Planta (1988) 176:173–182

Planta


# Formation of wheat protein bodies: Involvement of the Golgi apparatus in gliadin transport

**Woo Taek Kim[1], Vincent R. Franceschi[2], Hari B. Krishnan[2]*, and Thomas W. Okita[3]****

[1] Graduate Program in Plant Physiology,
[2] Department of Botany, and
[3] Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340, USA

**Abstract.** Developing wheat (*Triticum aestivum* L.) endosperm was examined using ultrathin sections prepared from tissues harvested at 5, 9, 16 and 25 d after flowering. Protein bodies were evident by 9 d and displayed a variety of membranous structures and inclusions. The Golgi apparatus was a prominent organelle at all stages, and by 9 d was associated with small electron-dense inclusions. By immunocytochemical techniques, gliadin (wheat prolamine) was localized within these vesicles and in homogeneous regions of protein bodies, but not in the lumen of the rough endoplasmic reticulum. The protein bodies appear to enlarge by fusion of smaller protein bodies resulting in larger, irregular-shaped organelles. The affinity of the Golgi-derived vesicles for gliadin-specific probes during the period of maximal storage-protein synthesis and deposition indicates that this organelle includes the bulk, if not all, of the gliadin produced. The involvement of the Golgi apparatus in the packaging of gliadins into protein bodies indicates a pathway which differs from the mode of prolamine deposition in other cereals such as maize, rice and sorghum, and resembles the mechanism employed for the storage of rice glutelin and legume globulins.

**Key words:** Gliadin – Golgi apparatus – Protein deposition – Storage protein – *Triticum* (protein bodies)

## Introduction

The major storage proteins of most cereal seeds are classified as either prolamines (insoluble in dilute salt solutions, soluble in alcohol-water) or glutelins (soluble in dilute alkali or acid). In wheat, the prolamine and glutelin proteins are called gliadins and glutenins, respectively. The gliadins are synthesized on the rough endoplasmic reticulum (rough ER) (Greene 1981), and accumulate in membrane-bound protein bodies. Biochemical analysis of purified protein bodies indicates that glutenins are also present in the protein bodies (Payne et al. 1986).

The formation of protein bodies in cereals has been studied extensively by ultrastructural analysis, and two distinct pathways have been described. The prolamines of maize (Khoo and Wolf 1970; Larkins and Hurkman 1978), rice (Oparka and Harris 1982; Krishnan et al. 1986) and sorghum (Taylor et al. 1985) are synthesized on the rough ER and deposited within the lumen of this organelle. Biochemical studies have shown that polyribosomes isolated from the protein bodies of maize synthesize the storage protein zein (Burr and Burr 1976). Direct connections of the protein-body membranes with rough ER are evident by electron microscopy, indicating that the rough ER is not only the location of synthesis but is also the site of deposition (Larkins and Hurkman 1978). On the other hand, the rice glutelins, the predominant storage proteins of this species, are packaged into protein bodies via the Golgi complex (Krishnan et al. 1986). Thus, the rice glutelin protein bodies are formed by a pathway described for the legume 7S globulins (Greenwood and Chrispeels 1985). The similarities in the packaging of these proteins into discrete organelles are consistent with the homology the basic subunits of these proteins display in their primary sequences (Zhao et al. 1983; Wen and Luthe 1985).

Despite numerous studies, contradictory hypotheses exist regarding the site of synthesis and deposition of the storage proteins in developing

* *Present address:* Department of Plant Pathology, University of Missouri, 108 Waters Hall, Columbia, MO 65211, USA
** To whom correspondence should be addressed

*Abbreviations:* ER = endoplasmic reticulum; IgG = immunoglobulin G; DAF = days after flowering

wheat (and barley) seeds. Miflin et al. (1981, 1983) had postulated that storage proteins were deposited in the rough ER lumen and then subsequently released into the cytoplasm as protein aggregates. Similarly, Campbell et al. (1981) reported protein bodies to arise directly through distension of the rough ER, analagous to the prolamines of maize. This was also supported by Briarty et al. (1979) who suggested there was an apparent absence of the Golgi apparatus during the active phase of storage protein accumulation. In contrast, other workers have supported a role for the Golgi apparatus in protein body formation. Parker and Hawes (1982) have unequivocally demonstrated the presence of the Golgi apparatus in developing wheat endosperm during the period of active storage protein accumulation. Bechtel et al. (1982) and Bechtel and Barnett (1986), in extensive ultrastructural surveys by conventional and freeze-fracture techniques, observed no direct connections between protein bodies and rough ER and suggested the Golgi apparatus played a central role in protein body initiation and development.

In order to resolve the question of the role of the Golgi apparatus in protein body formation in wheat endosperm, gliadin-specific antibodies and labeling with colloidal gold-conjugated protein A were employed. These techniques have been extensively utilized in the localization of seed lectins and reserve proteins (see Chrispeels 1985 for review). Using immunogold labeling, we show here that unlike the packaging of the prolamines of maize, rice and sorghum into distended rough ER, the bulk, if not all, of the wheat prolamines are delivered to protein bodies via the Golgi apparatus. Other ultrastructural aspects related to protein body formation and the possible significance of these observations are also addressed.

## Material and methods

*Plant material.* Wheat (*Triticum aestivum* L. cv. Yecovo Rojo) seeds (caryopses) were obtained from the Department of Agronomy, Washington State University. Seeds were planted in 15-cm plastic pots containing soil composed of 55% (v/v) peat, 35% pumice, 7.5% pumice sand and 2.5% sand, and grown in a greenhouse with supplemental light from a 1000-W overhead metal-halide lamp. Plants were supplemented every 7 d with Peter's (Allentown, Penn., USA) soluble fertilizer. Ears were tagged upon anthesis, and developing grains were subsequently removed from the plants at 5, 9, 16 and 25 d after flowering (DAF).

*Tissue preparation.* Seeds were sliced into 1–2-mm sections and fixed with 2.5% glutaraldehyde in 50 mM sodium-phosphate buffer, pH 7.2, for 1 h at room temperature and subsequently for 14 h at 4° C. Postfixation was performed at room temperature for 2 h with 2% (w/v) osmium tetroxide. Following the postfixation step, samples were rinsed ten times with excess buffer A (50 mM sodium phosphate), dehydrated with a graded acetone series, and embedded in Spurr's resin (Spurr 1969). Ultrathin sections were prepared with a diamond knife, attached to uncoated nickel grids (200 mesh; Polysciences, Warrington, Penn., USA), and post-stained with 2% (w/v) uranylacetate and 2% (w/v) lead-citrate solutions, or processed for immunocytochemical labeling (see below). Following the post-staining step, the specimens were examined on either an Hitachi (Tokyo, Japan) HS-8 or an Hitachi H-300 transmission electron microscope.

*Anti-gliadin immunoglobulin preparation.* Wheat prolamine were purified by extraction with 70% (v/v) ethanol of *T. aestivum* cv. Cheyanne seed meal (obtained from the Department of Agronomy, Washington State University), followed by electrophoresis on pH 3.1 sodium-lactate polyacrylamide gels (Tkachuk and Metlish 1980). The sodium-lactate gel was stained with Coomassie brilliant blue and gel slices containing $\alpha/\beta$ and $\gamma$-gliadin polypeptides were then rinsed extensively with 100 mM 3-amino-(2-hydroxymethyl)-1,3-propanediol (Tris)-HCl, pH 8.0. The gel slices were mashed and then soaked overnight in 1% sodium dodecylsulfate (SDS), 50 mM Tris-HCl (pH 8.0), 0.1 mM ethylenediaminotetraacetic acid (EDTA), and 150 mM NaCl. Eluted proteins were collected by precipitation with four volumes of acetone followed by centrifugation at $28000 \cdot g$ for 15 min. The protein pellets were dried in vacuo and repurified a second time by sodium-lactate gel electrophoresis. New Zealand rabbits obtained from local breeders were injected with about 500 µg of purified $\alpha/\beta$- and $\gamma$-gliadin for four consecutive times at two-week intervals. Antigen for the first injection was emulsified in Freund's complete adjuvant, whereas in subsequent injections, incomplete adjuvant was used. The immunoglobulin G (IgG) fraction of the sera was partially purified by 40% (w/v) $(NH_4)_2SO_4$ precipitation and passage through a diethylaminoethyl (DEAE)-cellulose column.

*Western blotting.* The 70% ethanol-soluble proteins obtained from endosperm tissues of a diploid wheat species, *T. urartu* Tum. (obtained from Dr. G. Waines, University of California, Riverside) were resolved by sodium-lactate gel electrophoresis at pH 3.1. Following electrophoresis, the gels were incubated for 30 min in 200 ml of 20 mM Tris-HCl, 150 mM glycine and 20% (v/v) methanol, and transferred to nitrocellulose filters (pore size 0.45 µm; Millipore Corporation, Bedford, Mass., USA) as described by Burnett (1981). The $\alpha/\beta$- and $\gamma$-gliadins were visualized by incubating blotted nitrocellulose membranes with DEAE-cellulose-purified anti-gliadin antibodies (35 µg/ml), followed by incubation in the presence of $2 \cdot 10^6$ Bq of $^{125}$I-protein A (specific activity $20 \cdot 10^6$ Bq/µg; New England Nuclear, Boston, Mass., USA). Immunoreactive polypeptides were detected by autoradiographic methods, using a DuPont (Wilmington, Del., USA) Cronex Lightening Plus intensifying screen for signal enhancement.

*Immunocytochemical staining.* Ultrathin sections, mounted on uncoated nickel grids, were first treated with 0.56 M sodium metaperiodate ($NaIO_4$) for 30 min, followed by incubation in 0.1 N HCl for 10 min in order to unmask the antigenic determinants (Craig and Goodchild 1984). Pretreated sections on grids were incubated in several drops of Tris-buffered saline (TBST; 10 mM Tris, 500 mM NaCl, 0.3% polyoxyethylenesorbitan monolaurate (Tween 20), pH 7.2) containing 1% bovine serum albumin fraction V (BSA) for 10 min. The grids were then incubated for 3 h with 200 µl of the DEAE-cellulose-purified $\alpha/\beta$-antibody diluted with TBST and 1% BSA. Non-specifically bound antibody was removed by washing the sections five times

on drops of TBST-BSA. The grids were then incubated for 1 h in 150 µl of 20-nm diameter protein-A gold (Janssen Pharmaceuticals, Piscataway, N.J., USA) diluted 1:30 in TBST-BSA. The sections were thoroughly washed with TBST-BSA, TBST and then distilled water, as described above, and then sequentially stained with 2% aqueous uranyl acetate and 2% lead citrate for 10 min. All of the above operations were carried out at room temperature. In later experiments, $NaIO_4$ was omitted from the labeling procedure with no appreciable loss in specificity, but with pronounced reduction in background labeling. For control treatment the grids were incubated with diluted pre-immune serum under the same conditions.

## Results

*Specificity of anti-gliadin immunoglobulin preparations.* The specificity of the partially purified IgG preparations, generated against either the α/β- or the γ-gliadin fractions of *T. aestivum* were examined by Western blot analysis. Previously, these antibody preparations were demonstrated to react only with the gliadins in whole-cell preparations (Reeves et al. 1986). In this study, electrophoretically resolved prolamine preparations from the diploid wheat species, *T. urartu,* were employed as test antigens and a marked degree of specificity was observed (Fig. 1). The antibody fraction prepared against the α/β-gliadins reacted strongly with both its respective antigen and with the ω-gliadins (lane A), whereas the reactivity pattern of the anti-γ-gliadin preparation was more restricted, with clear binding to only one band in the γ-gliadin region of the blot (lane B). A prolonged exposure of these autoradiograms (not shown) showed the existence of some cross-reactivity between gliadin classes. These results were consistent with the known degree of homology among various types of gliadin (reviewed by Kreis et al. 1985), and indicated that these antibodies, while specific for gliadin in general, could not distinguish the various gliadin classes in the immunocytochemical localization studies. Indeed, when tissue sections were treated with either antibody preparation prior to their exposure to colloidal-gold-conjugated protein-A, identical degrees and patterns of antibody binding were observed (data not shown) despite the fact that individual gliadin classes are known to be present at different levels in the developing endosperm (Reeves et al. 1986). Thus, for most subsequent studies, only one of the antibody preparations (anti-α/β-gliadin) was employed. Specificity at the cytochemical level was further evaluated by the incubation of thin sections with either the anti-α/β-gliadin preparation (Fig. 2) or the pre-immune serum (Fig. 3). When tissues were first treated with gliadin-specific antibody, protein A-gold was found to be densely bound to protein bodies,

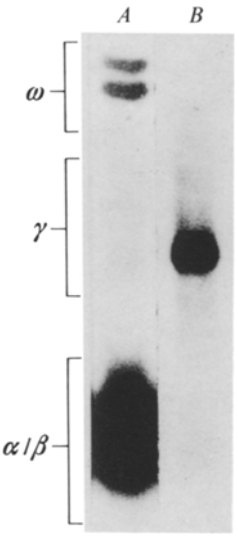
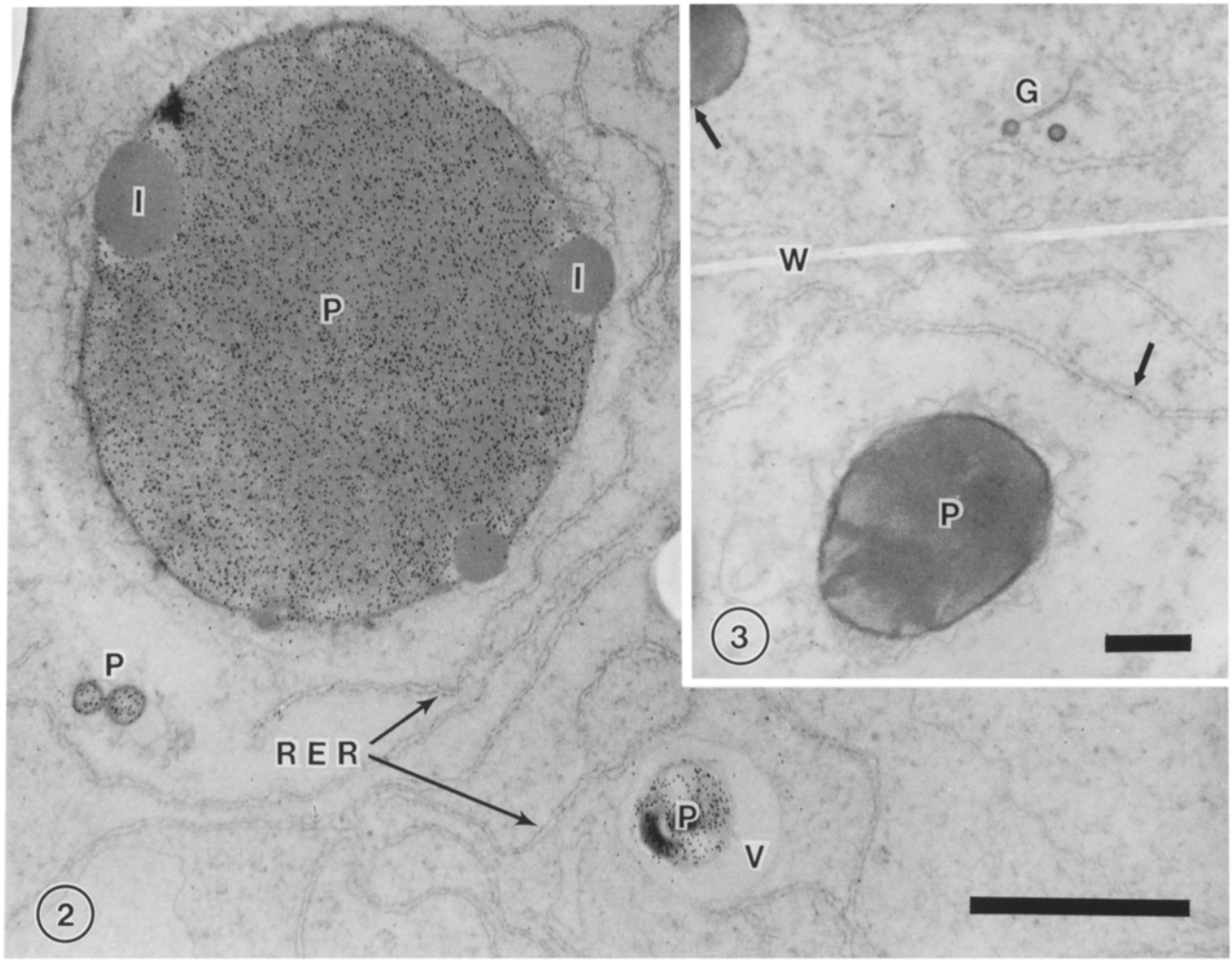



**Fig. 1.** Western blot analysis demonstrating the specificity of the α/β- and γ-gliadin IgG preparations from wheat (*T. aestivum*). Alcohol-soluble proteins of *T. urartu* were fractionated by sodium-lactate gel electrophoresis at pH 3.1 and the resolved proteins electrophoretically transferred to nitrocellulose filter. The nitrocellulose filter was then incubated with fractions of antisera raised against the purified α/β- (*lane A*) or γ-gliadins (*lane B*). The immunoreactive bands were visualized by incubation of the filter with $^{125}$I-protein A followed by autoradiography. Exposure time was 8 h with an intensifying screen at −80° C. ω, γ and α/β regions were defined based on the Coomassie-brilliant-blue staining pattern of identical sodium-lactate gels (not shown)

with a high degree of specificity (Fig. 2). However, very little protein A-gold was adsorbed to thin sections treated with pre-immune serum, and a specific localization of the probe was not apparent (Fig. 3).

*Protein bodies.* The cytoplasmic contents of the outer endosperm cells (three or four cell layers under the aleurone tissue) of wheat seeds at 9 DAF were characterized by an abundance of starch granules and numerous protein bodies of various dimensions (Fig. 2). The inner endosperm tissue was found to be quite similar in appearance, but with a heavier concentration of starch and consequently it was more difficult to section. Nonetheless, all of the results presented herein were observed at both tissue levels. In addition to antibody-reactive material, the large protein bodies, such as the one presented in Fig. 2, were found to contain one or more spherical, electron-opaque inclusions which contained material unreactive to the gliadin-specific probe employed in this study. Membranous structures could be also seen associated with these large protein bodies (Figs. 3, 14). Several of the smaller protein bodies appeared to contain an electron-lucent space, surrounded by




**Figs. 2, 3.** Immunolocalization of gliadin in wheat endosperm

**Fig. 2.** Low-magnification view of 9 DAF endosperm showing the specificity of the gliadin antibody. Protein-A gold particles specifically bind to various-sized protein bodies. Rough ER is extensive, but exhibits very little labeling. Note the electron-opaque inclusions which are unreactive to the gliadin-specific probe. *P*, protein body; *I*, inclusion; *RER*, rough endoplasmic reticulum; *V*, electron-lucent vesicle. × 16500; bar = 2 μm

**Fig. 3.** Control section incubated with pre-immune serum. The *arrow* points to background protein-A gold particles. *G*, Golgi apparatus; *W*, cell wall. × 12000; bar = 1 μm

**Figs. 4–10.** Protein-body formation and gliadin localization in wheat endosperm

**Fig. 4.** Small protein body surrounded by an electron-lucent space and a unit membrane (*arrows*). A membrane complex (*M*) is connected to the protein inclusion (*P*). × 23000; bar = 1 μm

**Fig. 5.** Membrane cisternae or tubules (*arrows*) continuous with the membrane of an electron-lucent vesicle. Note the lack of labeling on rough ER. × 25000; bar = 1 μm

**Fig. 6.** Golgi complex (*G*) at 9 DAF. Electron-dense Golgi-derived vesicles (*arrows*); Electron-lucent vesicles (*V*). × 30000; bar = 0.5 μm

**Fig. 7.** Demonstration of a direct connection between a labeled electron-dense vesicle and the Golgi complex (*arrow*). Labeled vesicles were occasionally associated with the larger electron-lucent vesicles ($V_2$). × 30000; bar = 0.5 μm

**Fig. 8.** Golgi-derived electron-dense vesicles with and without immuno-gold particles. Note the presence of small vesicular blebs at the surface of Golgi-derived vesicles (*arrows*). Direct connection between electron-dense vesicles and electron-lucent vesicles (*V*) and the Golgi complex are also evident. × 31000; bar = 0.5 μm

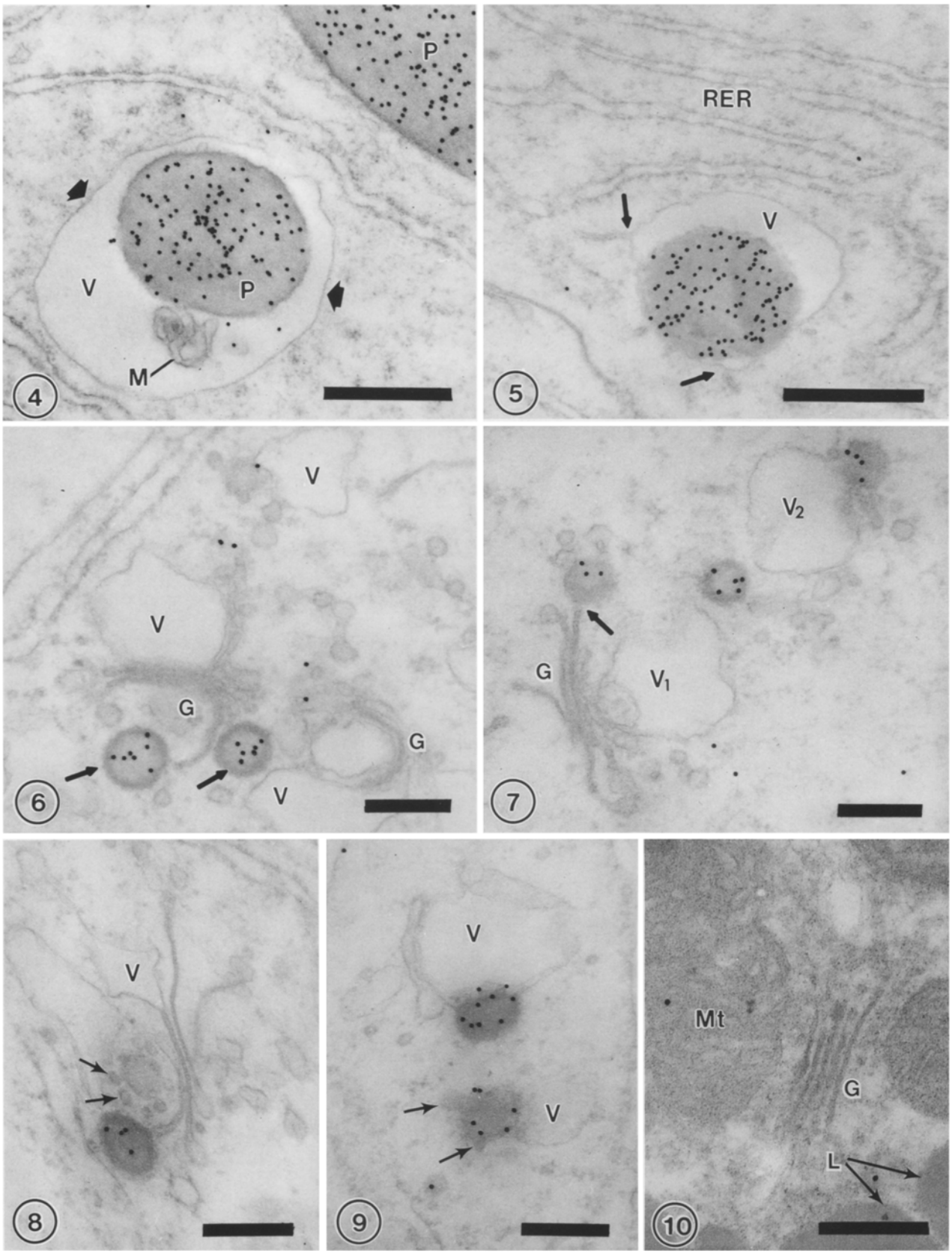



**Fig. 9.** Golgi-derived labeled electron-dense vesicles associated with membrane of electron-lucent vesicles (*V*). Small vesicular blebs are indicated by *arrows*. ×32000; bar=0.5 μm

**Fig. 10.** Golgi body (*G*) in aleurone layer of wheat seed at 9 DAF. Note the typical shape and absence of labeling. *Mt*, mitochondrion; *L*, lipid body. ×40000; bar=0.5 μm

a unit membrane (Figs. 2, 4, 5). Membrane networks or complexes were occasionally observed to be present within the electron-lucent space and appeared to be continuous with the surface of the protein inclusion (Fig. 4). More frequently, membrane cisternae reminiscent of Golgi cisternae were observed to be continuous with the unit membrane of electron-lucent vesicles having protein inclusions (Figs. 5, 12). In no instance, however, was continuity between the rough ER and the protein-body membrane or the electron-lucent vesicle membrane seen (Figs. 4, 5).

*Golgi apparatus.* This organelle was readily demonstrable in the endosperm tissues of all developmental stages examined. In tissue sections prepared at 5 DAF, each Golgi apparatus was found to consist of three to four parallel cisternae, with several associated small electron-lucent vesicles (see Fig. 16). At 9 DAF the electron-lucent vesicles were observed to be larger than in earlier stages, and electron-dense vesicles were found to be associated with the Golgi apparatus (Fig. 6). When tissues were pre-incubated with gliadin antibodies, many of these vesicles were found to bind the colloidal-gold-conjugated protein-A probe (Fig. 6). In many cases, direct connection between these electron-dense vesicles and cisternae of the Golgi complex were observed (Figs. 7, 8). Electron-dense vesicles were also often associated with larger electron-lucent vesicles (Figs. 7, 9). Some electron-dense vesicles, such as seen in Fig. 8, were not reactive to protein-A probe. The Golgi-associated electron-dense vesicles were occasionally seen to elaborate or be associated with numerous, relatively electron-lucent surface blebs (Figs. 8, 9, arrows) which did not appear to react with the protein-A probe (although their small relative size may not have allowed adequate surface area for reactivity to be apparent).

It was noteworthy that the Golgi apparatus at 9 and 16 DAF (Figs. 6–9) differed morphologically from that observed in less mature tissues (Fig. 16) or late stage of endosperm development (Fig. 18), even though the Golgi apparatus in the aleurone layer of the same tissue sections from 9 DAF tissue appeared to have a typical shape with several parallel cisternae (Fig. 10). These Golgi from endosperm tissue of mid-development stage were more highly curved and often had only two or three recognizable cisternae with less ordered stacking (Figs. 6–8). Large electron-lucent vesicles were often associated with one surface of the Golgi apparatus and may represent completely distended cisternae (Figs. 6, 7). Figure 8 is a possible example of an intermediate stage in cisternal distension.

*Enlargement of protein bodies.* Our observations indicate protein bodies may enlarge by more than one process. Protein bodies which contain electron-lucent space, such as presented in Fig. 11, may fuse with one another to form larger protein bodies (Fig. 12; see also Fig. 17). In many cases two protein inclusions appeared to be coalescing

**Figs. 11–15.** Enlargement and substructure of wheat endosperm protein bodies. All sections immunostained for gliadin

**Fig. 11.** Two adjacent vesicles (*V*) with gliadin-containing protein inclusions surrounded by electron-lucent space. ×20000; bar = 0.5 μm

**Fig. 12.** Apparent fusion of two gliadin-containing vesicles. Note the membrane cisternae (*arrows*) associated with membrane of the vesicle. ×33000; bar = 0.5 μm

**Fig. 13.** Four labeled protein masses (*P*) within a large vacuole (*V*). ×11000; bar = 1 μm

**Fig. 14.** Interconnection of two large protein masses (*P*), indicative of fusion of discrete protein bodies to form a larger irregular protein body. *I*, inclusion; *M*, membrane complex. ×14000; bar = 1 μm

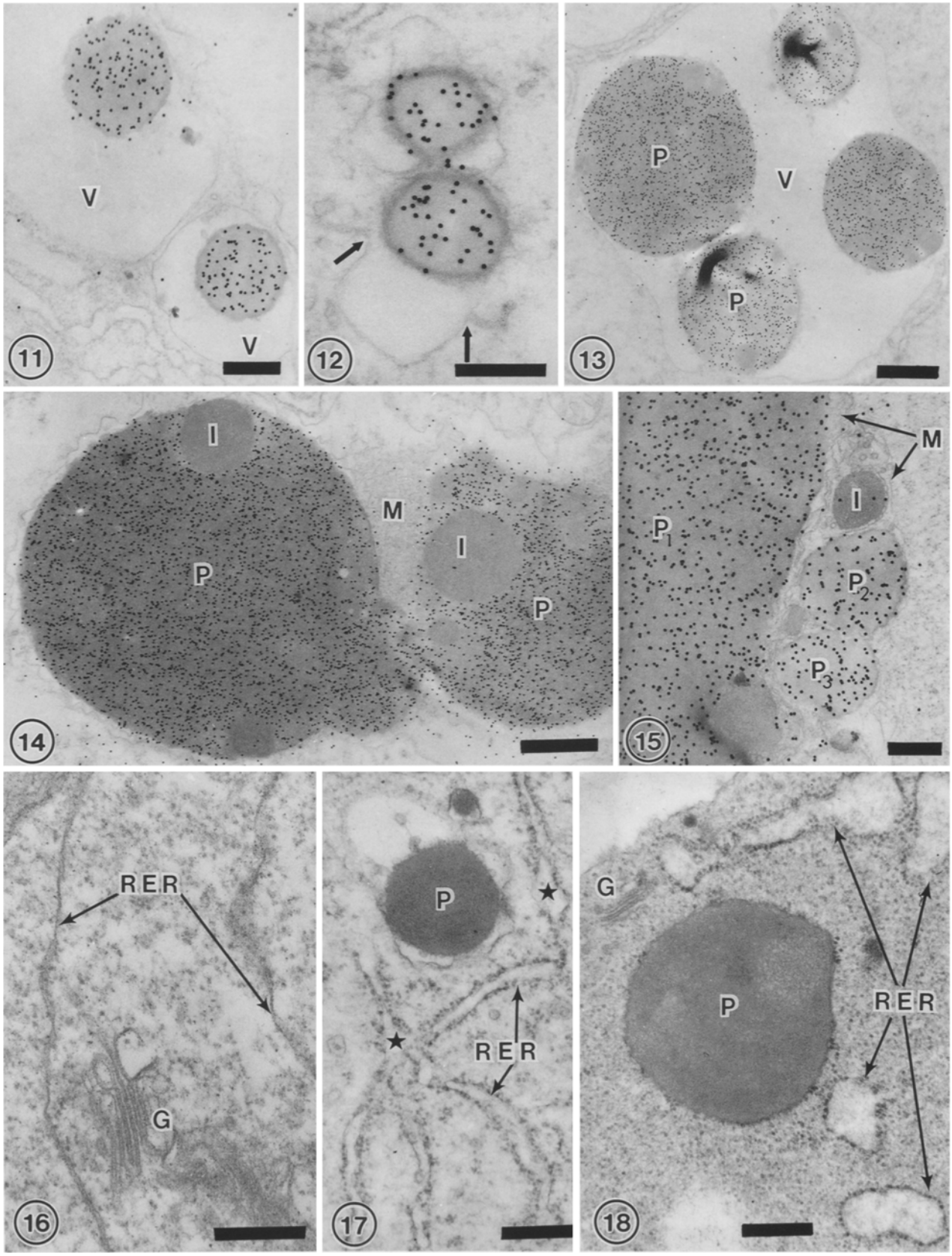
**Fig. 15.** Small protein masses ($P_2$, $P_3$) and an electron-opaque inclusion (*I*) at the periphery of a large mass (*P*). Note membrane (*M*) surrounding the edge of the protein body. ×22000; bar = 0.5 μm

**Figs. 16–18.** Rough ER in developing wheat endosperm. *G*, Golgi apparatus; *Mt*, mitochondrion; *P*, protein body; *RER*, rough endoplasmic reticulum

**Fig. 16.** Typical appearance of rough ER at 5 DAF. ×32000; bar = 0.5 μm

**Fig. 17.** At 16 DAF rough ER is more abundant, and distended regions of the rough ER are common (indicated by *asterisks*). ×25000; bar = 0.5 μm

**Fig. 18.** Distended and swollen rough ER at 25 DAF. ×25000; bar = 0.5 μm


V
V
11
12
P
V
P
13
I
M
I
P
P
14
M
I
P1
P2
P3
15
RER
G
16
P
RER
17
G
P
RER
18

within the same electron-lucent vesicle (data not shown), and this was probably the end result of fusion of two electron-lucent vesicles, each containing protein inclusions. In addition, several discrete protein bodies could be found inside a large vacuole (Fig. 13) which we suggest is the product of multiple fusion of vesicles with protein inclusions. Protein bodies which did not contain electron-lucent space appeared to fuse together and became larger and irregular-shaped protein bodies (Fig. 14). Unlabeled electron-opaque inclusions with a circular profile and of variable diameter were found primarily at the peripheral region of protein bodies during or after fusion (Figs. 2, 13–15). Complex membrane networks were commonly present in the vicinity of the point of fusion of individual protein bodies (Fig. 14). At higher magnification, it could be clearly seen that small protein bodies as well as electron-dense vesicles were associated with large protein bodies at their periphery (Fig. 15). Rough ER was never associated or connected to either type of protein body during fusion.

*Endoplasmic reticulum.* Specimens prepared from seeds harvested at 5 DAF contained relatively little ER (Fig. 16). At later developmental stages, rough ER was much more common, and in most instances contained a larger lumen (Fig. 17) as compared to rough ER in the younger endosperm cells. This morphologic trait did not appear to be an artifact caused by the osmolarity of the fixative, as the membranes of other organelles (including the nuclear envelope and Golgi apparatus) within the same cells were not distened. Such distended ER did not contain material reactive with gliadin-specific probes and was not connected with protein bodies. At 25 DAF, rough ER in this form typically observed during the earlier stages of development was very rare; however, large, ribosome-studded vesicular structures were a common feature (Fig. 18). These structures contained an electron-lucent lumen which possessed no demonstrable affinity for gliadin-specific probes (data not shown). In no instance were protein bodies observed to be enclosed within these structures. Other membrane structures, such as the Golgi cisternae, were not swollen (Fig. 18).

## Discussion

Our immunolabeling studies clearly demonstrate that gliadin, the predominant storage protein of wheat endosperm, is localized in the protein bodies of developing wheat seeds as early as 9 DAF. By the use of gliadin-specific antibody, we have demonstrated that the Golgi apparatus is involved in the packaging and transport of gliadin to the protein body during the most active period of protein biosynthesis and accumulation. This confirms the earlier suggestion by Parker and Hawes (1982) of a role for the Golgi complex in protein-body formation in wheat and disproves the mechanism proposed by Miflin et al. (1981). Although we could not demonstrate a direct localization of gliadin within rough ER, occasionally gliadin-specific probes were found associated with this organelle at slightly higher than background labeling (see Fig. 11). This would be consistent with the known site of gliadin synthesis at the rough ER (Greene 1981). It must be noted that this lack of specific labeling may reflect a low concentration of gliadin within these structures rather than a complete absence of this protein. Although the rough-ER lumen cannot be totally excluded as a site of gliadin accumulation, our results indicate that it is not the major mechanism by which discrete, condensed protein bodies are formed in wheat.

Instead of direct deposition and aggregation of gliadin into the lumen of rough ER, the first step in protein-body formation in wheat appears to be condensation of protein within Golgi vesicles. The fact that the undilated regions of Golgi cisternae were not labeled is likely related to the relatively low concentration of gliadin presented at the surface of a section through the thin cisternae. Even the relatively large protein accumulations at the dilated ends of the cisternae showed low, but specific, labeling (see Figs. 6–8). Subsequent enlargement of protein bodies by fusion of vesicles with each other or with other protein bodies was clearly indicated. Such fusion of protein bodies resulted in larger, irregular-shaped organelles. While multiple nucleation sites within a vacuole would also give rise to larger, irregular-shaped protein bodies, our results do not support such a mechanism. Very small protein inclusions indicative of a nucleation event were never seen in the hundreds of cells examined. The smallest inclusions were at least the size of those associated within Golgi apparatus.

Complex membrane networks were generally found to be present in the vicinity of larger protein bodies and were most elaborate along presumed fusion areas. The features of these membranes and their formation have been detailed by others (Parker 1980; Bechtel et al. 1982) and so were not a major concern of our studies.

As noted in *Results*, in many cases vesicles containing protein inclusions surrounded by an electron-lucent space had flattened cisternae or tubules

that were continuous with the vesicle membrane. These were similar in size to cisternae of the Golgi apparatus, and it is suggested that such vesicles may have arisen by dilation of individual Golgi cisternae. Such dilation was evident at one face of many Golgi complexes, and intermediate stages in cisternal dilation were also observed. We do not feel this is an artifact since the Golgi of adjacent cells (aleurone) never show this phenomenon. We suggest that these vesicles may fuse with smaller vesicles containing protein, such as is indicated in Fig. 7, to produce a vesicle containing a protein inclusion surrounded by an electron-lucent space. This would seem to be another variation in the mechanism of protein-body growth or enlargement.

Distinct morphological and electron-density differences, that would indicate different classes of protein bodies, were not evident in wheat endosperm. All protein bodies at each stage examined were reactive to gliadin antibody. However, as protein bodies enlarged, unreactive spherical inclusions become more common at the periphery of the gliadin-reactive protein mass. Similar inclusions have been previously reported by several authors (Buttrose 1963; Barlow et al. 1974; Parker 1980; Campbell et al. 1981). Parker (1980) presented evidence to indicate that these electron-opaque protein-body inclusions of wheat are in part composed of lipoprotein. Simmonds (1972), who noted similar inclusions in mature wheat endosperm, concluded that these structures contained glutenins. Our preliminary immunocytochemical study using antibody to the acidic subunit of rice glutelin which cross-reacts specifically to the low- and high-molecular-weight wheat glutenin (Okita et al. 1988) failed to show cross-reactivity between this antibody and the electron-opaque inclusions, indicating that they do not contain glutenins.

This work was supported in part by National Science Foundation, grant DCB-8502244 and DCB-8702182, and Project 0590, College of Agriculture and Home Economics Research Center, Washington State University. The authors express their gratitude to Professor Maarten Chrispeels (University of California/San Diego, La Jolla, USA) for his valuable suggestions to this manuscript. Facilities used in this study were provided by the Washington State University Electron Microscopy Center.

## References

Barlow, K.K., Lee, J.W., Vesk, M. (1974) Morphological development of storage protein bodies in wheat. In: Mechanisms of regulations of plant growth, pp. 793–797. Bieleski, R.L., Ferguson, A.R., Cresswell, M.M. eds. Royal Society of New Zealand, Wellington

Bechtel, D.B., Barnett, B.D. (1986) A freeze-fracture study of storage protein accumulation in unfixed wheat starchy endosperm. Cereal Chem. **63**, 232–240

Bechtel, D.B., Gaines, R.L., Pomeranz, Y. (1982) Early stages in wheat endosperm formation and protein body formation. Ann. Bot. **50**, 507–518

Briarty, L.G., Hughes, C.E., Evers, A.D. (1979) The developing endosperm of wheat – a stereological analysis. Ann. Bot. **44**, 641–658

Burnett, W.N. (1981) Western Blotting: electrophoretic transfer of proteins from SDS-polyacrylamide gels to unmodifed nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal. Biochem. **112**, 195–203

Burr, B., Burr, F.A. (1976) Zein synthesis in maize endosperm by polyribosome attached to protein bodies. Proc. Natl. Acad. Sci. USA **73**, 515–519

Buttrose, M.S. (1963) Ultrastructure of the developing wheat endosperm. Aust. J. Biol. Sci. **16**, 305–317

Campbell, W.P., Lee, J.W., O'Brien, T.P., Smart, M.G. (1981) Endosperm morphology and protein body formation in developing wheat grain. Aust. J. Plant Physiol. **8**, 5–19

Chrispeels, M.J. (1985) The role of the Golgi apparatus in the transport and post-translational modification of vacuolar (protein body) proteins. In: Oxford Surv. Plant Molec. Cell Biol., vol. 2, pp. 43–68, Miflin, B.J., ed. Oxford University Press, Oxford, UK

Craig, S., Goodchild, D.J. (1984) Periodate acid treatment of sections permits on-grid immunogold localization of seed vicillin in ER and Golgi. Protoplasma **122**, 35–44

Greene, F.C. (1981) In vitro synthesis of wheat (*Triticum aestivum* L.) storage proteins. Plant Physiol. **68**, 778–783

Greenwood, J.S., Chrispeels, M.J. (1985) Immunocytochemical localization of phaseolin and phytohemagglutinin in the endoplasmic reticulum and Golgi complex of developing bean cotyledon. Planta **164**, 295–302

Khoo, V., Wolf, M.J. (1970) Origin and development of protein granules in maize endoaperm. Am. J. Bot. **57**, 1042–1050

Kreis, M., Shewry, P.R., Forde, B.G., Forde, J., Miflin, B.J. (1985) Structure and evolution of seed storage proteins and their genes with particular reference to those wheat, barley and rye. In: Oxford Surv. Plant Molec. Cell Biol., vol. 2, pp. 253–317, Miflin, B.J., ed. Oxford University Press, Oxford, UK

Krishnan, B.H., Franceschi, V.R., Okita, T.W. (1986) Immunochemical studies on the role of the Golgi complex in protein body formation in rice seeds. Planta **169**, 471–481

Larkins, B.A., Hurkman, W.J. (1978) Synthesis and deposition of protein bodies of maize endosperm. Plant Physiol. **62**, 256–263

Miflin, B.J., Burgess, S.R., Shewry, P.R. (1981) The development of protein bodies in the storage tissues of seeds: subcellular separation of homogenates of barley, maize and wheat endosperm and pea cotyledons. J. Exp. Bot. **32**, 199–219

Miflin, B.J., Field, J.M., Shewry, P.R. (1983) Cereal storage proteins and their effect on technological properties. In: Seed proteins, pp. 255–319, Daussant, J., Mosse, J., Vaughan, J., eds. Academic Press, London

Okita, T.W., Krishnan, H.B., Kim, T.W. (1988) Immunological relationships among the major seed proteins of cereals. Plant Sci., in press

Oparka, N., Harris, N. (1982) Rice protein body formation: all types are initiated by dilation of the endoplasmic reticulum. Planta **154**, 184–188

Parker, M.L. (1980) Protein body inclusions in developing wheat endosperm. Ann. bot. **46**, 29–36

Parker, M.L., Hawes, C.P. (1982) The Golgi apparatus in developing endosperm of wheat (*Triticum aestivum* L.). Planta **154**, 277–283

Payne, P.I., Holt, L.M., Burgess, S.R., Shewry, P.R. (1986) Characterization by two-dimensional gel electrophoresis of the protein components of protein bodies, isolated from the developing endosperm of wheat (*Triticum aestivum*). J. Cereal Sci. **4**, 217–223

Reeves, C.D., Krishnan, B.H., Okita, T.W. (1986) Gene expression in developing wheat endosperm: accumulation of gliadin and ADPglucose pyrophosphorylase messenger RNAs and polypeptides. Plant Physiol. **82**, 34–40

Simmonds, D.H. (1972) Wheat grain morphology and its relationship to dough structure. Cereal Chem. **49**, 324–335

Spurr, A.P. (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. **26**, 31–43

Taylor, J.R.N., Schussler, L., Liebonberg, N. v. d. W. (1985) Protein body formation in the starch endosperm of developing *Sorghum bicolor* (L.) Moench seeds. S. Afr. J. Bot. **51**, 35–40

Tkachuk, R., Metlish, V.J. (1980) Wheat cultivar identification by high voltage gel electrophoresis. Can. Technol. Agric. **29**, 207–212

Wen, T.-N., Luthe, D.S. (1985) Biochemical characterization of rice glutelin. Plant Physiol. **78**, 172–177

Zhao, W.-M., Gatehouse, J.A., Boulter, D. (1983) The purification and partial amino acid sequence of a polypeptide from the glutelin fraction of rice grains: homology to pea legumin. FEBS Lett. **162**, 92–102

Received 28 March; accepted 20 June 1988