotyledons show that the change in the mobility of phaseolin occurs quite rapidly during a 2 h chase (Bollini *et al.* 1982) while the change in the mobility of PHA occurs gradually during a 24 h chase (A. Vitale and R. Bollini, personal communication). Post-translational changes resulting in small differences in the mobilities on denaturing gels have also been demonstrated for the α and α′ subunits of conglycinin (Sengupta *et al.* 1981; Beachy *et al.* 1981). If the chase-out time of conglycinin from the e.r. is similar to that of the pea storage proteins (Chrispeels *et al.* 1982*a*, *b*) or PHA (Chrispeels & Bollini 1982) one would expect these changes to occur in the protein bodies. With respect to phaseolin and conglycinin it is not clear whether the changes are caused by the removal of a small peptide, the removal of carbohydrate residues, or both. With respect to PHA we have found that the slow decrease in mobility seen on SDS gels is caused by the gradual removal of carbohydrate residues from the complex oligosaccharide side chains of PHA (A. Vitale and M. J. Chrispeels, unpublished observations).

Cotyledons were labelled for 1 h with [^{3}H]fucose, and the radioactivity chased for up to 24 h. The pulse-labelled cotyledons were used for the isolation of PHA from the Golgi and dense vesicle fraction. The chased cotyledons were used for the isolation of PHA from the protein body fraction. Phytohaemagglutinin was digested with pronase and protease K, and the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

resulting glycopeptides chromatographed on a 100 cm long Bio-Gel P4 column to determine the size of the complex glycopeptides. The results showed that the glycopeptides with complex side chains were the same size in the Golgi and the vesicles, but that their size started to decrease gradually after they arrived in the protein bodies. This gradual decrease was caused by the removal of peripheral GlcNAc resides that were added to the complex chains in the Golgi apparatus (unpublished observations from this laboratory). By using tunicamycin to block Asn-linked glycosylation we were also able to show that the unglycosylated polypeptides of PHA in the organelle fraction (e.r. and Golgi) are the same size as the polypeptides in the protein bodies after a long chase. The results support the conclusion that the slow change in mobility of the PHA polypeptides during a 24 h chase is because of the gradual removal of carbohydrate residues from the complex oligosaccharide sidechains.

Oligomer formation

A third type of processing that occurs in the protein bodies concerns the formation of oligomers. The formation of oligomers starts in the e.r. and may be completed there (e.g. for vicilin or PHA) or may be completed in the protein bodies (e.g. for legumin or glycinin). Isolation of e.r. from pulse-labelled pea cotyledons followed by an analysis on sucrose gradients of the sedimentation coefficient values of the storage proteins shows that vicilin is in the 7–8 *S* form, while legumin is in an 8 *S* form. The transport of legumin from the e.r. to protein bodies occurs in this 8 *S* form (probably a trimer of the M_r 60000–65000 precursors) and the formation of mature 12 *S* legumin is a very slow process which takes several weeks (Chrispeels *et al.* 1982*b*). Glycinin undergoes a similar maturation process (Barton *et al.* 1982).

Research in my laboratory is supported by the U.S. National Science Foundation and the U.S. Department of Agriculture. Collaboration with the Istituto Biosintesi Vegetali in Milano is supported by a grant from the North Atlantic Treaty Organization.

References

Abe, Y., Iwabuchi, M. & Ishii, S. I. 1971 Multiple forms in the subunit structure of concanavalin A. *Biochem. biophys. Res. Commun.* **45**, 1271–1278.

Allen, L. W., Svenson, R. H., Yachnin, S. 1969 Purification of mitogenic proteins derived from *Phaseolus vulgaris*: isolation of potent and weak phytohemagglutinins possessing mitogenic activity. *Proc. natn. Acad. Sci. U.S.A.* **63**, 334–341.

Badenoch-Jones, J., Spencer, D., Higgins, T. J. V. & Millerd, A. 1981 The role of glycosylation in storage protein synthesis in developing pea seeds. *Planta* **153**, 201–209.

Bailey, C. J., Cobb, A. & Boulter, D. 1970 A cotyledon slice system for the autoradiographic study of the synthesis and intracellular transport of the seed storage proteins of *Vicia faba*. *Planta* **95**, 103–118.

Bain, J. M. & Mercer, F. V. 1966 Subcellular organization of the developing cotyledons of *Pisum sativum*. *Aust. J. biol. Sci.* **19**, 49–67.

Barton, K. A., Thompson, J. F., Madison, J. T., Rosenthal, R., Jarvis, N. P. & Beachy, R. N. 1982 The biosynthesis and processing of high molecular weight precursors of soybean glycinin subunits. *J. biol. Chem.* **257**, 6089–6995.

Baumgartner, B., Tokuyasu, K. T. & Chrispeels, M. J. 1980 Immunocytochemical localization of reserve protein in the endoplasmic reticulum of developing bean (*Phaseolus vulgaris*) cotyledons. *Planta* **150**, 419–425.

Beachy, R. N., Jarvis, N. P. & Barton, K. A. 1981 Biosynthesis of subunits of the soybean 7 *S* storage protein. *J. mol. appl. Gen.* **1**, 19–27.

Beachy, R. N., Thompson, J. R. & Madison, J. T. 1978 Isolation of polyribosomes and messenger RNA active in *in vitro* synthesis of soybean seed proteins. *Pl. Physiol.* **61**, 139–144.

Bollini, R. & Chrispeels, M. J. 1978 Characterization and subcellular localization of vicilin and phytohemagglutinin, the two major reserve proteins of *Phaseolus vulgaris* L. *Planta* **142**, 291–298.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Bollini, R. & Chrispeels, M. J. 1979 The rough endoplasmic reticulum is the site of reserve-protein synthesis in developing *Phaseolus vulgaris* cotyledons. *Planta* **146**, 487–501.

Bollini, R., Van der Wilden, W. & Chrispeels, M. J. 1982 A precursor of the reserve-protein phaseolin is transiently associated with the endoplasmic reticulum of developing *Phaseolus vulgaris* cotyledons. *Physiologia Pl.* **55**, 82–92.

Bollini, R., Vitale, A. & Chrispeels, M. J. 1983 In vivo and in vitro processing of seed reserve protein in the endoplasmic reticulum: evidence for two glycosylation steps. *J. Cell Biol.* **96**, 999–1007.

Bollini, R. & Vitale, A. 1981 Genetic variability in charge microheterogeneity and polypeptide composition of phaseolin, the major storage protein of *Phaseolus vulgaris*; and peptide maps of its three major subunits. *Physiologia Pl.* **52**, 96–100.

Briarty, L. G. 1973 Stereology in seed development studies: some preliminary work. *Caryologia* **25**, Suppl. 289–301.

Brown, J. W. S., Bliss, F. A. & Hall, T. C. 1980 Microheterogeneity of Globulin-1 storage protein from French bean with isoelectrofocusing. *Pl. Physiol* **66**, 838–840.

Chrispeels, M. J. 1983 Incorporation of fucose into the carbohydrate moiety of phytohemagglutinin in developing cotyledons of *Phaseolus vulgaris*. *Planta* **157**, 454–461.

Chrispeels, M. J. 1983*b* The Golgi apparatus mediates the transport of phytohemagglutinin to the protein bodies. *Planta* **158**, 140–151.

Chrispeels, M. J. & Bollini, R. 1982 Characteristics of membrane-bound lectin in developing *Phaseolus vulgaris* cotyledons. *Pl. Physiol.* **70**, 1425–1428.

Chrispeels, M. J., Higgins, T. J. V., Craig, S. & Spencer, D. 1982*a* The role of the endoplasmic reticulum in the synthesis of reserve proteins and the kinetics of their transport to protein bodies in developing pea cotyledons. *J. Cell Biol.* **93**, 5–14.

Chrispeels, M. J., Higgins, T. J. V. & Spencer, D. 1982*b* Assembly of storage protein oligomers in the endoplasmic reticulum and processing of the polypeptides in the protein bodies of developing pea cotyledons. *J. Cell Biol.* **93**, 306–313.

Craig, S., Goodchild, D. J. & Miller, C. 1980 Structural aspects of protein accumulation in developing pea cotyledons. II. Three-dimensional reconstructions of vacuoles and protein bodies from serial sections. *Aust. J. Pl. Physiol.* **7**, 329–337.

Croy, R. D., Gatehouse, J. A., Evans, I. M. & Boulter, D. 1980 Characterization of the storage protein subunits synthesized *in vitro* by polyribosomes and RNA from developing pea (*Pisum sativum* L.). I. Legumin. *Planta* **148**, 49–56.

Davies, H. M. & Delmer, D. P. 1981 Two kinds of protein glycosylation in a cell-free preparation from developing cotyledons of *Phaseolus vulgaris*. *Pl. Physiol.* **68**, 284–291.

Derbyshire, E., Wright, D. J. & Boulter, D. 1976 Legumin and vicilin storage proteins of legume seeds. *Phytochemistry* **15**, 3–24.

Dieckert, J. W. & Dieckert, M. C. 1976 The chemistry and cell biology of the vacuolar proteins of seeds. *J. Food Sci.* **41**, 475–482.

Dorland, L., van Halbeek, H., Vliegenthart, J. F. G., Lis, H. & Sharon, N. 1981 Primary structure of the carbohydrate chain of soybean agglutinin. A reinvestigation by high resolution ^{1}H n.m.r. spectroscopy. *J. biol. Chem.* **256**, 7708–7711.

Dürr, M., Bailey, D. S. & Maclachlan, G. 1979 Subcellular distribution of membrane-bound glycosyl transferase from pea stems. *Eur. J. Biochem.* **97**, 445–453.

Elbein, A. D. 1981 The role of lipid-linked saccharides in the biosynthesis of complex carbohydrates. *Encycl. Pl. Physiol.* (New Ser.) **13 B**, 166–193.

Ereken-Tumer, N., Richter, J. D. & Nielsen, N. C. 1982 Structural characterization of the glycinin precursors. *J. biol. Chem.* **257**, 4015–4018.

Ericson, M. C. & Chrispeels, M. J. 1976 The carbohydrate moiety of mung bean vicilin. *Aust. J. Pl. Physiol.* **3**, 763–769.

Goldberg, R. B., Hoschek, G., Ditta, G. S. & Breidenbach, R. W. 1981 Developmental regulation of cloned superabundant embryo mRNAs in soybean. *Devl Biol.* **83**, 218–231.

Goldstein, I. J. & Hayes, C. E. 1978 The lectins: carbohydrate binding proteins of plants and animals. *Adv. Carbohyd. Chem. Biochem.* **35**, 128–340.

Graham, T. A. & Gunning, B. E. S. 1970 Localization of legumin and vicilin in bean cotyledon cells using fluorescent antibodies. *Nature, Lond.* **228**, 81–82.

Harris, N. 1979 Endoplasmic reticulum in developing seeds of *Vicia faba*. *Planta* **146**, 63–69.

Harris, N. & Boulter, D. 1976 Protein body formation in cotyledons of developing cowpea (*Vigna unguiculata*) seeds. *Ann. Bot.* **40**, 739–744.

Harris, N. & Oparka, K. J. 1983 Connections between dictyosomes, e.r. and g.e.r.l. in cotyledons of mung bean (*Vigna radiata* L.). *Protoplasma* (In the press).

Hasilik, A. & Neufeld, E. F. 1980 Biosynthesis of lysosomal enzymes in fibroblasts. Synthesis as precursors of higher molecular weight. *J. Biol. Chem.* **255**, 4937–4945.

Hasilik, A. & Tanner, W. 1978 Biosynthesis of the vacuolar yeast glycoprotein carboxypeptidase Y. *Eur. J. Biochem.* **85**, 599–608.

Higgins, T. J. V. & Spencer, D. 1977 Cell-free synthesis of pea seed protein. *Pl. Physiol.* **60**, 655–661.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Higgins, T. J. V. & Spencer, D. 1981 Precursors forms of pea vicilin subunits. Modification by microsomal membranes during cell-free translation. *Pl. Physiol.* **65**, 205–211.

Higgins, T. J. V., Chandler, P. M., Zurawski, G., Button, S. & Spencer, D. 1983*a* The synthesis and primary structure of pea lectin. *J. biol. Chem.* **258**, 9544–9549.

Higgins, T. J. V., Chrispeels, M. J., Chandler, P. M. & Spencer, D. 1983*b* Intracellular sites of synthesis and processing of pea seed lectin. *J. biol. Chem.* **258**, 9550–9553.

Hoffman, L., Ma, Y. & Barker, R. F. 1982 Molecular cloning of *Phaseolus vulgaris* lectin in RNA and use of cDNA as a probe to estimate lectin transcript levels in various tissues. *Nucl. Acids Res.* **10**, 7819–7828.

Hori, H., James, D. W. & Elbein, A. 1982 Isolation and characterization of the $Gk_3Man_9GlcNAc_2$ from lipid-linked oligosaccharides of plants. *Archs Biochem. Biophys.* **215**, 12–21.

Horisberger, M. & Vonlanthen, M. 1980 Ultrastructural localization of soybean agglutinin on thin sections of *Glycine max* var. Altona by the gold method. *Histochemistry* **65**, 181–186.

Hubbard, S. C. & Ivatt, R. J. 1981 Synthesis and processing of asparagine-linked oligosaccharides. *A. Rev. Biochem.* **50**, 555–583.

Hurkman, W. J. & Beevers, L. 1982 Sequestration of pea reserve proteins by rough microsomes. *Pl. Physiol.* **69**, 1414–1417.

Iglesias, J. L., Lis, H. & Sharon, N. 1982 Purification and properties of a D-galactose/*N*-acetyl-D-galactosamine-specific lectin from *Erythrina cristagalli*. *Eur. J. Biochem.* **123**, 247–252.

Jaffé, W. G., Levy, A. & Gonzales, D. I. 1975 Isolation and partial characterization of bean phytohaemagglutinin. *Phytochemistry* **13**, 2685–2693.

Juniper, B. E. Hawes, C. R. & Horne, J. C. 1982 The relationships between the dictyosomes and the forms of endoplasmic reticulum in plant cells with different export programs. *Bot. Gaz.* **143**, 135–145.

Kurokawa, T., Tsuda, M. & Sugino, Y. 1976 Purification and characterization of a lectin from *Wistaria floribunda* seeds. *J. biol. Chem.* **251**, 5686–5993.

Leavitt, R. D., Felsted, R. L. & Bachur, N. R. 1977 Biological and biochemical properties of *Phaseolus vulgaris* isolectins. *J. biol. Chem.* **252**, 2961–2966.

Lehle, L. 1981 Plant cells synthesize glucose-containing lipid-linking oligosaccharides similar to those found in animals and yeast. *FEBS Lett.* **123**, 63–66.

Lehle, L., Bowles, D. J. & Tanner, W. 1978 Subcellular site of mannosyl transfer to dolichylphosphate in *Phaseolus aureus*. *Pl. Sci. Lett.* **11**, 27–34.

Lotan, R., Lis, H. & Sharon, N. 1975 Aggregation and fragmentation of soybean agglutinin. *Biochem. biophys. Res. Commun.* **62**, 144–150.

Manen, J. F. & Pusztai, A. 1982 Immunocytochemical localization of lectins in cells of *Phaseolus vulgaris* L. seeds. *Planta* **155**, 328–334.

Mellor, R. B., Roberts, L. M. 1980 *N*-acetylglucosamine transfer reactions and glycoprotein biosynthesis in castor bean endosperm. *J. exp. Bot.* **31**, 993–1003.

Mialonier, G., Privat, J.-P., Monsigny, M., Kahlem, G. & Durand, R. 1973 Isolement, propriétés physico-chimiques et localisation in vivo d'une phytohémagglutinine (lectine) de *Phaseolus vulgaris* L. (var. rouge). *Physiol Vég.* **11**, 519–537.

Misaki, A. & Goldstein, I. J. 1977 Glycosyl moiety of the lima bean lectin. *J. biol. Chem.* **252**, 6995–6999.

Nagahashi, J. & Beevers, L. 1978 Subcellular localization of glycosyltransferases involved in glycoprotein biosynthesis in the cotyledons of *Pisum sativum* L. *Pl. Physiol.* **61**, 451–459.

Neumann, D. & Weber, E. 1978 Formation of protein bodies in ripening seeds of *Vicia faba* L. *Biochem. Physiol. Pflanz.* **173**, 167–180.

Öpik, H. 1968 Development of cotyledon cell structure in ripening *Phaseolus vulgaris* seeds. *J. exp. Bot.* **19**, 64–76.

Püchel, M., Muntz, K., Parthier, B., Aurich, O., Bassiner, R., Manteuffel, R. & Schmidt, P. 1979 RNA metabolism and membrane-bound polysomes in relation to globulin biosynthesis in cotyledons of developing field beans (*Vicia faba* L.). *Eur. J. Biochem.* **96**, 321–329.

Räsänen, V., Weber, T. H. & Gräsbeck, R. 1973 Crystalline kidney-bean leucoagglutinin. *Eur. J. Biochem.* **38**, 193–200.

Roberts, J. & Lord, L. M. 1981 The synthesis of *Ricinus communis* agglutinin. Cotranslational and post-translational modification of agglutinin polypeptides. *Eur. J. Biochem.* **119**, 31–41.

Sabatini, D. D., Kreibich, G., Morimoto, T. & Adesnik, M. 1982 Mechanism for the incorporation of proteins in membranes and organelles. *J. Cell Biol.* **92**, 1–22.

Schuler, M. A., Schmidt, E. S. & Beachy, R. N. 1982 Closely related families of genes code for α and α′ subunits of the soybeans 7 *S* storage protein. *Nucl. Acids Res.* **10**, 8225–8244.

Sengupta, C., Deluca, V., Bailey, D. S. & Verma, D. P. S. 1981 Post-translational processing of 7 *S* and 11 *S* components of soybean storage proteins. *Pl. molec. Biol.* **1**, 19–34.

Skudlarek, M. & Swank, R. T. 1979 Biosynthesis of two lysosomal enzymes in macrophages. *J. biol. Chem.* **254**, 9939–9942.

Spencer, D. & Higgins, T. J. V. 1980 The biosynthesis of legumin in maturing pea seeds. *Biochem. Int.* **1**, 502–509.

Staneloni, R. J., Tolmasky, M. E., Petriella, C., Ugalde, R. A. & Leloir, L. F. 1980 Pressure in a plant of a compound similar to the dolichyldiphosphate oligosaccharide of animal tissue. *Biochem. J.* **191**, 257–260.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Sun, S. M., Buchbinder, B. U. & Hall, T. C. 1975 Cell free synthesis of the major storage protein of the bean *Phaseolus vulgaris* L. *Pl. Physiol.* **56**, 780–785.

Thomson, J. A., Schroeder, H. E. & Tassie, A. M. 1980 Cotyledonary storage proteins in *Pisum sativum*. V. Further studies on molecular heterogeneity in the vicilin series of holoproteins. *Aust. J. Pl. Physiol.* **7**, 271–282.

Tumer, N. E., Thanh, V. H. & Nielsen, N. C. 1981 Purification and characterization of mRNA from soybean seeds. Identification of glycinin and β-conglycinin precursors. *J. biol. Chem.* **256**, 8756–8760.

Van Driessche, E., Smets, G., Dejaegere, R. & Kanarek, L. 1981 The immunohistochemical localization of lectin in pea seeds (*Pisum sativum* L.). *Planta* **153**, 287–296.

Van der Wilden, W., Herman, E. & Chrispeels, M. J. 1980 Protein bodies of mung cotyledons as autophagic organelles. *Proc. natn. Acad. Sci. U.S.A.* **77**, 428–432.

Varner, J. E. & Schidlovsky, G. 1963 Intracellular distribution of proteins in pea cotyledons. *Pl. Physiol.* **38**, 139–144.

Wang, J. L., Cunningham, B. A. & Edelman, G. M. 1971 Unusual fragments in the subunit structure of concanavalin A. *Proc. natn. Acad. Sci. U.S.A.* **68**, 1130–1134.

Yoo, B. Y. & Chrispeels, M. J. 1980 The origin of protein bodies in developing soybean cotyledons: a proposal. *Protoplasma* **103**, 201–204.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES