Lipid bodies in lipotubuloids of *Ornithogalum umbellatum* ovary epidermis contain diacylglycerol acyltransferase 2 (DGAT2) and lipase, incorporate $^3$H-palmitic acid and are connected with cuticle synthesis

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**ABSTRACT**

Lipid bodies of *O. umbellatum* ovary epidermis are surrounded by a network of microtubules and assembled in lipotubuloids i.e. cytoplasm domains containing ribosomes, ER cisternae and vesicles as well as some mitochondria, microbodies, Golgi structures and autolytic vacuoles. Lipid bodies in statu nascendi grow from 0.04 up to about 0.1 µm. In maturity they are about 0.1-0.4 µm in diameter and maintain this dimension during epidermis development, accompanied by lipotubuloid enlargement resulting in the higher number of lipid bodies. Using the immunogold technique with anti-diacylglycerol acyltransferase 2 and anti-lipase antibodies it was shown that mature lipid bodies were labeled with gold grains near a phospholipid monolayer. Autoradiography with $^3$H-palmitic acid demonstrated that lipid body surface is the site of this precursor incorporation into lipids. Selective labeling of lipotubuloid lipid bodies with silver grains observed after 2-h incubation of the ovary disappeared after 6-h postincubation in the non-radioactive medium. It can be suggested that the size of mature lipid bodies does not change because of the dynamic balance between synthesis and degradation of lipids inside them. It was shown that lipotubuloid growth dynamics was closely correlated with the growth of ovary epidermis cells. Lipids of lipotubuloids are probably building block of cuticle.

**KEYWORDS:** DGAT2, lipase, lipid bodies in statu nascendi, mature lipid bodies, $^3$H-palmitic acid, autoradiography, ovary epidermis, immunogold, cuticle synthesis

**INTRODUCTION**

Plant and animal cell lipid bodies are spherical and have simple construction. They are surrounded by a unique phospholipid monolayer (half lipid membrane; [1]), with adjacent structural proteins [2] among which oleosins are most common in plants while those of perilipin family (PAT family; [3]) in animals. Moreover, different functional proteins involved in lipid metabolism were also identified in the lipid body fraction [4]. The hydrophobic core of lipid bodies is mostly filled with triacylglycerols and free fatty acids that is why they used to be treated only as the source of energetic substances. However, during the last decade they were shown to be active organelles involved in lipid homeostasis, in intracellular signaling, in transient protein storage and their degradation, in protein lipidation, in membrane biogenesis and in eicosanoid synthesis [5-9]. Disturbances in their functioning cause numerous diseases both in animals and humans e.g. obesity, diabetes, arteriosclerosis, cardiovascular disease,
allergic inflammation, arthritis, mycobacterial infections, bacterial sepsis, acute respiratory distress syndrome [10]. Due to that and because lipid bodies are important in biotechnology [11-13] the research concerning them has become crucial.

Although up till now many papers concerning lipid bodies have been published, still numerous questions regarding their functioning need elucidation, e.g. biogenesis remains the subject of controversy. Using freeze-fracture replica immunogold labeling it has been shown that lipid bodies appear to develop externally to both ER membrane leaflets at specialized sites in which the ER enwraps the droplet [14]. However, it is generally accepted on the basis of old ultrastructural observations of seeds [15] that lipid bodies are formed in ER through the accumulation of triacylglycerols between phospholipid leaflets of ER membrane; simultaneously a phospholipid monolayer surrounding lipid bodies in statu nascendi appears [16-18].

The above described biogenesis of lipid bodies was also observed in *O. umbellatum* [19]. During the earliest stage of this process two monolayers of rough ER move apart, in the space between them barely detectable osmiophilic substance appears which later becomes more abundant and more osmiophilic. Lipid bodies in statu nascendi are still connected with ER till the moment they reach an intermediate form between a developing and mature lipid body. The latter are spherical and surrounded by a distinct half unit membrane. At that stage lipid bodies are no longer directly connected with ER but they are entwined by microtubules joining them with ER due to the fact that at one end they are connected with lipid bodies at the other with ER [19].

Lipid bodies in *O. umbellatum* ovary epidermis are assembled in the cytoplasm domain called lipotubuloid [20]. This term was introduced to point out the fact that they are not plastids as the former term “elaioplasts” introduced by Wakker [21] suggested. The lipotubuloid does not have its own membrane but great part of its surface is surrounded by tonoplast since it invaginates into a vacuole. Lipotubuloids were first noticed in *O. umbellatum*; however they were also described in *Haemanthus albiflos* [22] and in *Vanilla planifolia*, *Funkia Sieboldiana*, *Althaea rosea* [23]. Their presence seems to be more common which need elucidation.

The lipotubuloids mainly consist of lipid bodies surrounded by a system of microtubules which join neighboring lipid bodies. When growth of epidermis is finished, microtubules disappear before the lipotubuloid breaks up into single lipid bodies [24] which means that their role is that of a framework. Moreover, as it was shown in the former paper [19] the presence of gold grains at the microtubule surface following the immunogold reaction to diacylglycerol acyltransferase (DGAT) and phospholipase D suggests that the microtubules can serve as transmitters of these enzymes to the lipid bodies where lipid synthesis takes place. Since the microtubules connect the lipid bodies with ER, which contains enzymes necessary for lipid synthesis all factors indispensable for this synthesis may move to the lipid bodies through the microtubules. The latter also seem to be transmitters of lipid precursors because they are the first to be labeled with silver grains after the incubation in 3H-palmitic acid. Labeling of the lipid bodies appears only after 2-h incubation of the ovary [25]. The role of microtubules in lipid synthesis is also suggested by the fact that a microtubule inhibitor, propyzamide blocks 3H-palmitic acid incorporation into the lipotubuloids [19].

Moreover, the microtubules together with actin filaments [26] are also involved in the generation of specific rotary-progressive movement of lipotubuloids [27]. Despite the lack of their own membrane the lipotubuloids move in a cell as one body. It was shown that the progressive movement was cyclosis - dependent while the rotary one was autonomic. These movements are sometimes violent and their direction, rotation axis and dynamics may vary [27]. Rotation speed of a lipotubuloid reaches 3.14 µm/sec and is 6.2 times faster than that of cyclosis. What is more, the use of dinitrophenol (DNP) to block cyclosis stops this process earlier than it blocks rotation of lipotubuloids [20]. Intracellular movements play an important role in accelerating the exchange of molecules between organelles and a cell [28].

In lipotubuloids apart from lipid bodies and microtubules there are other organelles: numerous ribosomes, ER vesicles and cisternae as well as
single mitochondria, microbodies, Golgi structures and autolytic vacuoles. It is worth noting that in the recently studied species, *H. albiflos* [22], *V. planifolia*, *F. Sieboldiana* and *A. rosea* [23] the same organelles are there in lipotubuloids which implies their functional connection with lipid bodies and lipid metabolism.

The present paper is a continuation of earlier studies aiming at elucidation of the structure and function of lipid bodies and lipotubuloids. It was shown that mature, spherical lipid bodies maintained similar sizes through lipotubuloid development because they were the site of both synthesis and degradation of lipids. This was proved by the immunogold method revealing the presence of DGAT2 and lipase in them as well as by autoradiography studies with the use of $^3$H-palmitic acid. Lipotubuloid development is closely correlated with the growth of ovary epidermis cells and of the developing fruit. Lipids of lipotubuloids are probably building block in cuticle synthesis.

**MATERIALS AND METHODS**

Ovarian epidermis from different stages of flower and fruit development of *Ornithogalum umbellatum* was used. Three main stages of ovary development were distinguished (I - flower buds, II - blooming flowers and III - ovaries turned into fruits); each of them was additionally divided into three size categories (small, middle and large) which gives together nine stages (1-9). The epidermal cells were in the phase of intense elongation and non-dividing.

**Light microscope observations**

The living epidermis cut with a razor blade was examined in water and photographed with the use a Nikon DXM 1200 CCD camera. Measurements of lipotubuloid and epidermis cell sizes were made by means of computer software for cytophotometry (Cytophotometer v 1.2; Forel, Łódź, Poland). The correlation coefficient between epidermis cell and lipotubuloid sizes, and means ± SE were estimated with the use of Microsoft Excel 2007.

**The autoradiography in light microscope**

The best results were produced with the method employing OsO$_4$-fixed squashed preparation. The control material consisted of corresponding squashed preparation from which all the lipid material had been extracted. Whole ovaries were treated $^3$H-palmitic acid (at the final concentration - 12$m$Ci/ml, spec. activity: 31.0 Ci/mmol; Perkin Elmer Boston USA) for 2-h incubation. After washing with distilled water the epidermis cut off from the ovaries was fixed with 1% water solution of OsO$_4$ for 1-h. After fixation, the tissue sections were squashed onto Polysine$^\text{TM}$ (Menzel-Glaser) glass slide. Following freezing with dry ice, coverslips were removed. Prior to staining by the Unna method the OsO$_4$-fixed preparations were treated with 10% hydrogen peroxide for 15 min. After air-drying the preparation were coated with EM-1 emulsion (Amersham-Elkabe) for light microscopy, exposed for 6 days, developed and fixed according to the standard method. The number of autoradiographic grains over compartments of cell was counted.

**Electron microscopy**

Ovary epidermis was fixed in the mixture of freshly prepared 2.5% glutaraldehyde and 1% OsO$_4$ (1:1) in cacodylate buffer (pH 7.4) for 1h and post fixed in 1% OsO$_4$ in the same buffer at 4°C for 1h. After dehydration in the ethanol series, the material was embedded in the medium consisting of Epon 812 and Spurr’s resin. Ultrathin sections cut using Reichert Jung Ultracut ultra microtome with the glass knife were double stained with uranyl acetate and lead citrate according to Reynolds [47]. The sections were examined and photographed in JEOL JEM 1010 transmission electron microscope at 80kV acceleration voltage.

**Electron-microscopy autoradiography**

The ovaries of plants were incubated in $^3$H- palmitic acid (12$m$Ci/ml spec. activity 31.0 Ci/mmol; Perkin Elmer Boston) for 120 min. Contiguous epidermis sections fixed as described above (EM section), followed by alcohol dehydration and epon-embedding. Ultrathin sections were placed on grids and coated with Ilford L4 emulsion using the platinum loop [48]. After 7 months of exposure the preparations were developed in a phenidone containing developer [49] and contrasted according to Reynolds [47].

**Immunogold technique**

The plant material was prepared as it is described in EM section. Fixation only in the glutaraldehyde,
which is recommended in immunogold technique, results in destruction of lipotubuloids hence we added 1% OsO₄ to fixative mixture. Freshly cut ultrathin sections were mounted on nickel Formvar coated girds for EM investigations. Prior to immunogold reaction the sections were treated with 10% hydrogen peroxide for 15 min to remove osmium which changes antigen structure [30] and washed in distilled water and PBS (0.01M, pH 7.4, Sigma). Air dried grids with the 4 sections were blocked with 0.5% BSA and 0.05% Tween 20 in PBS for 20 min and then dried with tissue-paper and incubated with the primary rabbit polyclonal anti-DGAT2 (H-70) antibody (Santa Cruz Biotechnology USA) or anti-lipase antibody (Abcam, UK) in antibody diluent (DAKO) 1:100 (overnight at 20°C). Then the gird were washed 10 time for 5 min each in PBS and incubated with the secondary antibody (goat anti-rabbit IgG conjugated with 10 (DGAT2) or 20 (lipase) nm gold (no EMGAR10, or EM GAR 20) diluted 1:50 in the antibody diluent for 1.5 h in the same temperature and rinsed again in PBS and distilled water (10 times for 5 min each). Ultrathin sections were double stained, examined and photographed as above.

RESULTS

The size of lipid bodies during lipotubuloid development

Pictures of lipotubuloids in living cells at different developmental stages suggested that lipid bodies which form them were of similar sizes throughout this process (Figure 1A). Ultrastructural studies support this hypothesis. Diagrams representing the sizes of the population of lipid bodies at three lipotubuloid developmental stages showed that they fell into five size classes with the range of 0.04 to 0.5 µm at every stage (Figure 1B). The most numerous population at all stages was that of 0.1 to 0.4 µm (2-4 classes); these were mature lipid bodies. Only in intensively growing small lipotubuloids, lipid bodies in statu nascendi (0.04-0.1 µm, 1 class) prevailed which was connected with very active lipotubuloid growth resulting from the formation of new lipid bodies. At the stage preceding lipotubuloid disintegration when autolytic vacuoles were present, lipid bodies of these sizes (class 1) were the least numerous which means that their biogenesis became slowed down (Figure 1B).

 Autoradiographic studies showing the incorporation of radioactive palmitic acid

It was shown that lipotubuloids intensively incorporated ³H-palmitic acid into epidermis after immersion of the ovary into the radioactive solution. After 2-h incubation the number of silver grains per a lipotubuloid was about 240, which was about 82% of all grains visible over epidermis cells (Figure 2 A; Table 1). The observed silver grains were connected with ³H-palmitic acid incorporation into lipids since following their extraction with fat solvents autoradiograms were nearly unlabeled (Table 1).

Ultrastructural autoradiographic studies showed that the labeled ³H-palmitic acid was incorporated into the peripheries of lipid bodies where they were adjacent to the hydrophobic core/half unit membrane (Figure 2B-J). Autoradiograms of the ovaries incubated for 2 h and post-incubated for 6 h in a dark, humid chamber in non-radioactive medium did not show selective labeling of lipotubuloids. Silver grains were uniformly located over the whole cell. About 12% of the grains visible on the autoradiograms just after incubation still remained over the lipotubuloids (Table 1). This means that lipotubuloids did not accumulate the whole pool of lipids synthesized during incubation with radioactive palmitic acid or that the accumulation was short-lasting.

During 6-h postincubation about 88% of lipids which were synthesized during incubation were transferred to other cell compartments outside the lipotubuloid where they could be incorporated into the metabolized substances (which was reflected by decreased number of silver grains over autoradiograms) and into substances which were soluble or insoluble in lipid solvents. Lipid solvents only partly diminished the number of grains in these autoradiograms (Table 1). Cross sections of O. umbellatum ovary epidermis revealed that about 19% of grains (Table 1) still present after extraction were localized in the area corresponding to cuticular layer presented by Bird [29] (Figure 3A comp with Figure 3B); probably they correspond with cutine.
Lipid bodies contain DGAT2 and lipase, synthesize lipids

Figure 1. Percentage of particular lipid body size classes during three stages of lipotubuloid development. (A) Lipotubuloids in living cells at successive stages of their development (I – III). (B) Size classes of lipid bodies during different stages of lipotubuloid development. 1) 0.04 – 0.1 µm, 2) 0.11 – 0.2 µm, 3) 0.21 – 0.3 µm, 4) 0.31 – 0.4 µm, 5) 0.41 – 0.5 µm; bar = 10 µm.

Table 1. Number of silver grains over particular compartments of O. umbellatum ovary epidermis cell after incubation in 3H-palmitic acid under different experimental conditions ± SE.

<table>
<thead>
<tr>
<th>Labeled area</th>
<th>Incubation in 3H-palmitic acid</th>
<th>Incubation in 3H-palmitic acid after lipid extraction</th>
<th>Incubation in 3H-palmitic acid and 6 h post-incubation in non-radioactive medium</th>
<th>Incubation in 3H-palmitic acid and 6 h post-incubation in non-radioactive medium after lipid extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Cell</td>
<td>294 ± 14</td>
<td>4 ± 0.8</td>
<td>102 ± 5</td>
<td>19 ± 0.9</td>
</tr>
<tr>
<td>Lipotubuloid</td>
<td>240 ± 9</td>
<td>1 ± 0.5</td>
<td>12 ± 0.4</td>
<td>3 ± 0.8</td>
</tr>
<tr>
<td>The rest of cytoplasm and nucleus</td>
<td>54 ± 1,8</td>
<td>3 ± 0.9</td>
<td>90 ± 11</td>
<td>16 ± 1,1</td>
</tr>
</tbody>
</table>
over the half unit membrane (Figure 5B), but resulted in disappearance of the dark color filling the inside of lipid bodies, which became visible as light area surrounded by a phospholipid monolayer. Gold grains were also present near ER and over microtubules closely to lipid bodies (Figure 5B) and in the space between them. This localization of the gold grains showing DGAT2 together with the autoradiographic presentation of the ultrastructure proved that the final stage of triacylglycerole synthesis consisting in the transformation of DGA into TGA took place at the peripheries of lipid bodies.

**DGAT2 localization in lipid bodies**

Specificity of the antibody was confirmed with the western blot method which revealed the presence of one specific band of 44 kDa (Figure 5A).

Mature, spherical lipid bodies were filled with osmiophilic substance and surrounded with a distinct phospholipid monolayer (Figure 4A). Prior to immunogold reaction with the anti-DGAT2 antibody coupled with gold (10 nm) ultrathin sections were treated with H₂O₂ to remove osmium (Figure 4B), which changes the structure of antigen [30]. The presence of DGAT2 very often were localized over the half unit membrane (Figure 5B), but resulted in disappearance of the dark color filling the inside of lipid bodies, which became visible as light area surrounded by a phospholipid monolayer. Gold grains were also present near ER and over microtubules closely to lipid bodies (Figure 5B) and in the space between them. This localization of the gold grains showing DGAT2 together with the autoradiographic presentation of the ultrastructure proved that the final stage of triacylglycerole synthesis consisting in the transformation of DGA into TGA took place at the peripheries of lipid bodies.
Lipid bodies contain DGAT2 and lipase, synthesize lipids

Figure 3. Fragment of a cuticle cross section.
(A) *O. umbellatum* ovary epidermis after incubation in radioactive palmitic acid, post-incubated for 6 h in non-radioactive medium and extraction with a lipid solvent. Autoradiographic grains located in the cuticle layer.
(B) Schematic view of cuticle in higher plants according to Bird (2008). bar = 5 µm.

Figure 4. Fragment of *O. umbellatum* lipotubuloid.
(A) After OsO₄ and glutaraldehyde fixation.
(B) Fix as previously and treated with H₂O₂.
ER - endoplasmatic reticulum; mt - microtubules; lb - lipid bodies
bar = 200 nm.
Correlation between sizes of lipotubuloids and the cell dimension growth during ovary development

The earliest stage of lipotubuloid development takes place in tiny *O. umbellatum* flower buds in which an ovary is about 1 mm long. During development it grows up to 8 mm simultaneously becoming a fruit [31]. During that time ovary epidermis cells do not divide but grow, about 30 x (3000%). Now it was shown that this growth was directly proportional to the enlargement of lipotubuloids (Figure 7, correlation coefficient 0.98).

DISCUSSION

Our results concerning DGAT2 presence are in agreement with those of other authors. In adipocytes and CoS7 fibroblasts the immunogold method revealed DGAT2 at the lipid body peripheries [32]. On this basis the authors think that conversion of DAG into TAG i.e. the final stage of lipid synthesis, takes place in lipid bodies. Buers *et al.* [33] are of identical opinion on the basis of macrophage analyses with freeze-fracture replica immunogold methods.

Localization of lipase in lipid bodies and in lipotubuloid autolytic vacuoles

Specificity of the antibody used for detection of lipase was confirmed with the western blot method which revealed the presence of specific band of 54 kDa (Figure 6A).

Contrary to the products of the reaction showing DGAT2, 20 nm gold grains proving the presence of lipase were localized somewhat deeper in the lipid bodies, closely to the phospholipid monolayer. They were connected with the fine structure penetrating the core of lipid bodies (Figure 6B). Thus both enzymes, DGAT2 and lipase, were localized near the surface of lipid bodies.

Moreover, lipase was abundant in autolytic vacuoles of lipotubuloids (Figure 6C). These vacuoles were surrounded with a distinct tonoplast formed by a phospholipid bilayer and in addition to gold grains they contained membrane fragments and some shapeless bodies characteristic of autolytic vacuoles. The observed phenomenon is completely in agreement with the earlier results of histochemical reactions observed in light microscope with the use of method to reveal lipase and acid phosphatase [24].

Figure 5. Immunodetection of DGAT2 in *O. umbellatum* ovary epidermis.

(A) Western blot analysis; line 1 – SDS-PAGE electrophoretic separation of the ovary epidermis extract; line 2 – Western blotting of the ovary epidermis extract probed with the anti-human DGAT2 antibody; line 3 – molecular mass standards and their weights in kDa.

(B) Electron microscopy immunogold labeling; gold grains located at the lipid body surface.

ER - endoplasmatic reticulum; mt - microtubules; lb - lipid bodies; bar = 100 nm.
Lipid bodies contain DGAT2 and lipase, synthesize lipids

It has been suggested that DGAT2 may constitute a rate-limiting factor in TAG bioassembly in developing seeds [34]. In the case of *O. umbellatum* the autoradiographic studies with the use of \(^3\)H- palmitic acid are an additional argument indicating that the surface of lipid bodies is an active site of lipid synthesis. Silver grains showing incorporation of this precursor into lipids are localized over the lipid body half unit membrane. The location of gold grains reflecting DGAT2 presence was the same. Similarity of these data indicates that the lipid synthesis in *O. umbellatum* takes place at the surface of mature, spherical lipid bodies. In the case of *O. umbellatum* lipotubuloids the widely accepted fact that lipid synthesis occurs in ER was confirmed by us during biogenesis of new lipid bodies which was closely correlated with rough ER (comp. Introduction).

**Figure 6.** Immunodetection of lipase in *O. umbellatum* ovary epidermis. 
(A) Western blot analysis; line 1 – SDS-PAGE electrophoretic separation of the ovary epidermis extract; line 2 – Western blotting of the ovary epidermis extract probed with the anti-human lipase antibody; line 3 – molecular mass standards and their weights in kDa. 
(B) Localization of gold grains at lipid body surfaces and at microtubules. 
(C) Localization of gold grains inside autolytic vacuoles and at lipid body surfaces. 
av – autolytic vacuol; ER - endoplasmatic reticulum; mt - microtubules; lb - lipid bodies; bar = 100 nm (B), bar = 500 nm (C).
Lipase is also present in the mature, spherical lipid bodies. It was revealed in other plants with biochemical methods. Su et al. [35] showed and characterized lipase in lipid bodies of Chinese varietas of castor bean seeds. Lipase bound to the oil bodies was also observed in Olea europaea in olive fruit [36]. Eastmond [37] with the use of immunogold method revealed lipase near lipid bodies surface. In O. umbellatum lipid bodies gold grains indicating lipase were localized somewhat deeper than those showing the presence of DGAT. They were localized near fine structures connected with the phospholipid monolayer, invaginating slightly into lipid bodies core. These structures seem to correspond to hydrophobic anti-parallel beta-stretch domain of oleosin, structural proteins covering the lipid bodies surface [38, 39]. They were revealed at the lipid bodies surface with the immunogold method [40]. They protect the lipid bodies against merging during seed germination. It is suggested that the specific oleosin region may contain receptor sites for lipases [38, 39, 41-44]. Lipases are acylhydrolases that play a key role in fat digestion. Due to an opposite polarity between the enzyme (hydrophilic) and the substrates (lipophilic), lipase reaction occurs at the interface between the aqueous and oil phases [45, 46].

Since immunogold reactions show that DGAT2 and lipase are located near the phospholipid monolayer, newly synthesized lipids most probably become lipolyzed at the earliest because they are placed nearest to lipase. This possibility is suggested by autoradiographic studies with the use of 2-h incubation in $^3$H-palmitic acid followed by post-incubation. While just after incubation lipotubuloids were the most labeled structures in a cell, after 6-h post-incubation in dark silver grains were evenly dispersed over the whole cell [25]. Thus the lipids synthesized in lipotubuloids were used by the cell and partly metabolized because simultaneous decrease in epidermis radioactivity was observed. The other part of lipids are transformed into substances non extracted in lipid solvent. Cross sections of epidermis revealed that autoradiographic grains still present after extraction were localized in the area corresponding to the cuticle of cuticular layer. Autoradiographic labeling which disappeared after extraction with lipid solvent while they were visible after post-incubation over the whole cell may have indicated labeled waxes which are cuticle components soluble in organic solvent. Cross sections of epidermis revealed that autoradiographic grains still present after extraction were localized in the area corresponding to the cuticle of cuticular layer. Autoradiographic labeling which disappeared after extraction with lipid solvent while they were visible after post-incubation over the whole cell may have indicated labeled waxes which are cuticle components soluble in organic solvent. Light layer at the outside of cuticular layer seems to be cuticle proper [29] which was formed probably prior to isotope incubation. This result suggests that lipid components of lipotubuloids become building block of cutin and waxes. The question arises whether these changes take place in lipotubuloids or in other cell components and lipids from lipotubuloids are only building material. Moreover, it is understandable that the sizes of mature lipid bodies in lipotubuloids remained similar during ovary development since these lipids
which were *de novo* synthesized were after some time removed from the lipid bodies by lipolysis. Thus there must exist precise mechanisms regulating biogenesis of new lipid bodies, synthesis and lipolysis of lipids as well as enlargement of epidermis cell and cuticle synthesis, the mechanisms correlating these parameters with the growth of ovary and then fruit and seeds developing in it [31]. We have shown that the growth of ovary cells is closely correlated with the size of lipotubuloids (correlation coefficient 0.98) which to some extent depends on DNA endoreduplication. The higher nuclear polyploidy the bigger are the cells containing greater lipotubuloids although these values are not directly proportional. Moreover, the upper part of the ovary, containing bigger cells and bigger lipotubuloids than the lower part, exhibits higher GA\(_3\) level [31]. Thus the processes described in *O. umbellatum* ovary epidermis are under genetic control through nuclear factors and under hormonal control. This is in agreement with physiological and developmental studies concerning fat production regulation in the seeds of *Arabidopsis thaliana* and its mutants, which prove the existence of a very complicated network regulating seed development and storage of fats in them. This network consists of hundreds of regulatory transcriptional factors in a crosstalk with physiological signals [13].

**CONCLUSIONS**

Lipotubuloids are a very specific, dynamic, complicated set of metabolically active (although seemingly static) lipid bodies containing DGAT2 and lipase which cooperate with microtubules and other organelles. A lipotubuloid is somewhat independent in a cell which is reflected by its capability for autonomous rotary movement, however, it is closely correlated with the development of the ovary epidermis and cuticle synthesis during seed formation in a fruit. Studies concerning these structures may further elucidate lipid metabolism and their functional relation with a cell and its organelles as well as with an organ whose part it is.

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