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Seed oil bodies in maize and other species[1]

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Contents

Introduction

Most seeds contain storage oils in the form of triacylglycerols (TAG), which comprise 5-50% of the seed's total dry weight. The TAG are packed in subcellular organelles called oil bodies (lipid bodies, oleosomes, spherosomes). The spherical oil body is about 0.6-2.5 μm in diameter. It is surrounded by a "half-unit" membrane of one layer of phospholipids (PL) about 3 nm thick (Yatsu and Jacks, 1972) embedded with proteins. The acyl moieties of the membrane PL are believed to orient themselves toward the matrix such that they have hydrophobic interactions with the internal TAG. In mature seeds, oil bodies are stable, and they do not coalesce, even after prolonged storage.

Oil bodies are synthesized during seed maturation. They are degraded during seed germination, and the TAG are converted to sugars to support the growth of the seedling.

In this article, we present our findings on the structure and ontogeny of seed oil bodies in maize and other species. Where appropriate, the work of other laboratories is also described.

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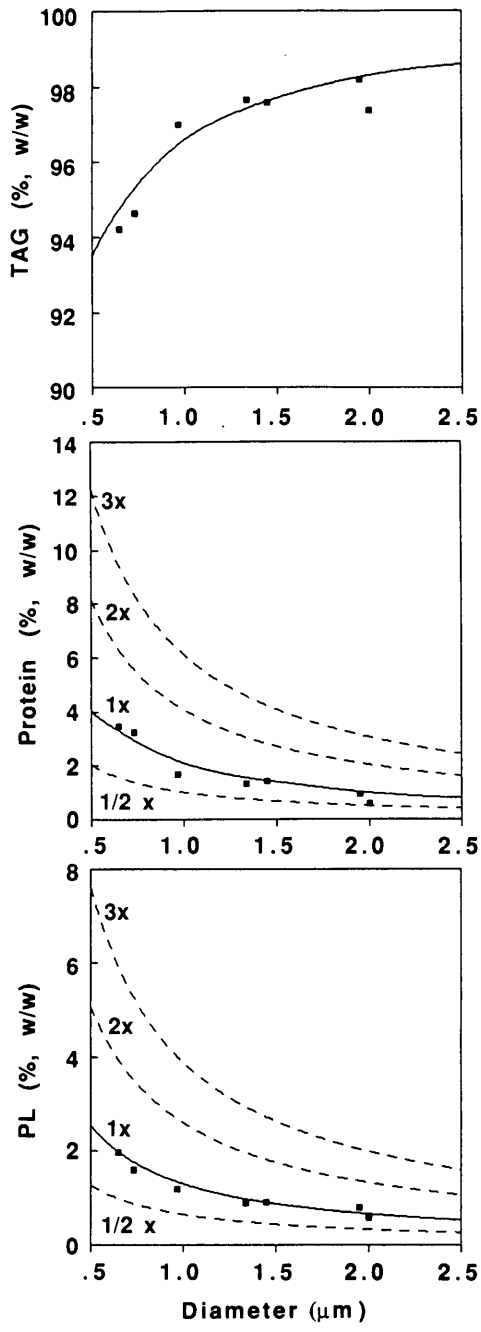

The Percent Contents of the Constituents of Isolated Oil Bodies are Correlated to the Organelle Size

In maize kernel, TAG are stored in the scutellum, the embryonic axis, and the aleurone layer. The oil bodies inside these three parts of the kernel have no appreciable differences in their structure, although they vary slightly in size. Inside the cells, oil bodies are usually pressed into irregular shapes by other cellular components. Isolated oil bodies always assume a spherical shape. They have an average diameter of 1.45 μm, as measured from electron micrographs of serial sections of a preparation of isolated oil bodies (Tzen and Huang, 1992).

Oil bodies isolated from mature maize kernel contain 97% neutral lipids (mostly TAG and a few percent of diacylglycerols), 1.4% proteins, 0.9% PL, and 0.09% free fatty acids (FFA). These relative amounts of the various constituents correlate with the diameter of the organelles (to be described). Of the acyl moieties in the TAG, about 60% are linoleic acid and 30% are oleic acid. The PL components include 64% phosphatidylcholine, 20% phosphatidylserine, 8% phosphatidylethanolamine, and 7% phosphatidylinositol. More than 90% of the proteins are represented by three proteins of 16, 18, and 19 kD. These proteins possess special secondary structures and are unique to the oil bodies. They are termed oleosins.

These data on the oil bodies from maize have been extended to oil bodies from other plant species (Tzen *et al.*, 1993). The average diameter of the organelles varies from species to species, ranging from 0.65 to 2.0 μm. As in the maize organelle, the relative amounts of TAG, PL, and proteins in the oil bodies of diverse species correlate with the organelle's diameter (Fig. 1). Also, oil bodies from the various species contain substantial amounts of the negatively charged phosphatidylserine and phosphatidylinositol, as well as FFA. In all, oil bodies from diverse species are very

Fig. 1. A comparison between experimentally determined and theoretically calculated values of oil body contents in relation to the average diameters of the organelles from seeds of diverse species. The species and their seed oil body diameters are: *Brassica*, 0.65 μm; mustard, 0.73 μm; cotton, 0.97 μm; flax, 1.34 μm; maize, 1.45 μm; peanut, 1.95 μm; sesame, 2.00 μm. The closed squares represent the experimentally determined values from the seven species. The curves show the theoretical values of the percent contents of TAG, proteins, and PL calculated according to a formula derived from an oil body model. The model denotes a spherical TAG matrix surrounded by a shell of one PL layer. The layer contains 80% PL and 20% oleosins. In each oleosin molecule, about 20% of the amino acid residues are embedded in the PL layer, 30% are located in the TAG matrix, and 50% are exposed to the exterior. The theoretical values representing half, two, and three layers of PL plus proteins in an oil body are shown in broken lines (Tzen *et al.*, 1993).

similar in their chemical and structural properties.

The Surface Protein, Oleosin, Contains an N-Terminal Amphipathic Domain, a Central Hydrophobic Anti-Parallel β-Structure Domain, and a C-Terminal Amphipathic α-Helical Domain

Maize oil body proteins can be resolved by SDS-PAGE into three oleosins of molecular masses 16, 18, and 19 kD. The 16 and 18 kD oleosins have been sequenced via their cDNA and genomic DNA. Oleosins from many other plant species have also been studied. Similar to those in maize, they all have low molecular masses, ranging from 15 to 26 kD. About 10 of them have been sequenced via their genomic or cDNA clones (Huang, 1992).

Analyses of the amino acid sequences of the oleosins from diverse species show that they all possess the following three structural domains (Fig. 2):

(a) An amphipathic domain of 40-60 amino acids is present in the N-terminal region. The secondary structure of this domain is unknown. According to the distribution of hydrophilic and hydrophobic residues along the polypeptide, most of the domain is likely to be associated with the oil body surface.

(b) A totally hydrophobic domain of 68-74 amino

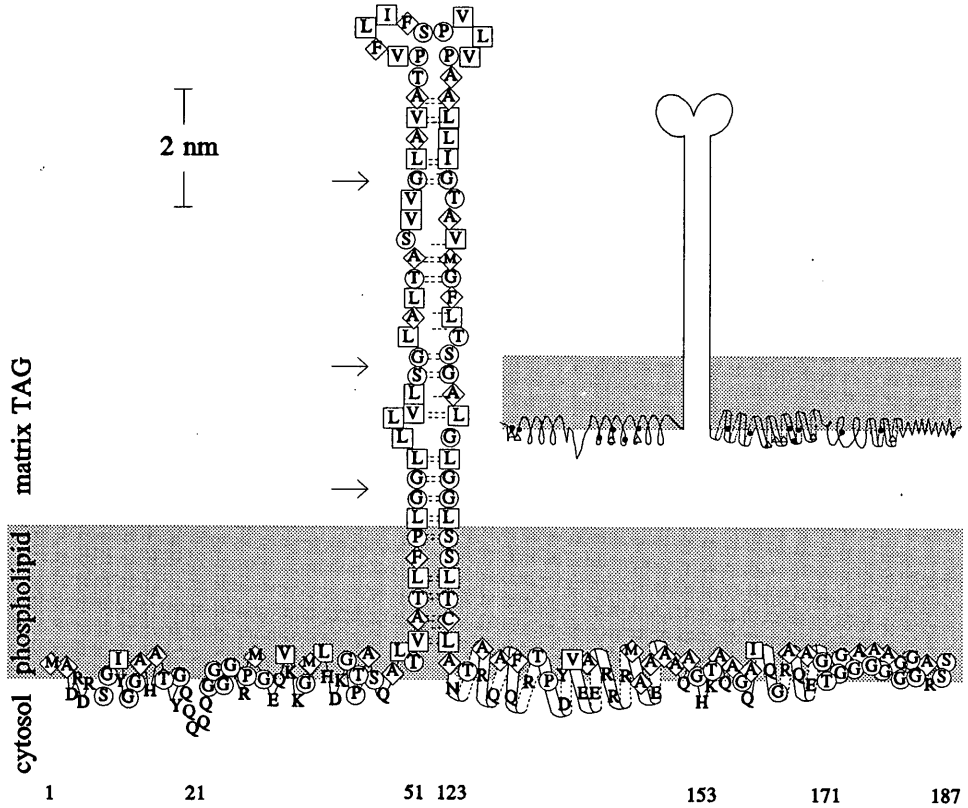

Fig. 2. A model of maize 18-kD oleosin on the surface of an oil body (Tzen *et al.*, 1992). The shaded area represents the PL layer with the head groups facing the cytosol. Symbols of amino acids are: *square* for very hydrophobic, *diamond* for hydrophobic, *circle* for amphipathic, and *no enclosure* for hydrophilic amino acids. The N-terminal domain (residues 1-50) is shown without any well-defined structure. The central hydrophobic domain (residues 51-122) is depicted as anti-parallel β-strands with a "proline knot" at the loop. The anti-parallel strands are aligned according to the best-fit pairing of amino acids of similar hydropathy indexes; *two dotted lines* represent an identical fit, and *one dotted line* denotes a close fit. Only the proposed secondary structure is shown. The strands are likely to fold and curl at the more flexible glycine or serine pairs (indicated by *arrows*) so that maximal hydrogen bonding occurs between the carbonyl groups and the imino groups of the peptide bonds. The third domain (residues 123-170) is an amphipathic α-helical structure interacting with the PL surface. The above three structural domains are present in oleosins of all species. The C-terminal extension (residues 171-187) of different length and sequence is present in oleosins from some species. The insert shows that the positively charged (*dark circles*) and the negatively charged (*open triangles*) residues are facing the PL and the cytosol, respectively.

acids is located at the center. The amino acid sequence of this domain, unlike those of the other two domains, is highly conserved among oleosins of diverse plant species. This domain is likely organized into an anti-parallel β - structure (Fig. 2). The organization is proposed based on thermodynamic considerations, the conservation of key amino acid residues along the stretch (especially the loop), and computer modeling. It is also in agreement with recent experimental findings that a sizable portion of the whole oleosin is in β-structure (Jacks *et al.*, 1990; Li *et al.*, 1992).

(c) An amphipathic α-helical domain of 33-40 amino acids is situated at or near the C-terminus.

Although oleosins of diverse plant species all contain the above three structural domains, their molecular masses range from 15 to 26 kD. In those oleosins having a higher molecular mass, the extra mass appears as an extension at the C-terminus or the N-terminus. The extension sequence at the C-terminus is amphipathic, and in some species (e.g., maize and soybean), it contains small repeats of short amino acid sequences. The extension sequence at the N-terminus (or a substantial modification) is less clear, owing to insufficient sequence data. It is assumed that the extension is not essential to the functioning of the oleosin.

At Least Two Oleosin Isoforms are Present in Diverse Species

Oleosins in different plant species, as well as within the same inbred line, exist in at least two isoforms (Tzen *et al.*, 1990). One isoform always has a molecular mass higher than that of the other isoform. Tentatively, they are termed high-molecular-mass and low-molecular-mass isoforms. The two isoforms are distinguishable immunologically under a defined condition of immunoblotting after SDS-PAGE. Also, the amino acid sequences of one isoform from diverse species are more similar among themselves than they are to those of the other isoform within the same species (Huang, 1992). A good illustration can be found in maize, in which the two isoforms (16 and 18 kD) have more similarities, in immunological properties and in sequence identities, to their respective isoforms in other species than to each other. In maize, the two isoforms are encoded by different genes rather than by different alleles of the same gene. The two oleosin isoforms are present in both monocotyledons and dicotyledons, and thus the duplication giving rise to their encoded genes must have occurred before the divergence of the two plant groups. At least the low-molecular-mass isoform is present in the gymnosperm pine. The benefit of having two isoforms of oleosins within the same seed, and presumably within the same oil body, is unknown.

Oleosins are Encoded by a Small Gene Family Which is Active During Seed Maturation

The proteins of oil bodies isolated from diverse species can be resolved into several oleosin protein bands by SDS-PAGE (Qu *et al.*, 1986; Tzen *et al.*, 1990). Some of the protein bands may be further resolved into additional protein bands by subsequent or different systems of electrophoresis. In general, oleosins in each species are encoded by members of a small gene family. This conclusion is supported by analyses of the oleosin genes. In maize, the oleosin gene family consists of three members, encoding the 16, 18, and 19 kD oleosins (Huang, 1992). The 16 and 18 kD oleosin genes do not contain introns. Their 5' upstream sequences contain putative regulatory sequences that are related to the expression of the genes in seeds during maturation. The two genes are not linked, and are located in single loci in two different chromosomes. Although less well-known than that in maize, the oleosin gene family in *Brassica* possesses a few members per haploid genome.

The expression of the maize 16 and 18 kD genes are coordinated, tissue-specific, and under developmental and hormonal controls (Vance and Huang, 1988; Qu *et al.*, 1990). The genes are expressed only in the scutellum, the embryonic axis, and the aleurone layer; these parts contain oil bodies. They are expressed only during seed maturation and are positively regulated by abscisic acid. In carrot, the 5' upstream sequence of the oleosin gene contains specific motifs to which nuclear proteins from maturing embryos bind (Hatzopoulos *et al.*, 1990). In *Brassica,* the oleosin gene in maturing seed and in growing microsporic embryos is also under a similar developmental and hormonal control (Taylor *et al.*, 1990).

The Surface of an Oil Body is Densely Packed With Oleosins and Carries Negative Charges at Neutral pH

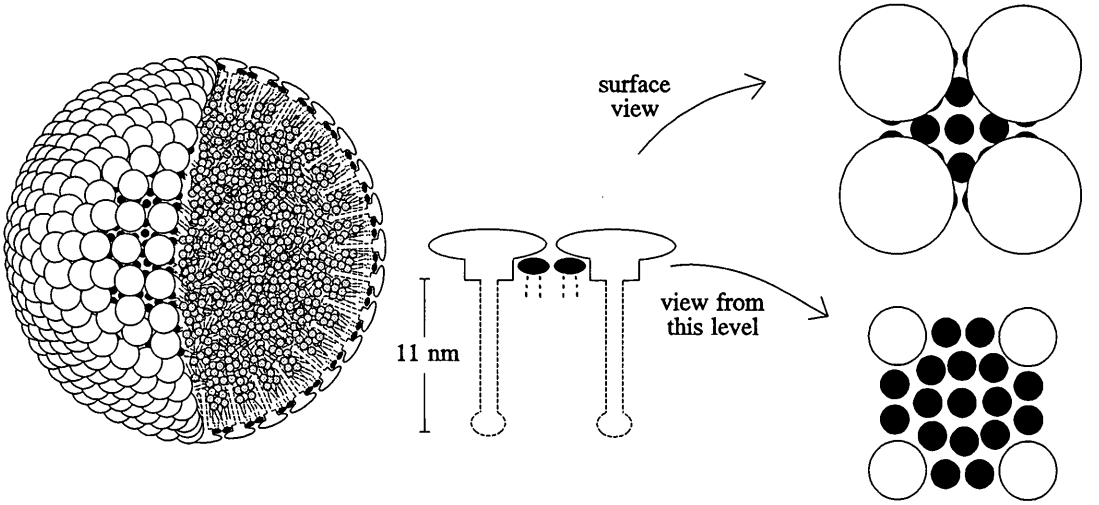

Fig. 3. A model of a maize oil body (Tzen and Huang, 1992). All molecules are drawn in roughly proportional sizes. The shape of the oleosin molecule is depicted as an 11-nm stalk (equivalent to the central hydrophobic anti-parallel β-strand domain) attached to an amphipathic and hydrophilic globular structure (equivalent to the respective portions of the N-terminal amphipathic domain and the C-terminal amphipathic α-helical domain). The stalk is likely to fold into a denser and shorter multistranded structure. *Solid lines* and *circles* represent hydrophilic components, and *dotted lines* denote hydrophobic components. *Dark spheres* attached to 2 lines and *shaded spheres* attached to 3 lines represent PL and TAG, respectively. (*left*) An entire oil body with a quarter section cut open. In order to show the whole organelle in this drawing, the dimension of the oil body in proportion to that of the molecules has been reduced by a factor of 24 ; the curvature of the surface molecules is much sharper (576 times) than for those of a native oil body; the molar proportion of TAG to PL and oleosin is drastically reduced. (*middle*) A small surface-section cut perpendicular to the surface, showing two oleosin molecules and two PL molecules. (*right*) A surface view of a structural unit consisting of 13 PL molecules and 1 oleosin molecule. In order to illustrate the structural unit clearly, the adjacent 3 oleosin molecules and 4 PL molecules are also shown. The upper figure is the surface view above the oleosin globe, and the lower figure is the view from the level of the PL head group.

Oil bodies of mature seeds inside the cells or in isolated preparations are remarkably stable and do not aggregate or coalesce. Stable oil bodies can be reconstituted *in vitro* from TAG, PL/FFA, and oleosins in their native proportions by a suitable sonication condition. The stability of the oil bodies is apparently contributed by the following two factors:

(a) Steric hindrance. When trypsin is applied to isolated oil bodies, it hydrolyzes the oleosins and induces the organelles to coalesce (Tzen and Huang, 1992). Phospholipase A2 (13 kD) or phospholipase C (90 kD) does not act on the surface PL of the oil bodies, and has no effect on the stability of the organelles. These phospholipases do, however, hydrolyze the surface PL if the oleosins are first removed by trypsin. Apparently, the oleosins shield the PL from hydrolysis by phospholipase. Our theoretical calculations indicate that the entire surface of an oil body is covered by oleosins, such that contact between and fusion of the PL and TAG of two adjacent oil bodies are prevented.

(b) Negatively charged surface. The charged amino acid residues in both the N-terminus and the C-terminus of oleosins on the oil body surface are highly oriented (Fig. 2). The positively charged residues face the negatively charged lipids (phosphatidylserine, phosphatidylinositol, and free fatty acid) on the boundary PL layer, whereas the negatively charged residues are exposed to the surface. A computation of all the charges associated with the various constituents in a maize oil body reveals an isoelectric point of about 6.2. This value is in agreement with the pH at which isolated oil bodies aggregate most rapidly, and with the isoelectric point of the organelles determined by isoelectric focusing. Thus, maize oil bodies have a negatively charged surface at neutral pH. Similar charge properties are also present in the oil bodies isolated from other plant species. The negatively charged surface prevents the aggregation of the oil bodies. If the oil bodies are induced to aggregate by lowering the pH of the suspension medium to their isoelectric point,

they still do not coalesce, presumably due to the steric hindrance provided by the shielding oleosins.

A Detailed Structure of an Oil Body has Been Projected Based on Its Size and the Percent Contents and Properties of the Constituents

A structural model of the maize oil body has been proposed based on its diameter, the relative amounts of TAG, PL, FFA, and oleosins, the predicted secondary structures of the oleosins, and other experimental findings (Fig. 3). On the surface of the oil body, the basic structural unit consists of 13 PL molecules and one oleosin molecule. About 2/5 of the oleosin molecule consists of the hydrophobic stalk of the central hydrophobic domain, which is embedded in the hydrophobic acyl moieties of PL and of the TAG matrix. The remaining 3/5 of the oleosin molecule covers or protrudes from the oil body. For simplicity, the covering portion and the protruding portion of the oleosin are depicted as a spherical structure. Together, they shield the PL shell such that the PL are not accessible to external phospholipases. The model represents the basic structure of an oil body in a mature seed, and it does not include minor components such as diacylglycerols (about 2-4% of the TAG), FFA (10% of the PL), and the surface TAG (a few percent of the PL, whose presence is due to molecular partitioning between the hydrophobic core and the hydrophilic exterior). Oil bodies from other species share this structural model established for the maize organelles, as judged from findings of the average diameter of the organelles, the relative proportions of the various constituents, the contents of the negatively charged phosphatidylserine, phosphatidylinositol, and FFA, the isoelectric points, the successful reconstitution of the organelles, and the structure of the oleosins on the organelle surface (Tzen *et al.*, 1993).

Oleosins Stabilize the Oil Bodies and Likely Provide Recognition Sites for Lipase Binding During Germination

We can now recapitulate the function of oleosins on the surface of the oil bodies. When we first initiated our study of the oleosins in 1986-87, we proposed the metabolic roles of the proteins to be the stabilization of the TAG droplets in a hydrophilic environment and the provision of a specific recognition signal for lipase binding during germination (Vance and Huang, 1987). The role of stabilizing the oil bodies has now been documented. The proposed role of lipase recognition site (see latter part of this article) has yet to be demonstrated. The site may be the C-terminal amphipathic α-helix domain, which is similar to that of a lipase-binding site in mammalian lipoproteins, and/or the N-terminal amphipathic domain.

During Seed Maturation, Oil Bodies are Synthesized on the Rough Endoplasmic Reticulum by Special Vesiculation

In situ electron microscopic observation of maturing seeds has led to the suggestion that the oil body is synthesized in the endoplasmic reticulum (ER) via vesiculation (Schwarzenbach, 1971; Wanner *et al.*, 1981). The electron micrographs reveal physical contact between the oil bodies and the ER. In view of the oil body being surrounded by only one layer of PL, and in consideration of the thermodynamics involved, the researchers suggested that TAG synthesized in the ER is sequestered in a region between the two PL layers of the ER membrane, such that a nascent oil body of a TAG matrix surrounded by one PL layer is produced. This model of oil body formation, however, was based on little biochemical information. In addition, it is difficult to judge if the observed physical contact between the oil body and the ER represents only a casual contact or a direct connection between the matrix TAG of a budding oil body and the in-between region of the PL bilayers of the ER membrane.

Nevertheless, the results of recent biochemical studies support the vesiculation model. The three major components of the oil bodies (TAG, PL, and oleosins) are all synthesized in the ER. Fatty acids are synthesized in the plastids, whereas the assembly of fatty acids into TAG occurs in the ER (Browse and Somerville, 1991). Specifically, the last enzyme of TAG synthesis, diacylglycerol acyltransferase (which is the only enzyme unique to TAG synthesis) is localized in the rough ER (Cao and Huang, 1986). Thus, the newly synthesized TAG should appear first in the rough ER. PL in plant tissues are known to be synthesized in the ER, although their synthesis in maturing seeds has not been vigorously studied (Moore, 1982). Presumably, the oil body PL are also synthesized in the ER during seed

maturation. Oleosins, at least those in maize embryos, are synthesized on mRNA associated with the ER (Qu *et al.*, 1986). There is no cleavable N-terminal signal sequence in the oleosins (Huang, 1992). Apparently, the oleosin does not enter into the lumen of the ER; otherwise, vesiculation would occur at the luminal side of the ER and the newly formed oil body would enter the intracellular secretory pathway. The signal for the attachment of the mRNA-oleosin polypeptide to the ER (or directly to the budding oil body) is unknown. It appears to be universal, since the maize oleosin is correctly targeted to seed oil bodies in *Brassica* transformed with the maize oleosin gene (Lee *et al.*, 1991). It may be an unknown secondary structure of the N-terminal sequence or the central hydrophobic domain. The amino acid sequence of the central hydrophobic domain of about 70 residues is highly conserved among the oleosins in dicotyledons and monocotyledons. The domain is likely to be unstable in the hydrophobic region of an ER membrane. Thus, the oleosin, once synthesized in the ER, would flow to a region that is more hydrophobic than a general region of the ER membrane. This more-hydrophobic region is between the two PL layers where the TAG accumulate. The expanding TAG-oleosin bud generates a low density of PL on the surface, and the deficiency attracts the inflow of PL from the ER. In addition, the newly synthesized oleosin in the ER may, as a consequence of their interaction, carry PL with it to the budding oil body. Alternately, the oleosin, once in the bud, may retain PL and TAG (which become less mobile) that are already there. This interaction between oleosin and PL/TAG during the formation of an oil body is made possible by the unique charge and polarity distributions in the oleosin. Based on experimentally determined localization of the syntheses of the various components and thermodynamic considerations, a model of oil body formation on the surface of the ER has been proposed (Fig. 4). The product is an oil body with a matrix of TAG surrounded by a layer of PL and oleosins. This model is simply an extension of the model first proposed based on electron-microscopic studies.

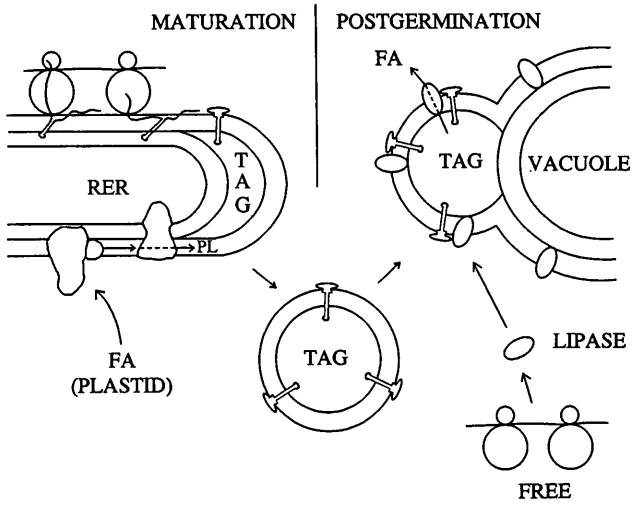

Fig. 4. A model of the synthesis and degradation of an oil body in maize embryo during seed maturation and postgermination. See text for explanation. RER, rough endoplasmic reticulum; FA, fatty acid; TAG, triacylglycerols; FREE, free polyribosomes (Huang, 1992).

A different model of oil body synthesis in *Brassica* has been proposed. According to this model, the oil body is synthesized first with the TAG, followed by an encasement with oleosin/PL. One of the major supports of this proposal is the report that in maturing *Brassica* seed, accumulation of TAG occurred before that of oleosins (Murphy *et al.*, 1989; Cummins and Murphy, 1990). Our recent analyses of maturing *Brassica* seed, however, showed that TAG and oleosins accumulate by indistinguishable developmental patterns (Tzen *et al.*, 1993). This model is considered unlikely in view of uncertainties in the other experimental observations, as well as of unfavorable thermodynamic analyses (Huang, 1992). It is possible that oil bodies are synthesized by different mechanisms in different species, but such species-dependent mechanisms have not been demonstrated, and the controversies arise from a difference in data interpretation.

During Seed Germination, TAG and Oleosins in the Oil Bodies are Degraded Whereas Their Phospholipids May be Retained

Oil bodies in seeds are degraded during germination and postgerminative growth. TAG in oil bodies are hydrolyzed to glycerol and fatty acids, which are then converted to carbohydrates for the growth of the seedling. In the maize embryo, lipase activity starts to appear 2 days after imbibition, concomitant with the decrease in total lipids (Wang and Huang, 1987). The activity reaches a maximum at about day 5-6. The enzyme is tightly bound to the surface of the oil bodies. It is synthesized on free polyribosomes without appre-

ciable co- or post-translational processing. Presumably, after its synthesis, the lipase attaches itself specifically to the surface of the oil bodies and not to other organelles. The recognition site in the lipase is unknown, and that on the oil body has been suggested to be the oleosins (Vance and Huang, 1987). At the late stage of seedling growth after most of the lipids have been depleted (day 5-10), lipase activity remains in the tissue, whereas other gluconeogenic enzymes such as the glyoxysomal catalase and isocitrate lyase rapidly decrease in activity. Apparently, the lipase present at day 6-10 is the remaining rather than newly synthesized enzyme, because lipase-specific mRNA has completely disappeared by day 7. During or after lipolysis, the surface structure of an oil body fuses with that of another oil body or of an enlarging vacuole to form a part of the central vacuole, as shown by electron microscopy (Wang and Huang, 1987). The lipase may follow the oil body surface structure in the fusion or may remain in the cytosol, either as clumps or in association with membrane vesicles; subcellular fractionation shows that the lipase sediments with diverse sedimentation coefficients, which may be those of lyzed tonoplast, clumped proteins, or cytosolic membrane vesicles. Oleosins do not follow the same retention pattern as that of lipase during seedling growth, but disappear rapidly concomitant with the total lipids (Fernandez *et al.*, 1988). It is likely that the cell does not need to degrade the harmless PL and lipase and incorporates them into the membrane of the enlarging vacuole, and that the very hydrophobic oleosins are unstable in a PL bilayer of the vacuole membrane and are degraded.

The maize system has been studied intensively because the maize lipase has been purified such that antibodies against the enzyme can be used for a vigorous biochemical study. Whether or not the findings on maize are applicable to other species remains to be seen. Maize scutellum is a storage tissue that does not perform a new role or survive in the seedlings after the storage food has been depleted. In the seeds of *Brassica, Sinapsis,* cotton, and many other genera, the storage cotyledons turn green and carry out photosynthesis after the food reserves have been depleted. Electron microscopy of *Brassica* and *Sinapsis* seedlings shows that the "ghosts" of the oil bodies after the TAG have been depleted remain in the cytosol (Bergfeld *et al.*, 1978; Wanner and Theimer, 1987). In cotton, the remaining oil body PL are believed to be used for membrane assembly of the enlarging glyoxysomes (Ni and Trelease, 1991).

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