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Update on Cell Biology

Oleosins and Oil Bodies in Seeds and Other Organs[1]

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Diverse organisms store lipids in subcellular particles as food reserves, which will be mobilized during a forthcoming period of active metabolism. These lipid particles can be found in the seeds, pollens, flowers, roots, and stems of flowering plants, the spores and vegetative organs of nonflowering plants, and algae. They are also present in some animal cells, fungi, and *Euglena*. Of all these subcellular storage lipid particles, those from seeds have been studied most extensively.

Seeds of most plant species store TAGs as food reserves for germination and postgerminative growth. The TAGs are present in small subcellular spherical oil bodies of approximately 1 μm in diameter. Each oil body has a matrix of TAGs surrounded by a layer of PLs. The small entities provide a large surface area per unit TAG, which would facilitate lipase binding and lipolysis during germination. Oil bodies inside the cells of mature seeds or in isolated preparations are remarkably stable and do not aggregate or coalesce. This stability is in contrast to the instability of artificial liposomes made from amphipathic and neutral lipids; the liposomes slowly coalesce after formation. Seed oil bodies are stable because their surface is shielded by a layer of unique proteins, termed oleosins.

Oleosins, in addition to being present in the storage oil bodies of seeds and pollens, have recently been found in the nonstorage tissue of floral tapetum. The preliminary findings suggest that the tapetum oleosins are similar to seed oleosins in structure and function. They may prevent subcellular lipid droplets from coalescing; these lipid droplets are to be deposited onto the surface of the maturing pollen.

OLEOSINS AND OIL BODIES IN SEEDS

Oleosins and Their mRNAs Are Abundant in Seeds

Oleosins in the seeds of diverse species are small proteins of about 15 to 26 kD (Huang, 1992; Murphy, 1993; Herman, 1995). They completely cover the surface of the small subcellular oil bodies. When a seed contains a high percentage of oils and its oil bodies are small (i.e. having a large surface area per unit TAG), the amount of oleosins is high and can account for several percent of the total seed proteins (Tzen et al., 1993). For example, the *Brassica* seed has about 40 to 45% (w/w) of TAGs localized in the smallest known seed oil bodies (0.6 μm in diameter), and 8% of the total seed proteins are oleosins. In spite of their abundance, oleosins are encoded by only two to a few genes per haploid genome. In comparison, seed storage proteins are encoded by members of larger gene families. The high ratio of oleosin amount per gene copy suggests that during seed maturation the genes are active, and presumably their mRNAs are abundant or stable. The inference that oleosin mRNAs are abundant is revealed in the numerous successes in cloning the oleosin genes via random sequencing of the abundant cDNAs from embryogenic tissues.

Whereas the oleosin genes in maize, soybean, and pine do not contain introns, those from Brassicaceae have one intron that occurs between the two exons encoding the central hydrophobic domain and the carboxylic α-helix stretch (see further). In general, the temporal expression of the oleosin genes in maturing seeds is quite similar to that of seed storage proteins and is regulated by factors (e.g. ABA) that promote seed maturation and embryogenesis (Huang, 1992; Plant et al., 1994; Zou et al., 1995). Expression of the oleosin genes during seed maturation can be detached from that of the lipid-synthesizing genes. This was demonstrated with maize kernels that have been bred to possess diverse oil contents; in these kernels, the oleosin genes are constitutively expressed independently of the oil contents (Ting et al., 1996).

The Hallmark of the Oleosin Molecule Is Its Highly Conserved Hydrophobic Anti-Parallel β-Stranded Domain That Anchors the Protein onto an Oil Body

Each oleosin molecule has a highly conserved central hydrophobic stretch of 72 amino acid residues. This stretch is the longest hydrophobic polypeptide found in proteins of diverse organisms. It is depicted as an anti-parallel β-stranded domain (Tzen et al., 1992; Fig. 1). The turn of the anti-parallel β-strands consists of 13 residues and is the most conserved region. The two anti-parallel strands are highly symmetrical in the pairing of residues of similar hydrophobicity on the opposite strands. There are several pairs of small residues (e.g. Ala and Gly) along the anti-parallel strands. At these pairs, the strands could bend for additional intrastrand interac-

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Abbreviations: PL, phospholipid; SRP, signal recognition particle; TAG, triacylglycerol.

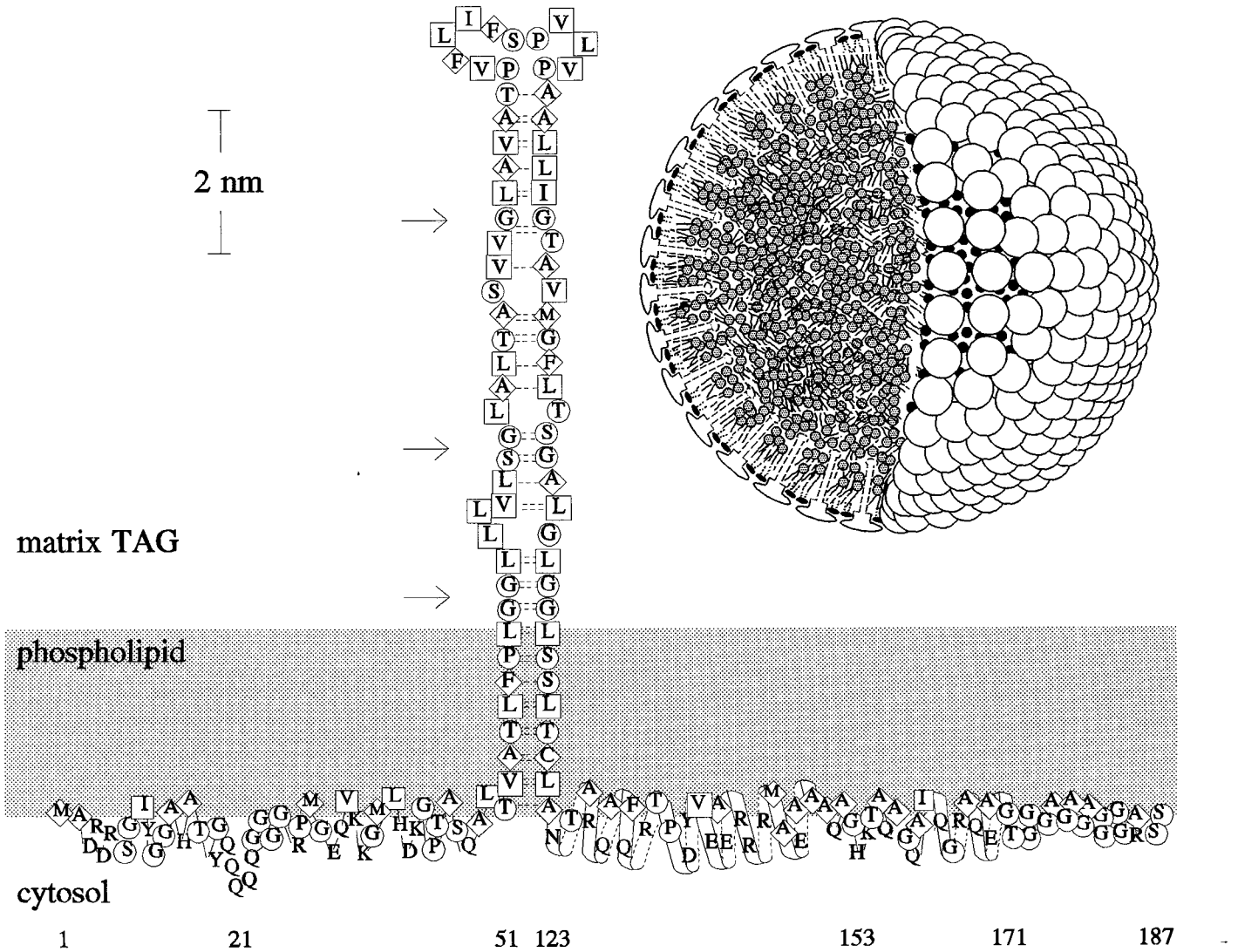

Figure 1. A model of a maize oleosin on the surface of an oil body. The shaded area represents the PL layer with head groups facing the cytosol. Amino acid residues are highlighted according to their decreasing hydrophobicity: square, diamond, circle, and no enclosure. The molecule can be divided into four portions: the amphipathic N terminus (residues 1–50), the central anti-parallel β strands (residues 51–122), the carboxylic α helix (residues 123–170), and the C-terminal extension (residues 171–187). Arrows indicate where folding of the anti-parallel strands could occur. The inset shows a model of an oil body with a quarter cut open. Mushrooms, dark spheres attached to two lines, and shaded spheres attached to three lines represent oleosins, PLs, and TAGs, respectively. Solid lines and circles denote hydrophilic components, and dotted lines represent hydrophobic components. All molecules are drawn approximately to scale, whereas the diameter of the oil body has been reduced by 24 times to magnify the surface structures. (Modified from Tzen et al., 1992.)

tions that would stabilize the hydrophilic peptide bonds in the hydrophobic TAG matrix. In addition, the bending could allow the anti-parallel strands, which would otherwise be too long (11 nm), to stay temporarily in the hydrophobic region (4 nm) of the double PL layers of the ER membrane during the synthesis of an oil body (to be described). On a mature oil body, the anti-parallel strands (11 nm long) would penetrate the monolayer of PLs into the TAG matrix and thus anchor the whole protein stably.

Flanking the Oleosin Hydrophobic Domain on the Oil Body Surface Are Amphipathic Stretches That Play Specific Biological Roles

Flanking the central hydrophobic domain is an amphipathic N-terminal stretch that has no apparent clear-cut secondary structures (Fig. 1). Among the N-terminal sequences of diverse oleosins, there are few similarities in residues and length. Flanking the central domain on the carboxylic side is an amphipathic α-helical stretch (Fig. 1).

Its amphipathicity allows the α helix to interact with the PLs on the surface of the oil body. Along the α helix, the alkaline residues are situated closer to the interior, presumably interacting with the phosphate moieties of PLs and other acidic amphipathic lipids on the surface, whereas the acidic residues are exposed to the exterior (Tzen et al., 1992). Together, the N-terminal, the central, and the α-helical portions anchor the oleosin stably on the surface of the oil body. The secondary structures of the three portions as described have been implicated based on considerations of the thermodynamics involved and experimental findings (Huang, 1992; Li et al., 1992; Tzen et al., 1992). With these secondary structures, an oleosin molecule can achieve maximal stability in the unique environment of the oil body surface (Tzen et al., 1992). Removal of the oleosin from the oil bodies by mild treatments and presentation of the protein in a proper medium may preserve its secondary structures (Huang, 1992; Li et al., 1992). These secondary structures are destroyed when the oil bodies and oleosin have been treated with a strong chaotic agent (9 M urea) (Millichip et al., 1996). In addition to the three major portions, an extension at the C terminus is present in some oleosins. The extensions of these oleosins vary greatly in residues and length. The great variabilities in the length of this extension and of the N-terminal stretch contribute to the difference in the size of oleosins.

Oleosins Are Present as Heteromers of Two Isoforms on Oil Bodies

Oleosins in seeds of diverse species exist in two isoforms (Huang, 1992). The two isoforms are distinguishable immunologically under defined conditions of immunological blotting after SDS-PAGE. This distinction is reflected in the difference in amino acid sequences (to be described). Both isoforms still maintain the characteristic structural features of an oleosin as described in the preceding paragraphs. Each isoform is represented by members from both monocots and dicots. Genetic studies of maize have revealed that the two isoforms are present in a 1:1 molar ratio irrespective of the relative dosage of the two isoform genes (Lee et al., 1995). The findings suggest that the two isoforms are present as heterodimers or heteromultimers on the surface of the oil bodies. The physiological significance of having two isoforms and their presence as heteromers is unknown. Speculations can be made that the formation of heteromers could provide more interactions among oleosins and thus a higher stability of the proteins on the surface of the oil bodies and might create a better receptor for lipase binding.

Oleosins Play the Roles of Stabilizing the Oil Bodies as Small Entities and Possibly Being a Receptor for Lipase Binding

Based on experimental tests and theoretical calculations (Huang, 1992), a model of the oil body has been proposed (Fig. 1). For simplicity, individual oleosin molecules are depicted as mushroom-shaped solitary molecules. It is likely that the abundant oleosins, which cover the whole surface of an oil body, interact among themselves to acquire extra stability; however, direct evidence of this interaction is lacking. Indirect evidence from genetic studies has suggested that the two oleosin isoforms are present as heterodimers or heteromultimers. Oleosins interact with and cover the underlining PL layer and the matrix TAGs. They form a steric barrier, preventing the PL layers of adjacent oil bodies from coalescing. The net negative charges of the oleosins exposed to the cytosol (Tzen et al., 1992) further prohibit the adhesion of oil bodies via electrical repulsion. Overall, the abundant oleosins maintain the seed oil bodies as small entities in vivo and in vitro. In the fruit mesocarp of olive, avocado, and oil palm, the large subcellular lipid particles (diameter larger than 10–20 μm), which are not mobilized by the plants, apparently do not have oleosins or oleosin-like molecules on their surface (Ross et al., 1993; Lee et al., 1994). In addition to playing the above structural role, oleosins may also serve as recognition signals on the surface of oil bodies for the binding of newly synthesized lipase during germination (to be described).

The Size of the Oil Bodies Is Controlled by the Relative Amounts of Oils to Oleosins Synthesized during Seed Maturation

The size of the spherical oil bodies in situ is apparently species specific, and the average diameter ranges from 0.6 to 2.0 μm (Tzen et al., 1993). The size of the oil bodies is controlled at least in part by the relative amounts of oils to oleosins synthesized during seed maturation. This was illustrated with maize kernels that were bred to possess diverse oil contents (Ting et al., 1996). In these kernels, the oleosins are constitutively produced independently of the oil contents. As a consequence, high-oil kernels have a high ratio of oils to oleosins (i.e. matrix/surface layer), and their oil bodies are larger and spherical. Low-oil kernels have a low ratio of oils to oleosins, and their oil bodies are smaller and have an irregularly contoured surface (i.e. more surface area per unit TAG).

Oil Bodies Are Synthesized in the ER by a Budding Process during Seed Maturation

During seed maturation, oil bodies are synthesized in the ER by a budding process (Fig. 2), which has been postulated based on experimental evidence and considerations of the thermodynamics involved (Huang, 1992). TAGs are synthesized by enzymes in the ER and are sequestered, due to their hydrophobicity, between the two PL layers of the ER membrane, thereby forming a bud. PL synthesized by enzymes in the ER also diffuse to the surface of this budding TAG particle. Simultaneously, oleosins are synthesized on polyribosomes bound to the ER without appreciable co- or posttranslational processing. The newly synthesized oleosin moves to the budding TAG particle, presumably guided by the central hydrophobic domain. Alternatively, the oleosins being synthesized on polyribosomes are inserted directly into the budding particle. The budding particle, which has a TAG matrix surrounded by

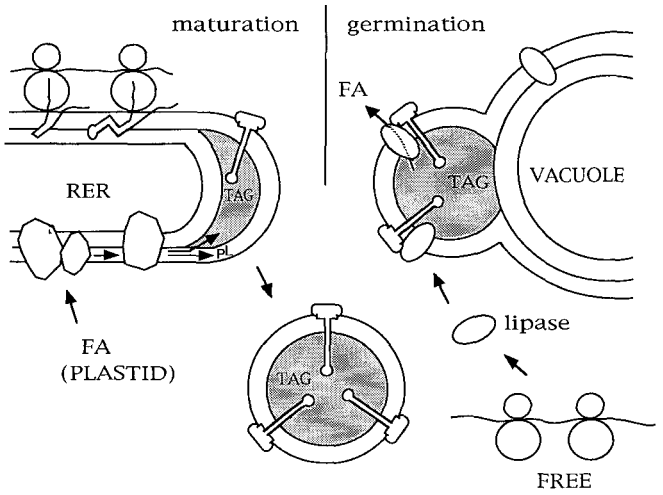

Figure 2. A model of the synthesis and degradation of an oil body in maize embryo during seed maturation and germination. RER, fatty acid (FA), and free polysome (FREE) are shown.

a layer of PLs and oleosins, is released into the cytosol as a mature oil body.

There is no cleavable N-terminal signal sequence in oleosin. Apparently, the oleosin does not enter the lumen of the ER; otherwise, budding would occur at the luminal side of the ER and the newly formed oil body would enter the intracellular secretory pathway. The latter scenario occurs in the secretion of lipoproteins in mammals (Rosseneu, 1992). It is not clear which parts of oleosin are used to target the protein to the ER or budding oil body. A targeting signal at the N-terminal end of the oleosin is not apparent. At this region, there are no appreciable similarities in the length, the amino acid residues, and the charge distribution among oleosins of diverse species. The targeting signal on the oleosin appears to be universal, however, since the maize oleosin is correctly targeted to seed oil bodies in transformed *Brassica* (Huang, 1992).

Fusion of a GUS protein to the C terminus of the oleosin also allows the targeting of the fusion protein to the oil bodies in transformed *Brassica* (Van Rooijen and Moloney, 1995). Omission of the C-terminal α-helical domain still permits the correct targeting of the truncated protein to the oil bodies in the transformant. Omission of the N-terminal stretch or the central domain results in the loss of the proteins in the transformants (Van Rooijen and Moloney, 1995). During in vitro translation, newly synthesized oleosins are targeted co-translationally to canine microsomes but not to mature oil bodies (Hills et al., 1993; Loer and Herman, 1993). It is unknown whether the oleosins would be targeted directly to the budding oil bodies which, unlike the mature oil bodies, are not saturated with oleosins. When the 3′ sequence of the oleosin mRNA encoding the C terminus of the protein has been removed, the in vitro synthesized truncated oleosin is still targeted to the ER (Thoyts et al., 1995). Addition of SRPs inhibited the in vitro translation of oleosin mRNA; this preliminary result suggests that SRPs may be involved in the targeting of oleosin to the ER. Additional tests are needed to interpret definitively the above in vitro experimental results. On the mRNA-ribosome complex, partly synthesized oleosin with its long hydrophobic stretch may insert into any added membrane or form hydrophobic interaction with any added hydrophobic component (SRPs are slightly hydrophobic).

Overall, it can be speculated that the N-terminal domain, by virtue of its yet-to-be-defined unique secondary structure, is necessary for targeting the protein to the ER or the budding oil bodies and that the central domain, by virtue of its hydrophobicity, functions to move the protein from the ER to the budding oil body and for allowing the protein to stay tightly on the oil body.

In the maturing seeds of maize and *Brassica*, oleosins are synthesized coordinately with the storage oils (Tzen et al., 1993). These detailed analyses disagree with an earlier report stating that in maturing *Brassica* seed oils accumulated before the oleosins; the report suggested that oleosins were added to immature oil bodies that had already been detached from the ER (Murphy, 1993). In maize and barley, the oleosin mRNAs encoding different isoforms accumulate simultaneously (Aalen, 1995; Lee et al., 1995). In *Brassica* and sunflower (Cummins et al., 1993; Thoyts et al., 1995), the oleosin mRNA of different genes may or may not accumulate coordinately among themselves. Because of this differential accumulation of mRNAs of various oleosin genes, the temporal appearance of a particular oleosin mRNA should not be used to represent that of the total oleosin proteins. In addition, there is apparently a strong control at the posttranscriptional level of the amount of oleosins synthesized. Specifically, in maize, the two oleosin isoforms are synthesized in a 1:1 molar ratio irrespective of the respective mRNA amounts (Lee et al., 1995). The apparent interaction of the two isoforms on the surface of the ER or oil bodies may dictate the translational efficiency or the stability of the mRNA, or it may exclude excess oleosins of one isoform.

Oil Bodies Are Degraded during Seed Germination

During germination of the maize kernel, lipase is synthesized de novo on free polyribosomes without any appreciable co- or posttranslational processing (Huang, 1992). The newly synthesized enzyme binds specifically to the oil bodies. The recognition signal on the oil bodies for lipase is likely to be the oleosins (Fig. 3), which are absent in other organelles. This role of oleosins in lipase binding has been proposed but has yet to be demonstrated. During or after lipolysis, the PL layers of the oil bodies fuse with the vacuolar membrane, eventually forming the large central vacuole.

OLEOSINS IN TAPETUM AND POLLEN

Oleosin mRNAs Are Present in Tapetum and Pollen

About 10 different oleosin mRNAs from the anther (possibly tapetum) of Arabidopsis, and the tapetum and the pollen of *Brassica*, obtained by cDNA cloning or genomic DNA cloning followed by northern blotting, have been reported (deOliveira et al., 1993; Roberts et al., 1993; Robert

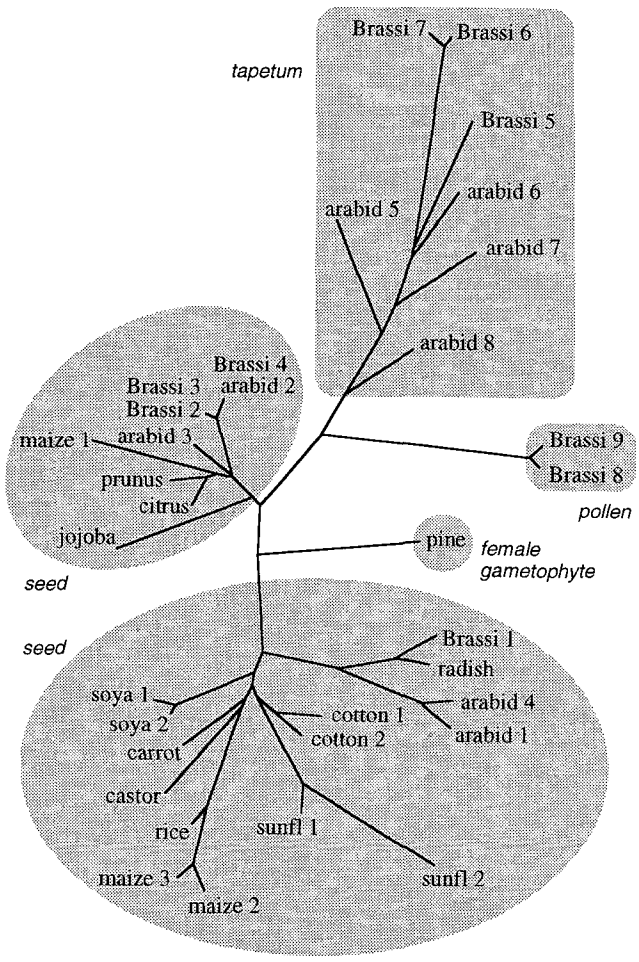

Figure 3. An unrooted phylogenetic tree of oleosins. The oleosin numbers after the names of plants follow approximately the chronological appearance of their genes in the Genetics Computer Group data bank. The large and small shaded ovals denote the two seed oleosin isoforms. The large and small shaded rectangles represent the tapetum and pollen (male gametophytic) oleosins, respectively. The shaded circle indicates the female gametophytic oleosin. The tree is expanded from that in Lee et al. (1994) courtesy of Dr. Gerry Learn (University of Washington, Seattle). arabid, Arabidopsis; Brassi, *Brassica;* sunfl, sunflower.

et al., 1994). Their deduced amino acid sequences contain the highly conserved, long hydrophobic stretch that is characteristic of seed oleosins. A minor departure from this generalization is that the two *Brassica* tapetum sequences have only about three-fourths the length of the hydrophobic stretch found in seed oleosins. The 10 tapetum and pollen sequences, with one exception, have an extension at the C terminus. The extensions are highly variable in length, ranging from about 50 to 400 amino acid residues. Largely because of this variable length, the sizes of the predicted oleosins vary from 14 to 60 kD. The extensions contain some short repeats of residues, which include Gly, Ala, Ser, or Pro. The secondary structures of the extensions are unknown. The presence of a C-terminal extension is not a unique characteristic of tapetum and pollen oleosins; extensions with similar features (short repeats rich in Ala or Gly), although substantially shorter in length, are also present in some seed oleosins (e.g. of soybean and maize).

The Oleosins of the Tapetum and Pollen May Be Present on the Surface of Intracellular Storage Oil Bodies and Lipid Globules Destined for the Pollen Surface

None of the *Brassicaceae* tapetum and pollen oleosin mRNAs has been linked directly to an authentic protein, and the subcellular locations of the predicted oleosins are unknown. Two of the four predicted Arabidopsis oleosin amino acid sequences may possess a signal peptide at the N terminus, and if so, the proteins may be exported (de Oliveira et al., 1993). Researchers have speculated about the subcellular locations and functions of these floral oleosins based on the characteristics of seed oleosins and the subcellular structures of *Brassica* tapetum and pollen (Polowick and Sawhney, 1990; Murgia et al., 1991; Owen and Makaroff, 1995):

1. In the tapetum cells, small oil bodies morphologically similar to those in seeds are present. They disappear just before the lysis of the tapetum cells. The metabolic role of these oil bodies, which presumably are of a storage nature, is unknown. These small oil bodies are likely to be covered by oleosins, which prevent them from coalescing.

2. In the tapetum cells, clusters of 10 to 40 spherical globules each of 0.2 to 2.0 μm in diameter are present. The clusters are located in the plastids. The globules are very electron-dense after osmium fixation and are likely to be of a lipidic nature. Larger lipid particles are also present in the cytoplasm. It has been speculated that the lipids in the plastid globules and the lipid particles will be released from lysed tapetum cells and deposited onto the surface of the pollen, forming the pollenkitt. Oleosins may be present on the surface of the plastid globules and the lipid particles, preventing them from coalescing before their release onto the pollen surface. In addition, the oleosins, by virtue of their Gly-/Ala-rich C-terminal extension, may assist the globules in penetrating the lysed tapetum cell wall and/or attaching to the pollen surface and, when present in the pollenkitt, may interact with the stigma.

3. In mature pollen, oil bodies of a storage nature similar to those in seeds are present. They are presumably mobilized during pollen tube growth. Their surface is likely to be covered by oleosins, which prevent the oil bodies from coalescing.

EVOLUTION OF OLEOSINS

An Unrooted Phylogenetic Tree of All Reported Oleosins Has Several Distinct Lineages

A phylogenetic tree of the 35 to 40 reported oleosins is constructed based on analyses of the amino acid sequences of the highly conserved 72 residues of the central hydrophobic stretch and its adjacent moderately conserved residues (Lee et al., 1994). The tree is unrooted, since no single oleosin can be assigned to the basal lineage (Fig. 3).

Two major seed oleosin lineages representing the two isoforms are apparent. Each lineage has both monocot and

dicot members. The Brassicaceae tapetum oleosins form another lineage. The two *Brassica* pollen oleosins make up a rather distinct lineage; they are of male gametophytic origin, which is different from the sporophytic origin of the seed and tapetum oleosins. Similarly, pine female gametophytic oleosin represents a separate lineage.

After the Appearance of TAGs in Early Eukaryotes, Oleosins Evolved, Which Stabilized the Hydrophobic Lipids, and Underwent Modifications to Acquire Additional Functions

Prokaryotes, in general, do not store TAGs as food reserves. A minor exception occurs in *Actinomyces*, which produces TAGs under certain nutritional conditions. TAGs likely evolved as efficient food reserves in early eukaryotes by the addition of one enzyme, diacylglycerol acyltransferase, which diverted diacylglycerols from the ubiquitous PL metabolic pathway to TAGs. Initially, the hydrophobic TAGs were stored between the two PL layers of the ER membrane, where the acyltransferase was located. Today, seeds of some species on occasion still have some TAGs present in the ER membrane. The presence of excess TAGs in the ER membrane would interfere with the normal functioning of the ER. This problem was overcome with the evolutionary appearance of oleosins, which had a long hydrophobic stretch. They were synthesized on bound ER, and from there they extracted the TAGs to produce solitary oil bodies. It is assumed that algae and primitive plants have oleosins associated with the storage TAGs. It is less certain if *Euglena*, yeast, and animals also have oleosins.

The hydrophobic stretch of 72 residues in the oleosin is the longest one found in any prokaryotic and eukaryotic protein. The mechanism by which it has evolved is intriguing. A postulation can be made partly based on the length of 72 residues being approximately 4 times that of a transmembrane polypeptide and on the occurrence of several relatively hydrophilic residues at the center of the stretch. Initially, a short hydrophilic polypeptide joining two transmembrane hydrophobic polypeptides, of possibly an ER membrane protein, became hydrophobic through DNA sequence mutation. A continuous hydrophobic stretch resulted, which consisted of about half of the final 72 residues. This primitive hydrophobic stretch could stabilize an oil body, although not efficiently. Its length was doubled by one of the following DNA sequence mutations: (a) duplication of the DNA segment encoding the primitive hydrophobic stretch, (b) mutation of an adjacent pair of transmembrane polypeptides similar to that which produced the primitive hydrophobic stretch, and (c) continuous mutation of the cytosol-exposed hydrophilic residues flanking the primitive hydrophobic stretch to hydrophobic residues. Once evolved, the 72-residue hydrophobic stretch has been preserved because of structural constraints. More modifications have occurred in the regions of oleosin exposed to the cytosol.

Both gametophytes and sporophytes of seed plants contain TAGs and oleosins. Therefore, oleosins evolved before the diversification of the two plant parts. The two plant parts would have their oleosin genes evolved independently, or they would share the use of the same genes. In the male gametophyte (pollen) of *Brassica*, the oleosins are different from those in the sporophytic seed (Roberts et al., 1995). However, in pine seed, both the female gametophytic tissue and the sporophytic tissue apparently use the same oleosin gene for oleosin synthesis (Lee et al., 1994).

Like many other structural proteins, oleosins have undergone more extensive modifications than enzymatic proteins. This statement is made based on the fact that oleosins in different organs and species have diverse sizes and sequences. It is also revealed in an Arabidopsis 10-kb genomic DNA, which contains four tandemly arranged oleosin genes, three of which have been shown to produce mRNA in the anther (deOliveira et al., 1993). Presumably, the four genes are recent descendants of a common gene via DNA duplications. Their predicted product oleosins are quite different in their amino acid sequences (arabid 5, 6, 7, and 8 in Fig. 3), and the C termini have very diverse sequences and lengths (ranging from 0–400 residues). In spite of these drastic changes, all four genes maintain the following characteristics of the Brassicaceae seed oleosin genes. They all possess an intron that occurs between the two exons encoding the central hydrophobic domain and the carboxylic α-helix stretch, and their encoded oleosins all contain the highly conserved long hydrophobic stretch.

PERSPECTIVES

Oleosin has unique structural features that allow the protein to interact with other molecules on the surface of an oil body. The long hydrophobic anti-parallel strands apparently penetrate deeply into the TAG matrix and act as a stable anchor. The cytosol-exposed regions interact with the surface lipids of the oil bodies; the interaction generates additional stability. These regions are possibly involved in serving specific functions, such as a targeting signal for the ER or budding oil body (the N terminus), a receptor for lipase (the carboxylic α helix), and an interacting element with the cell wall of tapetum or pollen (the C-terminal extension). Although the function of oleosins being a structural protein has been well established, the hypotheses of oleosins having the above additional features or functions need to be tested. The recent findings of oleosin mRNA in tapetum and pollen should be extended to the identification of the proteins and their subcellular locations and functions. Some of this information is obtainable by expressing the cloned genes in bacteria, raising antibodies against the oleosins, and probing their subcellular locations by immunocytochemistry. Additional information could be obtained using genetic mutants. All of the tapetum and pollen oleosin mRNA sequences had been obtained from one family of Brassicaceae. We need more sequence data from other plant species to assess what sequence characteristics are specific to the organs rather than related only to the Brassicaceae family. Information about the proteins in primitive nonseed plants will be useful to our understanding of the evolution of the oleosin structures and functions. It is possible that in higher plants oleosins with the basic characteristics plus additional structural features are present on the surface of small lipidic particles other

than those in seeds and antheridia. Examples include the cutin particles in epidermal cells and secretory lipid particles in secretory glands, which are to be transported to the outside of the cell.

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