Immunogold method evidences that kinesin and myosin bind to and couple microtubules and actin filaments in lipotubuloids of *Ornithogalum umbellatum* ovary epidermis

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Abstract Lipotubuloids in ovary epidermis of *Ornithogalum umbellatum* which are a domain of cytoplasm containing a lot of lipid bodies, microtubules and actin filaments, ribosomes, endoplasmic reticulum as well as scarce mitochondria, microbodies, dictyosomes, autolytic vacuoles, exhibit progressive-rotary motion. The immunogold method demonstrated that microtubules and actin filaments of lipotubuloids might be connected with one another by myosin and kinesin. It was supposed that collaboration of motor proteins with actin filaments and microtubules makes autonomic high peripheral speed rotary motion of lipotubuloids in epidermis cells possible. Moreover, myosin was also detected in Golgi bodies in lipotubuloid. In lipotubuloids, the immunogold method demonstrated immunosignals after the use of an antibody to dynein light chains but spectroscopy mass analysis showed that in *O. umbellatum* epidermis lacked dynein heavy chains.

Keywords Actin filaments · Kinesin · Lipotubuloids · Microtubules · Myosin · Rotary motion

Introduction

In plant and animal cells, the cytoskeleton consists of two major networks of protein polymers, actin filaments and

microtubules. However, classic intermediate filament proteins generating support in animal cells are absent from plant genomes (Collings 2009).

The cytoskeleton is involved in many fundamental cellular processes including morphogenesis, cell divisions and vesicle trafficking. It performs various functions with the assistance of interacting molecular motor proteins, signaling molecules or structure-supporting elements. Although actin filaments and microtubules fulfill many functions independently, they often act in a coordinated manner (Petrášek and Schwarzerová 2009). Intracellular transport along actin filaments and microtubules depends on the associated motor proteins, myosins connected with actin filaments and kinesins and dyneins connected with microtubules. In higher plants, homologs of both myosins and kinesins are represented by gene families (Lee and Liu 2004), whereas dynein homologs are lacking in *Arabidopsis* (Lawrence et al. 2001). However, examination of the whole genome sequence of *Oryza sativa* has revealed that four dynein heavy chains are present in this monocot (King 2002). A high molecular weight polypeptide, cross-reacting with the antibodies to the dynein heavy chain, was also demonstrated in *Triticum aestivum* root meristem cells in the subset Golgi complex (Shanina et al. 2009). These results indicate that the apparent lack of dynein sequences in *Arabidopsis* is not a general feature of the angiosperm genomes.

Some evidence suggests that organelle movement and positioning involve interaction of microtubules, while the actomyosin system generates motility and reorientation of microtubules (Sainsbury et al. 2008). In animal cells, actin-based vesicle-transport motor, MyoVA, can interact directly with a microtubule-based transport motor, KhcU. Localization of MyoVA and KhcU overlaps in a cell (Huang et al. 1999). In animal cells, cross-talk between the

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mitotic actin cortex and plus ends of astral microtubules was demonstrated (Kunda and Baum 2009). In mouse melanoma cells (B16-F1), microtubules function as platforms for assaying actin polymerization in vivo (Oelkers et al. 2011).

Reports of actin filament and microtubule associations in plants are supported by imaging of fixed tissues, pharmacological studies and the existence of common binding partners. Localization of actin filaments on microtubular mitotic apparatus was demonstrated immunocytochemically in tobacco BY-2 cells (Yasuda et al. 2005). The structural cross-talk between actin and tubulin components was observed in the zone of distal elongation of *Arabidopsis thaliana* roots (Shevchenko 2009). A plant-specific kinesin binds to actin filaments and interacts with cortical microtubules in cotton fibers (Preuss et al. 2004), and in *A. thaliana* root cells (Collings et al. 2006). Dynamic bridges are a calponin-domain kinesin from rice which link actin filaments and microtubules in both cycling and non-cycling cells (Frey et al. 2009). SB401 pollen-specific protein from *Solanum berthaultii* binds to and couples microtubules and actin filaments (Huang et al. 2007). Kinesin GhKCH2 interacts with both microtubules and microfilaments in living plant cells of *Arabidopsis* protoplast as well as in biological processes they co-ordinate together in cotton cells (Xu et al. 2009). In *Arabidopsis*, the type II formin 14 interacts with microtubules and microfilaments and regulates cell divisions (Li et al. 2010). Using spinning disc confocal microscopy in live cells of dual-labeled lines, coupled with pharmacological studies, it was deduced quantitatively that actin filaments and microtubules interact dynamically in *A. thaliana* (Sampathkumar et al. 2011).

The coalescence of short actin filaments and long microtubules was observed in ovary epidermis cells of *O. umbellatum* in EM microscopy (Kwiatkowska et al. 2005). These actin filaments, lying parallel to the surface of microtubules, are sensitive to cytochalasin B and are connected with rotary motion of lipotubuloids, i.e. a cytoplasmic domain containing aggregates of lipid bodies surrounded by microtubules which also include abundance of ribosome and endoplasmic reticulum, as well as single dictyosomes, mitochondria, microbodies and autolytic vacuoles (Kwiatkowska 1972; Kwiatkowska et al. 2009, 2012). Lipotubuloids do not have their own membrane but on the large area they are surrounded by a tonoplast as they invaginate into vacuoles. They move as one body because microtubules running in different directions surround and connect adjacent lipid bodies stabilizing their position.

Lipotubuloids were also observed in other plants: *Haemantus albiflos* (Kwiatkowska et al. 2010) *Althaea rosea*, *Funkia Sieboldiana*, *Vanilla planifolia* (Kwiatkowska et al. 2011a).

In the present study, we examined the localization of motor proteins in lipotubuloids. We used the immunogold labeling method to identify motor proteins, kinesin and myosin as well as dynein light chains. The immunogold technique, first introduced to EM observation by Faulk and Taylor (1971), has been recently used to investigate motor proteins (Romagnoli et al. 2007; Lam et al. 2008; Wei et al. 2009; Brozzi et al. 2012; Lenartowska et al. 2012; Loubéry et al. 2012). Axons studied with double labeling immunogold technique showed preferential binding of a kinesin-1 motor to GTP-tubulin-rich microtubules (Nakata et al. 2011).

Circulation of cytoplasm in ovary epidermis cell is multidirectional, different in each cytoplasm strand which led to the suggestion that it may be the source of lipotubuloid rotation, as they are surrounded by cytoplasm stream going in various directions. However, only the progressive movement of lipotubuloids depends on cyclosis, since its blockade with 2,4 dinitrophenol (DNP), which stops ATP synthesis, results in the blockade of this movement while consecutive 2–3 slowing down rotations of lipotubuloids can be observed during 90 s following cyclosis immobilization. Moment inertia may be ignored since the time for stopping the process, for bodies of this size order rotating in fluid, is only 10^{-7} s (Kwiatkowska 1972). This means that lipotubuloids have their own force driving rotation.

Using EM *in planta*, we demonstrated for the first time that myosin as well as kinesin bound to and mechanochemically coupled microtubules to actin filaments. Moreover, the Western blot indicated that dynein heavy chains were absent; however, the immunogold technique demonstrated the presence of dynein light chains in lipotubuloids of *O. umbellatum* epidermis.

Materials and methods

Plant materials

Ovary epidermis from fully developed flowers of *O. umbellatum* was used. The epidermal cells were in the phase of an intense elongation with a single lipotubuloid in each one.

Intracellular movement in *O. umbellatum* ovary epidermis

The observations in vivo were documented by means of film pictures at a frequency of 12 or 6 stills per second, which resulted in double or quadruple acceleration of the pictures of the motion during projection at a frequency of 24 stills per second.

Electron microscopy

Ovary epidermis was fixed in the mixture of freshly prepared 2.5 % glutaraldehyde and 1 % OsO₄ (1:1) in cacodylate buffer (pH 7.4) for 1 h and post fixed in 1 % OsO₄ in the same buffer at 4 °C for 1 h. 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 a glass knife were double stained with uranyl acetate and lead citrate according to Reynolds (1963). The sections were examined and photographed in JEOL JEM 1010 transmission electron microscope at 80 kV acceleration voltage.

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 and hence we added 1 % OsO₄ to the fixative mixture. Freshly cut ultrathin sections were mounted on nickel Formvar coated grids 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 (Bendayan and Zollinger 1983) and washed in distilled water and PBS (0.01 M, pH 7.4, Sigma). Air-dried grids with four 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-kinesin antibody (Thermo Scientific, cat. number PA1-19392) or mouse monoclonal anti-myosin antibody, light chains 20 kDa (Sigma, cat. number M4401) and goat polyclonal anti-dynein light chain (Santa Cruz Biotechnology, cat. number sc-15942) in antibody diluent (DAKO) 1:20 (kinesin, myosin) and 1:25 (dynein); overnight at 20° C. Then, the grids were washed ten times for 5 min each in PBS and incubated with the secondary antibody goat anti-rabbit IgG conjugated with 10 nm gold (kinesin) or goat anti-mouse IgG conjugated with 20 nm gold (myosin) and rabbit anti-goat IgG conjugated with 10 nm gold (dynein) (no EM.GAR10, or EM.GMHL20 and EM.RAG10; Polysciences) 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.

Double labeling immunogold technique

Ovary epidermis was fixed as above. The plant material was prepared as it is described in EM section, and fixation was the same as described above.

Further steps were similar to the single labeling immunogold technique. The sections on grids were incubated

overnight at 20 °C with the mouse monoclonal anti-myosin antibody (Sigma) diluted in an antibody diluent (pH 8.0, DAKO) at 1:20 dilution, and were incubated with the antibody (goat anti-mouse IgG conjugated with 10 nm gold [no. EM.GMHL10, Polysciences]) diluted 1:50 in the antibody diluent for 1.5 h at the same temperature. Next, the above procedure was repeated for rabbit polyclonal antibody to kinesin diluted in an antibody diluent (pH 8.0, DAKO) at a 1:20 dilution, and incubated with the final antibody (goat anti-rabbit IgG conjugated with 20 nm gold [no. EM.GAR20, Polysciences]) diluted 1:70 in the antibody diluent for 1.5 h at the same temperature.

Western blot technique

Proteins from ovary epidermis were extracted according to Wang et al. (2006) and then fractionated on 3–8 % NuPAGE Novex Tris–Acetate gels and blotted onto Nitrocellulose Membrane 0.45 µm pore size (Invitrogen). *O. umbellatum* motor proteins: kinesin, myosin and dynein were detected with the rabbit polyclonal anti-kinesin antibody (Thermo Scientific), mouse monoclonal anti-myosin antibody (Sigma) and goat polyclonal anti-dynein antibody (Santa Cruz Biotechnology) diluted 1:50 (kinesin) and 1:200 (myosin and dynein) using Chromogenic Western Blot Immunodetection Kit (Invitrogen). Instead of the primary antibody, non-immune goat serum at the same concentration as the specific antibody was used as the control.

Analysis of protein extracts of *O. umbellatum* ovary epidermis regarding the presence of dynein heavy chains was carried out in Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics, PAS, Warszawa, Poland.

Statistics

To estimate the percentage of kinesin and myosin as potential motor proteins in lipotubuloids, the number of gold grains was estimated for: (1) kinesin and myosin appearing separately, (2) colocalization of both proteins cooperating with a single microtubule and (3) kinesin and myosin adjacent to heterodimers. The sum of gold grains for the above-mentioned items was taken as 100 %. Micrographs of 30 lipotubuloids from different epidermal cells of *O. umbellatum* ovary were analyzed.

Results

Character of intracellular movement in *O. umbellatum* ovary epidermis

Figure 1 shows a drawing of a living epidermis cell presented in a phase contrast (a), and of a lipotubuloid as it

Fig. 1 Fragment of a living epidermis cell of *O. umbellatum* ovary: **a** drawing of a cell in a phase contrast with a lipotubuloid which turned around several times during 10–12 s, changing direction and axis of rotary motion; long arrows—the direction of lipotubuloid rotation, short arrows—direction of cytoplasm movement; **b** lipotubuloid as it was seen in EM (Kwiatkowska 1972, modified); *g* Golgi apparatus, *lb* lipid body, *m* mitochondrion, *n* nucleus, *v* vacuole

was seen in EM (b). Progressive movement of a lipotubuloid in an epidermis cell is presented in Fig. 2a–d. As can be seen in Fig. 2a, the lipotubuloid passes along a cell wall in adjacent cytoplasm but it can also move in a cytoplasm strand, reach the opposite wall, change direction or stop for some time in different parts of a cell, most often near a nucleus (Fig. 2b–d). Rotations of a lipotubuloid accompany progression or may appear when it stops in different places inside a cell. Lipotubuloid rotations can be very dynamic with peripheral rotary speed from 5.2 to 31.4 $\mu\text{m/s}$ (this is 6.2-fold faster than the maximum rotary speed of the cytoplasm in epidermis cells), they can change direction, rotation axis and speed.

Lipotubuloid motor proteins

Kinesin localization

Specificity of the antibody used for detection of kinesin was confirmed with the Western blot method which revealed the presence of a specific band of about 360 kDa (Fig. 3a).

10-nm gold grains indicating kinesin were localized near microtubule walls which were best visible on microtubule cross sections (Fig. 3b, c); sometimes two grains were localized at opposite sides of a microtubule. On longitudinal sections of microtubules, 3–5 gold grains were aligned at each side of an actin filament localized between two microtubules (Fig. 3d, e). Actin filaments parallel to

Fig. 2 Progressive movement of lipotubuloids in epidermis cells—schematic picture; *c* cytoplasm, *l* lipotubuloid, *n* nucleus, *v* vacuole, arrows indicate direction of lipotubuloid movement

microtubules were much better visible in preparations not treated with H_2O_2 (Fig. 4a, b), which is used to unmask the receptors of the tested substances in the immunogold technique. Immunosignals indicating kinesin presence were unevenly dispersed near lipotubuloid microtubules. Their localization indicated that they joined actin filaments and microtubules.

Myosin localization

Specificity of the antibody was confirmed with the Western blot method which revealed the presence of one specific band of about 20 kDa (Fig. 5a).

Fig. 3 Detection of kinesin: **a** Western blot analysis; line 1—SDS-PAGE electrophoretic separation of the epidermis cell extract; line 2—pre-immune serum; line 3—Western blotting of the epidermis cell extract probed with the anti-kinesin antibody; line 4—molecular mass standards and their weights in kDa; *Proteins revealed (**b–f**) immunogold localization; 10-nm gold grains near microtubule walls in the cross sections (**b–d**) and in longitudinal sections at each side of an actin filament (*short white arrow*) placed between two microtubules (**e, f**) with schemes of kinesin connection with microtubules (**b, f**); *mt* microtubules, *Bar* = 50 nm

Fig. 4 Microtubules and actin filaments (*arrows*) in a lipotubuloid of *O. umbellatum* ovary epidermis; *lb* lipid bodies, *mt* microtubules, *t* tonoplast, *Bar* = 0.1 μm

20-nm gold grains indicating myosin were localized between two microtubules in a lipotubuloid mostly separately (Fig. 5b, c) or in couples at opposite sides of microfilaments (Fig. 5d, e). They were also visible between cross-sectioned microtubules (Fig. 5d, e). Similarly as kinesin, myosin joined actin filaments with microtubules.

Figure 6 shows a dictyosome of Golgi apparatus in a lipotubuloid (comp. Fig. 1b) which was labeled with gold grains indicating the presence of myosin near cisternae membranes at each side of a dictyosome or near a middle cisternae.

Double labeling immunogold test for kinesin and myosin

The results of the double-immunogold test for myosin (10-nm grains) and for kinesin (20-nm grains) evidence that both motor proteins can be bound to the same microtubule (9 % of total labeling) (Fig. 7a–c). Sometimes 10-nm and 20-nm gold grains were joined together which probably means that myosin and kinesin may form an integrated motor (8 % of total labeling) (Fig. 7d). Labeling for kinesin and myosin alone accounts for 54 and 29 % of the total labeling, respectively.

The fact that microtubules and actin filaments appeared close to one another (Fig. 4) as well as co-localization of gold grains indicating the presence of kinesin and myosin near microtubules (Fig. 7) enabled us to propose models of co-operations between these motor proteins and cytoskeleton filaments in lipotubuloid (Fig. 8).

Localization of dynein light chains

Specificity of the antibody used for detection of dynein light chains was confirmed with the Western blot method which revealed the presence of a specific band of about 14 kDa (Fig. 9a). In lipotubuloids, immunosignals demonstrating localization of dynein light chains were visible in dictyosomes, as well as on lipid bodies periphery, in microtubules and cytoplasm (Fig. 9b, c).

Since analysis of proteins separated in polyacrylamide gel using spectroscopy mass method (Mass Spectrometry

Fig. 5 Detection of myosin: **a** Western blot analysis line 1—SDS-PAGE electrophoretic separation of the epidermis cell extract; line 2—pre-immune serum, line 3—Western blotting of the epidermis cell extract probed with the anti-myosin antibody; line 4—molecular mass standards and their weights in kDa; *Proteins revealed (**b–e**) immunogold localization; 20-nm grains near microtubules in longitudinal section (**b–d**) and cross-section (**e**); *mt* microtubules; *Bar* = 50 nm

Fig. 6 20-nm gold grains demonstrating myosin presence in Golgi structure of *O. umbellatum* lipotubuloid; *g* Golgi apparatus, *lb* lipid bodies, *mt* microtubules; *Bar* = 100 nm

Laboratory, Institute of Biochemistry and Biophysics, PAS, Warszawa, Poland) did not reveal heavy chains of dynein, light chains were not connected with lipotubuloid movement but with other functions.

Discussion

The presence of motor proteins, kinesin and myosin, revealed with the immunogold method in this researches closely corresponds with earlier findings regarding the characteristic lipotubuloid movement (Kwiatkowska 1972; Kwiatkowska et al. 2006, 2009). The activity of both motor

proteins together with two cytoskeletal elements, microtubules and actin filaments, seems to be the driving force generating specific, variable, very dynamic and complex, autonomic rotation of lipotubuloids which depends on ATP (which was proved by the experiments with DNP); according to Gunning and Hardham (1982), it is unique to cells.

Direct interactions between actin filaments and microtubules through motor proteins are found in eukaryotic cells but to date they have not been demonstrated with the immunogold technique. Kinesin and myosin interact with microtubules and actin filaments, respectively. Alternatively, myosin and kinesin motors interact to create an integrated motor, which uses both actin filaments and microtubules as a transport track (Petrášek and Schwarzerová 2009). Although all types of interactions were described in animal organisms, to date only kinesin-mediated interaction between microtubules and actin filaments was identified in plants (Preuss et al. 2004; Xu et al. 2009; Klotz and Nick 2012). The fact that our observations demonstrated that both myosins and kinesins connected actin filaments with microtubules is innovatory.

In general, motors do not work in isolation but in a group (Guérin et al. 2010). Using single-molecule in vitro techniques, Ali et al. (2008) showed that a functional consequence of myosin V's diffusion on microtubules was a significant enhancement of the processive run length of kinesin when both motors were present on the same cargo. The electrostatic interaction of myosin V with microtubules increases the processivity of kinesin. Thus, we

Fig. 7 Double immunogold localization of myosin (10-nm grains) and kinesin (20-nm grains) connected with microtubules: **a** longitudinal-sectioned (*black arrow*) and **b–d** cross-sectioned microtubules (*white arrows*); both grains **d** lie close together; *Bar* = 50 nm

Fig. 8 Models of motor protein connections with microtubules; **a** kinesin connects microtubules with actin filaments, **b** kinesin and myosin connect microtubules with actin filaments, **c, d** two types of connections of kinesin and myosin with a microtubule (a microtubule cross section). Scheme prepared on the basis of current micrographs and papers: Goode et al. (2000), Rodriguez et al. (2003), Schroeder et al. (2010)

Fig. 9 Detection of light chains of dynein: **a** Western blot analysis: line 1—SDS-PAGE electrophoretic separation of the epidermis cell extract; line 2—pre-immune serum; line 3—Western blotting of the epidermis cell extract probed with the anti-dynein light chain antibody; line 4—molecular mass standards and their weights in kDa; * Proteins revealed; **b** immunogold studies demonstrating presence of light chains of dynein in Golgi structure and **c** in lipid bodies and cytoplasm; *g* Golgi apparatus, *lb* lipid bodies, *white arrow*—cross section of microtubules; *Bar* = 100 nm

suppose that the presence of both kinesin and myosin connected with the same microtubules and actin filaments might mutually enhance their motor activity making a lipotubuloid rotate with the speed up to 31.4 $\mu\text{m/s}$, i.e. 6-fold greater than that of cyclosis (Kwiatkowska 1972).

Our earlier studies on lipotubuloid movement in *O. umbellatum* revealed that in a vigorously rotating lipotubuloid microtubules exhibited different widths or diameters on longitudinal (Kwiatkowska 1972) or cross (Kwiatkowska et al. 2009) sections, respectively. The use of OsO_4 for direct fixation of microtubules, due to its quick penetration, allowed to maintain their structure identical to that observed in vivo (Omoto and Kung 1980). Differences in size were even visible in the microtubules consisting of the same number of protofilaments (Kwiatkowska et al. 2009). In such a case, two subpopulations of microtubules differing in diameters, were observed while after blocking the movement with DNP only one population of microtubules with medium-size diameters was present. Upon DNP removal, the two populations reappeared and rotation of lipotubuloids was restored. Analysis of the structure of microtubule walls on the cross sections varying in diameter showed that the differences resulted from different distances between neighboring protofilaments and from the degree of expansion or compression of tubulin units (Kwiatkowska et al. 2006, 2009). A hypothesis has been put forward that changes in microtubule diameter

(Kwiatkowska et al. 2006, 2009) might be connected with the driving force of lipotubuloid movement.

Cao et al. (2004) revealed that in vitro co-sedimentation of microtubules, actin filaments and myosin-Va resulted in microtubules with varying widths, simultaneously myosin-Va bound actin filaments with microtubules. In lipotubuloids, myosin and kinesin also connected microtubules and microfilaments. It seems possible that motor proteins together with actin filaments may drive lipotubuloid rotation changing microtubule conformation, i.e. making narrow ones wide and vice versa.

Flexibility of microtubules connected with the fact that microtubule subunits are bound more strongly inter-protofilaments than within-protofilaments in vitro was shown by many authors (Chrétien et al. 1998; Nogales 1999; Nogales et al. 1999; Löwe et al. 2001; Meurer-Grob et al. 2001; Kis et al. 2002; Sept et al. 2003; Krebs et al. 2005; Tuszyński et al. 2005; Wang and Nogales 2005; Nogales and Wang 2006; Drabik et al. 2007; Hunyadi and Jánosi 2007).

It seems obvious that the progressive-rotary movement of lipotubuloids is crucial for the functioning of epidermis cells which exhibit very active, 30-fold growth during ovary development and its transformation into a fruit. These processes are correlated with DNA endoreduplication and lipotubuloid activity as well as they are regulated by gibberellins (Kwiatkowska et al. 2007, 2011b).

The way a lipotubuloid moves in a cell (see Fig. 2) suggests that this movement is not accidental but is controlled by signals from a cell directing this lipotubuloid to the site, where nutrients and cuticle building blocks are most necessary (Kwiatkowska et al. 2012). It seems possible since microtubules and actin filaments play a receptor-like role for Rho proteins which are involved in many important biological processes, e.g. cell growth (Mucha et al. 2011). Recently, formation of wide microtubules, “macrotubules” was found in plant cells under changing level of reactive oxygen species (ROS) which function as signaling molecules (Livanos et al. 2012).

Phospholipase D (PLD) is another element involved in cytoskeleton-mediated signaling (Ho et al. 2009). Gold grains decorating microtubules which indicate the presence of PLD were observed with the immunogold technique in *O. umbellatum* lipotubuloids (Kwiatkowska et al. 2012).

The progressive-rotary movement undoubtedly enables intensive transport of components present in lipotubuloid lipid bodies into epidermis cells and cuticle. Cytoplasmic flow accelerates the distribution rate of soluble materials in relation to the slow rate of diffusion (Esseling-Ozdoba et al. 2008). This is supported by the results concerning the involvement of intracellular motion in spreading various substances in a cell (Verchot-Lubicz and Goldstein 2010).

The latest autoradiographic and immunocytochemical research revealed that lipids synthesized in lipotubuloids were stored in them only for some time. On the surface of lipotubuloid lipid bodies, similarly as of lipid droplets in oleate-loaded COS-7-cells (Kuerschner et al. 2008; Stone et al. 2009) in macrophages (Buers et al. 2009) and murine cells (McFie et al. 2011) there is DGAT2. This enzyme is involved in triacylglyceride synthesis which was evidenced by autoradiographic studies with ^3H -palmitic acid which is incorporated into the surface layer of lipotubuloid lipid bodies (Kwiatkowska et al. 2012). Lipids forming in them became lipolyzed by lipase and the resulting products were transported out of a lipotubuloid into cytoplasm and following appropriate biochemical reactions were deposited into cuticle (Kwiatkowska et al. 2011b).

The presence of dynein light chains, which was revealed in lipotubuloids with the immunogold technique, is another interesting issue. However, it is not connected with a cell and lipotubuloid movement. Dynein light chain coding genes are present in all eukaryotic genomes, they code extremely conservative 10 kDa proteins (Pfister et al. 2006). Lack of dynein heavy chains observed in Western blot indicates that in *O. umbellatum* lipotubuloids dynein light chains did not form dynein. They are core elements of eukaryotic cells probably binding with hundreds of proteins (Rapali et al. 2011). The presence of dynein light chain molecules in fibroblast Golgi structures (Tai et al. 1998)

support our observations revealing them by immunogold method as gold grains in lipotubuloid Golgi structures. Other authors believe that Golgi structure movements depend on myosin XI (Avisar et al. 2009, 2012). The latter idea is in agreement with our finding showing gold grains, thus indicating myosin presence in Golgi cisternae. A hypothesis can be put forward that dictyosomes move within a lipotubuloid, being a set of cooperating organelles, which plays a key role in *O. umbellatum* ovary epidermis development (Kwiatkowska et al. 2012).

Author contribution M. Kwiatkowska designed the experiments and prepared manuscript, J.T. Polit designed scheme of the figure in manuscript, K. Popłowska prepared figures, D. Stępiński was involved in English version preparation of manuscript, A. Wojtczak made immunogold analysis. All the authors were responsible for experimental part and writing the manuscript.

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