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PROTEIN BODIES OF SEEDS: ULTRASTRUCTURE, BIOCHEMISTRY, BIOSYNTHESIS AND DEGRADATION

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Abstract—Typical organelles for protein storage occur in seeds, protein bodies are found in haploid, diploid or triploid tissues and are single membrane bound. In some plants, they exhibit inclusions (globoid and crystalloid), but not in Gramineae endosperm or in Leguminosae cotyledons. A relationship between species and protein body ultrastructure can be put forward. The chemical composition is based mainly on storage proteins and phytic acid but, hydrolytic enzymes (protease and phytase), cations and ribonucleic acids are also present. Other minor biochemical components include oxalic acid, carbohydrates (excluding starch) and lipids. The locations of the storage proteins, enzymes and phytin are described. Protein body ontogeny during seed maturation has given rise to much controversy: are they plastidic or vacuolar? Recent studies on the location of proteosynthesis show that protein bodies are probably synthesized in endoplasmic reticulum lumen and that the Golgi apparatus plays an important role in storage protein synthesis. During germination protein bodies swell and fuse, giving rise to the cell central vacuole, while the integrity of the membrane is maintained. Protein bodies may be considered as being an example of tonoplast origin from endoplasmic reticulum.

INTRODUCTION

Cellular organelles containing storage proteins are found in storage tissue, within the animal as well as the vegetable kingdom, particularly in reproductive organs. These organelles are now usually called protein bodies. They are found in haploid, diploid and triploid tissues [1] not only in angiosperm seeds, but also among gymnosperms [2–4] and in animal oocytes [5, 6]. This review deals with angiosperm protein bodies which occur in seeds. The main storage tissue of monocots is a triploid tissue (the endosperm) which is constituted of the starchy endosperm and the aleurone layers (outer layers). Both these tissues contain protein bodies which present some differences which can be correlated with the difference in function. The starchy endosperm is merely a storage tissue while the aleurone layer has an important role in enzyme secretion during germination processes. In dicot seeds there are usually no special storage tissues and the protein bodies are located within the cotyledons which are a part of the embryo. In monocots also, protein bodies occur in the embryo, but as their numbers are small compared to those in endosperm, we shall deal only with protein bodies in the latter.

Protein bodies were first demonstrated in 1856 by Hartig [7] who named these organelles 'aleurone grains' after the Greek word for flour. This author carried out the first isolation of protein bodies from oilseeds. In 1872, Pfeffer [8] confirmed the protein nature of these organelles and showed the presence of internal inclusions within protein bodies, the globoid and the crystalloid. The presence of phosphorus within protein bodies was reported in 1905 by Posternak [9], working on seeds of fir, sunflower, legumes and cereals.

Meanwhile, several authors [10, 11] had shown the great quantity of protein bodies (or aleurone grains) in the aleurone cells of numerous Gramineae. In 1908, Guilliermond and Beauverie published important data on 'aleurone grain' cytology [12], particularly about the inclusions, as did Dangeard in 1921 [13]. All these authors examined the origin and degradation of protein bodies during seed maturation and germination [14–17]. Further progress came with the development of new methods for studying protein bodies: electron microscopy and isolation methods (ultracentrifugation) which permitted biochemical investigation [18–20]. The more recent work has been reviewed by Miege [21] and Ashton [22].

In the literature, many terms are used to designate protein bodies such as aleurone grains, aleurone vacuole, protein grain, protein vacuole, proteoplast. In our view, 'protein body' must be preferred because this term is very general and does not imply a precise location or a specific origin of the storage material under consideration.

The modern methods used in protein body studies are either based on microscopy (EM, freeze etching, SEM and light microscopy) which permit *in situ* study, or the classical biochemical methods used for isolation of protein bodies. Isolation is carried out either with aqueous solvents (cereals and legumes) or with non aqueous solvents (oilseeds). There is also differential centrifugation or density gradient centrifugation. The

latter method is generally the one which gives the best results, since protein bodies are characterized by a specific density of 1.20–1.30 g/cm^3 [23]. Microscopic studies need a fixation step, which has been studied specifically for seeds [24].

Protein body ultrastructure

Protein bodies are small, more or less spherical organelles. They vary in size from one plant to another and from one tissue to another. Diameters have been reported from 0.1 to 25 μm, the average value being a few microns. Protein bodies are bound by a membrane which seems to be single in most cases. Some authors have described a double membrane indicating a plastid origin for protein bodies. This point will be discussed later in the section on ontogeny of protein bodies.

According to their specific or histological origin, one can distinguish various internal structures of protein bodies. Some (e.g. pea cotyledon, *Pisum sativum*) have a homogeneous or granular structure without inclusion embedded within a homogeneous proteinaceous matrix [25], while others (e.g. rice endosperm, *Oryza sativa*) show a granular concentric pattern of electron density with minute granules 150 Å in diameter [26]. Other protein bodies exhibit internal inclusion which can be of two types; the globoid and the crystalloid as in castor bean (*Ricinus communis*) endosperm [27]. As we shall see, the globoid is a phytic acid storage inclusion, while the crystalloid is proteinaceous. All authors are agreed about the lack of membrane bounding the crystalloid, which appears to be an ordered partly crystalline protein deposit [28]. On the other hand, the boundary of the globoid is still the subject of dispute. Some authors claim there is evidence of a membrane surrounding the globoid in the case of flax (*Linum usitatissimum*) [29], cucumber (*Cucumis sativus*) [29], mustard [30], castor bean (*Ricinus communis*) [31] and wheat (*Triticum aestivum*) scutellum [32]. Other authors show a boundary which is not typical for biological membrane, for the protein bodies of aleurone cells of rice [33], the aleurone cells of barley (*Hordeum vulgare*) [34], squash seeds [35], castor seeds [27] and cotton (*Gossypium hirsutum*) seeds [36]. Finally, a group of authors [37–39] claim there is no evidence for any membrane like boundary at the interface between the globoid and the proteinaceous matrix, in Yucca seed protein body, in cotton seed and in squash (*Cucurbita maxima*) cotyledon. Fig. 1 illustrates the structure of the typical protein body with two types of inclusions.

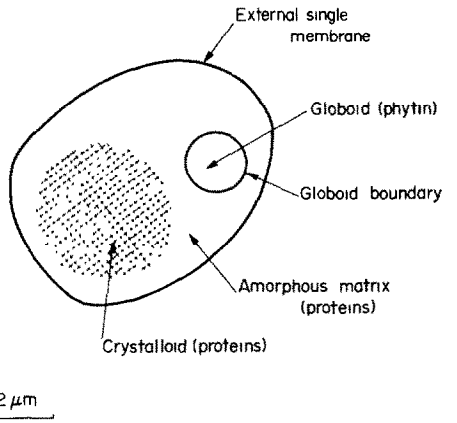

Fig. 1. Schematic view of a typical protein body, as exemplified by a complete protein body with two kinds of inclusions as in castor bean endosperm (from ref. [27]).

In contrast to Rost [40] who failed to compare protein bodies from various species, most authors agree that there is a relationship between the presence of inclusion and the species, when studying protein bodies from homologous tissues. Table 1 shows that legume species lack an inclusion, except for *Arachis hypogaea* which exhibits two types of protein bodies, some with one or several globoids and some which are homogeneous. All other angiosperm seeds studied possess at least a globoid and often the second type of inclusion, i.e. a crystalloid. This proteinaceous inclusion is only present when a

Table 1. Protein body structure in angiosperm seeds

	Species	References	Protein body diameter (μm)	Inclusions
Leguminosae	Pea (*Pisum*)	[19, 25, 41, 42]	1–3	No Inclusion
	Mung bean (*Phaseolus aureus*	[43], [44]	1–2	
	French bean (*Phaseolus vulgaris*)	[45], [46]	2–22	
	Lima bean (*Phaseolus lunatus*)	[47]	—	
	Kidney bean (*Lablab purpureus*)	[45]	1–5	
	Broad bean (*Vicia faba*)	[48–51]	1–5	
	Lupin (*Lupinus luteus*)	[52]	—	
	Soybean (*Glycine max*)	[53–58]	2–8	
	Peanut (*Arachis hypogaea*)	[59–61]	1–10	
Other Dicotyledons	Mustard (*Sinapi alba*)	[30, 52, 62–64]	1–10	Globoid
	Rapeseed (*Brassica campestris*)	[65]	2–4	
	Cotton (*Gossypium hirsutum*)	[36], [38]	4–6	
	Flax (*Linum usitatissimum*)	[30], [29]	5–10	Globoid and Crystalloid
	Castor bean (*Ricinus communis*)	[13, 27, 31, 52, 66–68]	5–12	
	Squash (*Cucurbita maxima*)	[35], [39, 69], [70]	2–6	
	Cucumber (*Cucumis sativus*)	[29]	5–10	
	Hempseed (*Cannabis sativa*)	[28]	1–4	
Monocotyledons	Yucca (*Species*)	[37, 71]	4–6	
	Gramineae Aleurone layer	See Table 2	1–5	
	Endosperm		1–20	No Inclusion

Table 2. Gramineae protein bodies: ultrastructural differences between aleurone layer and starchy endosperm protein bodies

Species	Aleurone layers: Diameter (μm)	Aleurone layers: Structure and references	Endosperm: Diameter (μm)	Endosperm: Structure and references
Barley	2–3	Two kinds of inclusions [72]	2	No inclusion. Lamellar structure [77]
	4–5	One globoid and one crystalloid [73]	1–2	No inclusion. Lamellar structure [78]
	2	One globoid and one crystalloid [34]	1–2	No inclusion [79]
	3–4	One globoid and one crystalloid [74]		
	3–5	Globoid and internal cavity [75]		
		Globoid and internal cavity [76]		
Wheat	2–3	Two kinds of inclusions [80–82]	0.1–8	No inclusion. Granular structure [80]
	2–4		2–3	No inclusion. Homogeneous [20]
	2–3		1–5	No inclusion. Homogeneous [83]
			1–10	No inclusion, but electron dense areas [84, 85]
			2.5–5	[79]
Rice	1.5–4	Globoid [86]	2–5	No inclusion. Homogeneous [88]
	1–3	Globoid [87]	2–5	No inclusion. Homogeneous [89]
			1.5–4	Concentric lamellar structure [26]
				Concentric lamellar structure [90]
Maize			1–2	No inclusion. Homogeneous [91], [92], [93], [94, 95], [79]
Sorghum			0.5–3.5	No inclusion. Concentric lamellar structure [79]
Millet			1–2	
Babala			1.5–4	
Proso millet			2.5	Central core and lamellar structure [96]

globoid is seen within a protein body. In case of Gramineae seeds, it is now clear that two different kinds of protein body exist: in the aleurone cells these organelles have inclusions, while in the starchy endosperm no such inclusions can be seen (Table 2). In this latter tissue several authors have found a granular concentric structure in case of barley, wheat, rice, sorghum and various millets. Only maize exhibits a homogeneous protein matrix. This difference between aleurone cells and endosperm protein bodies is now quite evident and in this particular case there is good reason to call the protein bodies of the aleurone cells 'aleurone grains' when comparing them with those of the starchy endosperm. As we shall see later, the protein composition of these two types of protein bodies is also different. It is evident that these differences can be related to the functional differences of these two tissues; endosperm is a storage tissue, while aleurone cells contribute to the germination processes by secreting numerous enzymes.

Biochemical composition of protein bodies: location and function

Many authors have been able to purify protein bodies after their separation from the other cell organelles. This approach has provided more precise biochemical data than can be obtained by cytochemistry which is nevertheless an essential approach for locating the biochemical compounds within protein bodies. There are numerous differences in biochemical composition between protein bodies from various species. On average, however, protein bodies are composed of storage proteins (*ca* 70–80 % of dry wt), salts of phytic acid (*ca* 10 % of dry wt), hydrolytic enzymes, cations and ribonucleic acids. Less important and more variable is the presence of such compounds as carbohydrates, oxalic acid salts, lipids and tocopherol.

Storage proteins. In dicotyledon seeds, storage proteins are located within protein bodies (see Table 3). Most authors have separated protein bodies while some like Graham and Gunning [98] have located globulins (legumin and vicilin) within protein bodies by immunological procedures. It is worthy of note that, when a crystalloid exists, it is a storage protein deposit [27, 28, 67, 68] and that a globoid is not a proteinaceous inclusion [38]. In monocotyledon seeds it is important to distinguish between aleurone layer protein bodies which contain few or no prolamines (storage protein) and less protein than endosperm protein bodies [61, 86, 101]. Table 4 summarizes literature data on cereal endosperm protein bodies, which consist essentially of prolamines.

Phytic acid. As myoinositol hexaphosphate (phytic acid) discovered by Posternak [9], is a chelating reagent for cations it is a form of cation storage as well as phosphorus storage. Some authors [66, 106, 107] have suggested that phytic acid could represent energy storage, since ADP or GDP could be phosphorylated into ATP or GTP using phytic acid through a phosphoinositol phosphotransferase catalysed reaction. This theory has been ruled out for various reasons. Raison and Evans [108] demonstrated on a thermodynamic basis that inositol hexaphosphate does not possess a phosphate group of sufficient high energy to be a precursor of nucleoside triphosphate. Also Mayer [109] could not detect any transphosphorylation reaction in extracts of pea and lettuce; the same conclusion was reached by Williams [142] for the wheat grain.

Phytin is principally located in globoids, when these inclusions are present in protein bodies. Its proportion can reach 60–80 % of the dry wt of globoids. That phytin is located within the globoid has been demonstrated for a range of plants: *Cucurbita maxima* [110], *Gossypium hirsutum* [38], *Linum usitatissimum* [29], *Cucumis sativus* [29], *Arachis hypogaea* [61], *Ricinus communis* [31, 52, 111]. In cases of protein bodies from Gramineae aleurone layers, phytin is also located in the globoid: in *Hordeum*

Table 3. Protein body proteins in dicotyledon seeds

Species	Organelle protein concentration*	Main proteins	Location	References
Broad bean (*Vicia faba*)	88%	Vicilin-Legumin (*Globulins*)		[97]
	—	Vicilin-Legumin		[98]
Kidney bean (*Lablab purpureus*)	—	Globulins		[99]
Pea (*Pisum sativum*)	—	Globulins	determined in whole	[19]
Soybean (*Glycine max*)	83%	Glycinin (*Globulin*)	protein body	[55]
	63%			[54]
	77%			[100]
Peanut (*Arachis hypogaea*)	60–80%	Arachin (*Globulin*)		[55]
	69%			[61]
Mustard (*Sinapis alba*)	93%	Globulins		[62]
Cotton (*Gossypium hirsutum*)	60%		Protein body	[38]
	4.4%		Globoid	
Hempseed (*Cannabis sativa*)	—	Edestin (*Globulins*)	Crystalloid	[28]
Castor bean (*Ricinus communis*)	4.8%		Globoid	[31]
	—	Globulins	Crystalloid	[67]
	—	Albumins	Amorphous matrix	[27]
	—	Globulins	Crystalloid	[68]

* Percentage on organelle (protein body or inclusions), dry wt basis.

vulgare [10, 74], in *Oryza sativa* [33, 86] and in *Sorghum bicolor* [101]. Since there is no globoid in the protein bodies of the endosperm of grass seeds, it is not clear where the phytin is deposited. In developing wheat endosperm Morton and Raison [106] have found areas of higher electron density within protein bodies, while Tronier *et al.* [78] and Ory and Henningsen [77] have considered that the lamellar concentric structure of barley protein bodies is due to alternating deposits of protein and phytin. This hypothesis has not still been supported by experimental evidence. In Leguminosae, there appears to be no definite location of phytin, which could be because of the existence of a phytin–protein complex. Phytin synthesis during seed maturation has been shown to take place within the protein body, and to proceed by myoinositol phosphorylation [111].

Enzymes. Germination implies hydrolysis of storage material which proceeds by the action of many enzymes, several of which are associated with protein bodies. Insoluble storage proteins are hydrolysed by endopeptidases into soluble peptides which are later hydrolysed to amino acids by peptidases [22]. At least one acid endopeptidase is usually associated with protein bodies (Table 5). Proteolytic activity sometimes occurs once germination has begun [116]. These authors [116] have shown that the induction of proteolytic activity in mung bean protein bodies implies a *de novo* proteosynthesis while others [115] have suggested that proteolytic enzymes are present but inactive in sorghum protein bodies before germination begins.

Phytase (acid phosphatase) occurs widely within protein bodies (see Table 5). Acid phosphatase surround the globoid, creating an interface between phytin and the proteinaceous matrix [29]. This boundary could be seen as a membrane, which would explain the controversy about its existence. Some carbohydrate hydrolases have been located within protein bodies: α- and β-glucosidases, α-mannoside, N-acetyl-β-glucosaminidase [44, 115, 117]. In barley aleurone layer, Jacobsen and Knox [121] have shown evidence for an α-amylase bound to the external membrane of protein bodies, while Gibson and Paleg [112] have found the same enzyme bound to wheat aleurone layer protein bodies. Ribonuclease is sometimes associated with various protein bodies; this

Table 4. Protein body storage proteins in Gramineae endosperm

Species	Major storage proteins	Total proteins percentage on protein body dry wt basis	References
Barley	Hordeins (*Prolamines*)	70%	[78]
		54%	[79]
Wheat	Gliadins (*Prolamines*)	72%	[102]
		72%*	[84]
		51%	[79]
Maize	Zeins (*Prolamines*)	79%	[79]
		>75%	[91]
		—	[94]
		—	[93]
Rice	Oryzenins (*Glutelins*)	82%	[103]
		58% (41%: Oryzenins)	[90]
Sorghum	Kafrin (*Prolamines*)	69%	[101]
		—	[104]
		—	[105]

* On carbohydrate free basis.

Table 5. Acid proteases and phosphatases associated with seed protein bodies

Species	Proteases	Phosphatases
GRAMINEAE		
Wheat	Protease [79] [112]	None [79]
Barley	Acid protease [77–79]	Acid phosphatase [77]
	Carboxypeptidase, aminopeptidase [113]	Phytase [78] [79]
Maize	Protease [79]	Phytase [79]
Rice		Phytase [114]
Sorghum	Protease [79, 115]	Phytase [79, 115]
LEGUMINOSAE		
Mung bean	Vicilin peptidophydrolase [116]	
	Carboxypeptidase, protease [44]	
Pea	Protease [117]	Phosphatase [117]
Broad bean	Protease [97]	Phosphatase [97]
OTHER DICOTS		
Sunflower	Protease [118]	
Cotton	Acid protease [119]	Acid phosphatase [119]
Flax		Acid phosphatase [29]
Cucumber		
Hemp	Protease [28]	
Vetch	Protease [120]	

has been shown for sorghum [115], wheat endosperm [85] and pea cotyledons [117].

As suggested by Bain and Mercer [41], the location of hydrolases within protein bodies protects the rest of the cell from their activity during germination. Some authors [e.g. 117] have compared protein bodies to animal lysosomes but, as the enzymes react inside protein bodies, this comparison is not valid [115].

Inorganic elements. Using chemical analysis after protein body isolation or dispersive X-ray analysis associated with scanning electron microscopy, many authors have shown that some cations are present within protein bodies as complexes with phytic acid. These cations are Ca^{2+}, Mg^{2+}, K^+ [29, 33, 110, 122]. Other ions are associated with protein bodies but not bound to phytic acid: Mn^{2+} [61, 86] which is linked to storage proteins [123] as seems to be the case for chloride and sulphur [110]. Na^+ has been detected in castor bean, mustard and lupin protein bodies but in very low amounts [52]. Fe^{3+}, Zn^{2+} and Cu^{2+} are also present in rice [86] and other plants [61, 101]. Protein bodies are therefore not only protein and phosphorus storage organelles but also provide a store of cations.

Minor constituents. Small amounts of carbohydrates of variable composition are found in protein bodies but are generally absent from the globoids. According to Jacobsen *et al.* [74], they may be associated with some storage proteins, in the form of glycoproteins [22]. Starch is never associated with protein bodies except by contamination. Lipids are sometimes detected in significant amounts within protein bodies: in wheat endosperm protein bodies their percentage is as little as 1 % dry wt [84] but it is ten fold greater in rice endosperm [26, 90, 103] and in soybean protein bodies [55]. Komoda *et al.* [124] pointed out that tocopherol is present in soybean protein bodies. Oxalic acid is not frequently found within protein bodies: Nougarede [125] and Prokof'ev *et al.* [66] have reported its presence in Umbelliferae protein bodies and Sharma and Dieckert [61] in peanut (*Arachis hypogaea*) globoids.

Ribonucleic acids. As RNA is a plastid component, its presence within protein bodies would support a plastid origin for them. As Table 6 shows, there is no doubt that ribonucleic acid is associated with protein bodies. In the case of broad bean (*Vicia faba*) and barley, the results of Prokof'ev *et al.* [66] explain the absence of RNA. These authors clearly show that the percentage of RNA increases during seed maturation, reaches a maximum when protein synthesis is also maximum, and decreases to negligible amounts at maturity. This result also explains the high amounts of RNA found in developing wheat endosperm protein bodies (85, 106, 126]. The association of RNA with protein bodies is supported by the concomitant presence of ribonuclease. Morton and Raison [126] showed that RNA isolated from developing wheat endosperm is functional Barlow *et al.* [127] obtained the same results with wheat protein bodies, Yokoyama *et al.* [58, 100] for soybean protein bodies and Larkins and Dalby [128] for maize endosperm protein bodies. As we shall see later, ribonucleic acids are associated with ribosomes strongly bound to protein bodies.

Origin and degradation of protein bodies

Ontogeny during seed maturation. The origin of protein bodies has been a controversial subject for as long as a century. Are storage proteins synthesized in the cytoplasm and then transported into protein bodies which would be then considered as vacuoles or are they synthesized within the storage organelles which would then be considered as plastids? The general view of a single membrane bounding the protein body would support the first hypothesis, while the presence of functional ribonucleic acids associated with them would support the second.

Light microscopy was not adequate for analysing protein body ontogeny so that older authors [8–13, 15, 16] concluded that protein bodies were vacuoles in which storage proteins precipitated, following the loss of water during seed maturation. The vacuolar origin was supported by the discovery of oxalic acid within protein bodies [11, 129] but some authors disagreed with this hypothesis. As early as 1921 Mottier [130] thought that proteosynthesis took place within plastids, while Rendle [131] and Wieler [132] conceived it as

Table 6. Ribonucleic acids associated with seed protein bodies

Species	Amount of RNA (on a protein body dry wt basis)	References
Peanut	0.29%	[60]
Castor bean	Decreases during maturation	[66]
	1.6–0.1 % in globoid	[31]
Soybean	1.2%	[55]
	0.51%	[58]
	0.5%	[54]
Maize	—	[93]
Wheat	10.4% (endosperm)	[85, 106, 126]
Rice	— (endosperm)	[26]
	0.02% (endosperm)	[90]
	0.23% (aleurone layer)	[87]
Broad bean	no RNA found	[97]
Barley	no RNA found	[77]

cytoplasmic. The vacuolar hypothesis was largely supported by the early electron microscope investigations which showed a single membrane around protein bodies. Today, the simplistic view of protein bodies as dehydrated vacuoles must be discarded. As a matter of fact, several authors have shown that the storage of proteins takes place prior to dehydration of the seed [30, 49, 133].

Using electron microscopy, Briarty and Boulter [49] obtained evidence that proteins were synthesized in the endoplasmic reticulum or the Golgi apparatus but were unable [133, 134] to demonstrate a relationship between these structures and the vacuoles. On the other hand, Buttrose [80] showed that wheat aleurone layer protein bodies develop close to the Golgi apparatus. In cotton seed embryo, Engleman [135] found a relationship between protein bodies and cytoplasmic proteosynthesizing structures, while Klein and Pollock [47] produced evidence of a relationship between the lamellar endoplasmic reticulum and protein bodies of lima bean cotyledons. Bailey *et al.* [48] showed in broad bean, by autoradiographic studies, that proteosynthesis was associated with endoplasmic reticulum and that the storage proteins were quickly transported within protein bodies. Bain and Mercer [41] have also shown in the pea that storage proteins are synthesized by the endoplasmic reticulum surrounding vacuoles, in which they accumulate along the external single membrane. Righetti *et al.* [136] describe an analogous scheme for maize endosperm protein body ontogeny. In protein bodies with globoids, the early storage protein deposits are located around this inclusion which is formed first. Nevertheless proteosynthesis may be associated with the endoplasmic reticulum or the Golgi apparatus in mustard, flax, castor and cotton [30, 31, 63, 67, 137].

Another group of authors have put forward the idea that proteosynthesis is not associated with cytoplasmic structures but takes place within protein bodies. As early as 1962, Morton and coworkers demonstrated proteosynthesis associated with developing wheat endosperm protein bodies. They gave the name 'proteoplasts' to protein bodies exhibiting a double membrane [20]. Morton and Raison [106] proposed that protein bodies were formed within a structure called the 'protein-forming plastid'. Morton and Raison [126] and Morton *et al.* [85] showed that isolated protein bodies are able to synthesize storage protein *in vitro* and that there are two different proteosynthetic systems, one for storage proteins and one for cytoplasmic proteins. Finally, these authors showed that there are specific ribosomes strongly bound to protein bodies [84].

This new concept has been criticized. Wilson [138] could not reproduce Morton's experiments with maize endosperm and claimed that the results were due to bacterial contamination. By contrast, Barlow *et al.* [127] succeeded in reproducing Morton's results and Larkins and Dalby [128] obtained *in vitro* zein synthesis with isolated protein bodies from maize endosperm. Burr and Burr [93] showed clearly by electron microscopy that, in maize endosperm, ribosomes are bound externally to the single membranes of protein bodies. As they are isolated with the protein bodies. storage proteins can be synthesized *in vitro* using protein body isolates. These ideas have been supported by Khoo and Wolf [92] and Righetti *et al.* [136]. Isolation and characterization of ribonucleic acids from protein bodies are in fairly good agreement with this concept [58, 87, 100].

In our present state of knowledge, it is reasonable to conclude that proteosynthesis does not take place within protein bodies. It is not clear if protein bodies are plastids or if they are formed from the endoplasmic reticulum. Studies by Hoshikawa [89] and by Harris and Juliano [88] on rice endosperm make the second hypothesis more probable. They showed that the protein body double membrane might be an artifact. According to them, protein bodies develop within an endoplasmic reticulum lumen and so are surrounded by ER membranes. If this hypothesis is true, the membrane of protein bodies would be initially an ER membrane which would explain the protein body surrounding ribosomes. Recent work on protein body development has indicated an important role for the Golgi apparatus in protein synthesis (D. Boulter, personal communication). According to Miege [21], there could be three kinds of protein bodies differing in their origin. There is no convincing evidence for this proposal and it is now more likely that protein bodies lack a vacuolar or plastid origin. Seed protein bodies are quite different from proteoplasts in orchid roots [139] or in bean root tips [140]: these organelles exhibit a typical structure with double membrane, starchy inclusions and internal ribosomes. Seed protein bodies can no longer be compared with oocyte protein bodies, which are not synthesized within the cell where they are stored, since they are transported into the oocyte by a process akin to pinocytocis [5].

Degradation during seed germination. As early as 1908, Guilliermond [12] postulated that, during germination, protein bodies give rise to the central vacuole. In 1922, Dangeard [14] confirmed this hypothesis. Since that time numerous authors have studies the fate of protein bodies during germination. The later results have been reviewed by Ashton [22]. Briefly, after swelling of the protein body, autolysis of storage proteins and phytin begins while the external membrane remains unbroken. Protein bodies become little vacuoles which fuse to give the central vacuoles. Hydrolysis of proteins can be internal or peripheral and fusion may begin before complete protein degradation. In all cases the result of degradation is the same: the central vacuole of the cell.

CONCLUSION

Seed storage tissue protein bodies exhibit various ultrastructural patterns according to their origin. In Leguminosae cotyledons and in Gramineae endosperm they have no typical inclusions while in other plants or tissues they show two types of inclusions, the globoid and the crystalloid. The membrane surrounding the protein body is single. Though some authors have shown a double membrane, it is now clear that this is probably artefactual. No membrane is found around the crystalloid and no typical membrane occurs around the globoid, which is separated from the proteinaceous matrix by an enzymatic interface.

Storage proteins are located within the proteinaceous matrix in protein bodies without inclusions. When a crystalloid exists within protein bodies, it seems to be the globulin location, while the amorphous matrix may be the albumin location. Phytic acid and most of the cations are concentrated within globoids or are bound to proteins in homogeneous protein bodies. Several hydrolytic enzymes are enclosed within protein bodies whose

function is to degrade storage compounds (proteins and phytin) during germination without reacting with the rest of the cell. The protein bodies have autolytic properties which are nevertheless quite different from lysosomal properties, since protein body hydrolases do not react externally with cytoplasmic compounds during germination. During germination, the protein body gives rise to the cell central vacuole, the membrane retaining its integrity. The mode of activation of the internal hydrolase is still uncertain and its study would be of interest in order to understand germination processes.

Protein body ontogeny has become clearer during the last few years. It is now more and more evident that protein bodies are neither dehydrated vacuoles nor plastids. It is probable that they develop in the endoplasmic reticulum lumen, the Golgi apparatus playing an important role in their ontogeny. Proteosynthesis is external to protein bodies but some of the ribosomes synthesis using their external ribosomes independently brane with great affinity. It is not unlikely that, following synthesis, protein bodies could proceed with proteosynthesis using their external ribosomes independently from endoplasmic reticulum, as *in vitro* experiments suggest. The processes in protein body development and degradation fit in with the hypothesis of an origin from Golgi apparatus and endoplasmic reticulum [141].

Surprisingly in Gramineae seeds, the precise locations of the numerous fractions of storage proteins (prolamines and glutelins) are quite unknown. It would be interesting to know whether all protein bodies in the same tissue contain all the storage proteins or whether these proteins are differentially distributed among these organelles. It is essential to have a thorough knowledge of the distribution of storage protein fractions (inside or outside protein bodies) in order to obtain a more precise definition of these molecules. It would be worth knowing if lamellar concentric structures in Gramineae endosperm protein bodies are due to alternation of different proteins or to phytin–protein alternation.

Finally, it is clear that seed protein bodies are not comparable with real proteoplasts or with oocyte protein bodies. They have a peculiar biology which make them quite different from other cell protein organelles.

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