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1. Introduction

Diatoms (BACILLARIOPHYTA) are unicellular eucaryotes with species-specific silica external skeleton forming intracellularly. The main part of ultrastructural studies are devoted to siliceous valve morphogenesis (Reimann, 1964; Drum & Pankratz, 1964; Schmid & Schultz, 1979; Pickett-Heaps et al., 1990; Pickett-Heaps, 1998; Van de Meene & Pickett-Heaps, 2002) and involvement of cytoskeleton elements in the control of nuclear division and formation large structures on the silica frustule (Pickett-Heaps & Kowalski, 1981; Pickett-Heaps et al., 1988; Tippit & Pickett-Heaps, 1977; Tippit et al., 1980; Pickett-Heaps, 1983).

According to Round et al. (1990), BACILLARIOPHYTA consists of three classes: centric diatoms with radial and bipolar symmetry of frustules (class Coscinodiscophyceae Round & Crawford) and two classes of pennate diatoms differing in presence (Bacillariophyceae Haeckel) or absence of a raphe (Fragilariophyceae Round).

For the last decade systematics of diatoms based on the morphological data (Round et al., 1990) has been subject to revision (http://www.algaebase.org) due to the appearance of molecular phylogeny and understanding of its data (Medlin et al., 2000; Medlin, 2009).

Molecular-phylogenetic analysis revealed that BACILLARIOPHYTA are divided into three clades. Clade 1 includes centric diatoms with radial symmetry of frustules, clade 2a consists of centric diatoms with bi- and multipolar symmetry of frustules and the order Thalassiosirales Glezer & Makarova, and clade 2b includes all pennate diatoms (Medlin, 1997).

Despite the limited number of papers describing diatom cell structure and lack of all major taxa, researchers attempted to detect characteristic features of cell organelle structure for different taxonomic groups of diatoms several times. For example, Mann (1996) and Schmid (2001) considered pyrenoid structure to be as a source of taxonomic and phylogenetic information. They discovered that pyrenoids of diatoms of phylogenetic clade 2 were more diverse than pyrenoids of clade 1. Lately, data on thin structure of chloroplasts and pyrenoids were enlarged and character sets were specified for different diatom phylogenetic clades (Bedoshvili et al., 2009).

Medlin and Kaczmarska (2004) systematized data on arrangement of Golgi bodies in diatom cells and defined three types. Type 1 is typical of centric diatoms with radial symmetry (except the order Aulacoiseirales Crawford). In this case, dictyosomes are associated with ER cistern and mitochondrion and form the so-called G-ER-M unit (Coscinodiscus Ehrenberg – Schmid, www.intechopen.com
1987; **Stephanopyxis** (Ehrenberg) Ehrenberg – Medlin et al., 2000; **Ellerbeckia** Crawford – Schmid & Crawford, 2001). Thalassiosirales and Aulacoseirales, most of pennates and bipolar centrics have Golgi arrangement of type 2. Dictyosomes encircle the nucleus and form a perinuclear shell around it (Medlin & Kaczmarska, 2004). **Stephanodiscus niagare** Ehrenberg is a pattern (Drum et al., 1966). Modifications of this type of Golgi arrangement were revealed in cells of **Synedra ulna** (Nitzsch) Ehrenberg (Schmid, 1989) and **Pinnularia nobilis** (Ehrenberg) Ehrenberg (Drum, 1966) where dictyosomes are located along the long nuclear extension towards the poles or periphery of cell. In the cell of **Pinnularia nobilis**, dictyosomes are paired. Type 3 was found in **Biddulphiopsis titiana** (Grunow) von Stosch & Simonsen (Coscinodiscophycidae Round et Crawford, clade 1). In this species only outer nuclear envelope forms filose tentacles along which Golgi bodies are paired (Medlin & Kaczmarska, 2004).

This work was aimed at studying cell ultrastructure of seven diatom species from different orders and phylogenetic clades. Ultrathin cell sections were analysed paying attention to a nucleus form, Golgi arrangement and its number, form and number of mitochondria. An attempt was made to reveal specific features of diatom cell ultrastructure of different taxa.

2. Materials and methods

2.1 Cultures

The cell culture of **Synedra acus** subsp. **radians** (Kützing) Skabitschevsky (Fragilariophyceae Round, Fragilariales Round, clade 2b) was isolated from Listvennichnyi Bay of Lake Baikal and was grown by T.A. Safonova on the medium DM1 (Thompson et al., 1988). **Cymbella ventricosa** Agardh (Bacillariophyceae Hauckel, Cymbellales Mann, clade 2b) was isolated from Irkutsk Reservoir and was grown by T.N. Basharina on the same medium. Cultures of **Thalassiosira proschkinae** Makarova (Coscinodiscophyceae, Thalassiosirales Glezer et Makarova, clade 2a), **Athega ussurensis** Stonik, Orlova et Crawford (Coscinodiscophyceae, Chaetocerotes Round et Crawford, clade 2a), **Ditylum brightwellii** (West) Grunow (Coscinodiscophyceae, clade 2a), and **Chaetoceros muelleri** Lemmermann (Coscinodiscophyceae, Chaetocerotes Round et Crawford, clade 2a) were kindly provided by N.A. Aizdaicher from A.V. Zhirmunsky Institute of Marine Biology FEB RAS (Vladivostok). Sampling of **Aulacoseira baicalensis** (K. Meyer) Simonsen (Coscinodiscophycidae, Aulacoseirales Crawford, clade 1) was carried out in Listvennichnyi Bay of Lake Baikal during its vegetation in spring.

2.2 Transmission electron microscopy

For transmission microscopy cells in their logarithmic phase were fixed by 2,5 % glutar aldehyde (Sigma-Aldrich, Germany) on PBS (pH 7.0) for 12 h and then by 1% osmium tetroxide (Merck KGaA, Germany) on the same buffer (2 h). Then the cells were placed in 1 % agarose (Helicon, Moskow, Russia). Dehydration was made in ethanol solution series (Reahimkomplekt, Angarsk, Russia) and then in ethanol and acetone dehydrated with copper sulphate (Reahimkomplekt, Angarsk, Russia) for 5 min in each solution. Dehydrated material was soaked in three mixtures with resin Araldite 502 Kit (SPI, USA) and acetone and in pure resin for 12 h. After this, the material was placed in resin with catalyst DMP-30 (SPI, USA) and polymerised in a thermostat at 60°C (for 3 days). Ultrathin sections were cut with a diamond knife ULTRA 35° (Diatom, Switzerland) on the ultramicrotome Ultracut R (Leica, Austria). Sections were stained with Reynolds’s lead citrate (Reynolds, 1963). Samples were analysed under a transmission electron microscope Leo 906 E (Zeiss, Germany) at www.intechopen.com
accelerating voltage 80 kV. Microphotographs were made by CCD camera MegaView II (Zeiss, Germany) and processed with the MegaVision programme.

3. Results

All organelles in the cell of *Thalassiosira proschkiniae* are tightly pressed to one another. Large nucleus is located in the cell centre between the lobes of the only chloroplast (fig. 1A). Poorly-expressed nucleolus is displaced on the nucleus periphery and pressed to the nucleus envelope (fig. 1B). Nuclear pores are turned to the cytoplasm free of chloroplast (fig. 1B). Golgi arrangement is perinuclear (fig. 1C). Large mitochondria (one or two per cell) are of diverse shape on the sections: ring-shaped (fig. 1D), complicated (fig. 1E) or oblong (fig. 1F).

A big vacuole occupies the main volume of *Aulacoseira baicalensis* cell and the nucleus and cell organelles are pressed to the cell wall (fig. 2A). There is a well-defined nucleolus in the centre of the nucleus (fig. 2B); Golgi bodies (it can be seen no more than 4; fig. 2B) are situated on the side of the nucleus directed to the cell periphery. Nuclear pores are directed to Golgi bodies (fig. 2C). There are vesicles budding from the outer nuclear membrane (fig. 2C). The analysis of serial sections of mitochondria revealed that they have either an intricate branchy form (fig. 2D) or a length up to 4.5 µm (fig. 2E). Mitochondria are frequently situated near the forming silica valves (fig. 2F) or lipid drops tightly pressed to them (fig. 2G).

The nucleus of *Chaetoceros muelleri* is shifted to girdle bands and the Golgi bodies are pressed to nuclear membrane (fig. 3A, B). Nuclear pores are directed to either Golgi bodies (fig. 3A) or cell periphery (fig. 3C). Nucleolus is poorly expressed pressing to the nuclear envelope (fig. 3C). The analysis of serial sections showed that the only mitochondrion in the cell can be of diverse shape: complicated (fig. 3D), elongate, sometimes branchy or roundish (fig. 3E). Invaginations were frequently observed (fig. 3F).

A nucleus with a well-defined nucleolus is seen in the cell centre of *Attheya ussurensis* (fig. 4A). Nuclear pores are directed to the Golgi bodies on most of the sections (fig. 4B). Up to four Golgi bodies are located near the nucleus (fig. 4C). A great number of small vesicles around Golgi bodies attest to their activity (fig. 4D). Mitochondria on some sections (fig. 4E) have a commissure of outer and inner membrane looking like a pore.

The nucleus of *Synedra acus* subsp. *radius* of a complicated form with lobes and invaginations (fig. 5A) is located diagonally, ER tanks being inside the invaginations (fig. 5B). There is a roundish or oval nucleolus in the nucleus centre (fig. 5B, C). Multiple Golgi vesicles are often observed budding from the outer nuclear membrane (fig. 5D). Six Golgi bodies are located along the nucleus; nuclear pores turn to them (fig. 5E). Multiple mitochondria of up to 2.7 µm long and to 0.4 µm wide are on the cell periphery (fig. 5F).

The nucleus of *Cymbella ventricosa* is located in the wide part of the cell and pressed to girdle bands (fig. 6A). There is a nucleolus in the nucleus centre (fig. 6B). Up to five Golgi bodies are around the nucleus (fig. 6A, C). A great number of vesicles and ER tanks are detected in the cytoplasm (fig. 6D). Long and narrow mitochondria reach 5 µm on the cell periphery (fig. 6E).

*Ditylum brightwellii* has a roundish nucleus in the cell centre (fig. 7A). There is a nucleolus in the nucleus centre (fig. 7B), multiple nuclear pores are aligned regularly on the nuclear envelope (fig. 7C). Multiple Golgi bodies are near the nucleus (fig. 7C). The G-ER-M units are also observed (fig. 7D).
Fig. 1. The cell structure of *Thalassiosira proschkinae*. TEM. A – cell section; B – nucleus section, the nucleus is indicated by black arrowhead, nuclear pore – white arrowhead; C – Golgi bodies section; D-F – sections of mitochondrion of different shape. Legend: Chl – chloroplast; G – Golgi bodies; m – mitochondrion; N – nucleus.
Fig. 2. The cell structure of *Aulacoseira baicalensis*. TEM. A – cell cross section; B – nucleus and Golgi bodies section; C – Golgi bodies section; D-G – mitochondria sections; D – mitochondrion longitudinal section; F – section of the mitochondrion near the forming valve (indicated by the black arrowhead); G – section of the mitochondrion near the lipid drop. Legend: G – Golgi bodies; Li – lipid drop; m – mitochondrion; N – nucleus.
Fig. 3. The cell structure of *Chaetoceros muelleri*. TEM. A – cell section (the nuclear pore is showed by the arrowhead); B – Golgi bodies section; C – nucleus section (black arrowhead – the nucleolus; white arrowhead – the nuclear pores); D-F – sections of mitochondria of different shape. Legend: Chl – chloroplast; G – Golgi bodies; m – mitochondrion; N – nucleus.
Fig. 4. The cell structure of *Attheya assurenxis*. TEM. A – cell cross section (the nucleolus is indicated by arrow); B – nucleus section (the arrows show the nuclear pores); C – nucleus and Golgi bodies section (arrows show the Golgi bodies); D – Golgi bodies section (black arrowheads – the nuclear pores); E – mitochondrion longitudinal section; F – mitochondrion cross section (arrows indicate the comissures of outer and inner mitochondrion membranes). Legend: Chl – chloroplast; G – Golgi bodies; m – mitochondrion; N – nucleus.
Fig. 5. The cell structure of *Synedra acus* subsp. *radians*. TEM. A – longitudinal section of the nucleus with the invagination (dotted line), the nucleolus is shown by the arrowhead; B – cross section of the nucleus invagination with the smooth ER tank inside (arrow); the arrowhead indicates the nuclear pore; C – the nucleus longitudinal section (the nucleolus is shown by the arrow); D – cross section of nucleus and Golgi bodies, the vesicles budded from the nuclear outer membrane are indicated by the arrows; E – nucleus and Golgi bodies longitudinal section, the arrowheads indicate the nuclear pores; F – mitochondrion longitudinal section. Legend: Chl – chloroplast; G – Golgi bodies; m – mitochondrion; N – nucleus.
Fig. 6. The cell structure of *Cymbella ventricosa*. TEM. A – cell cross section; B – nucleus cross section (the nucleolus is shown by the arrow); C – cross section of nucleus and Golgi bodies; D – cytoplasm section (the ER tanks are shown by the arrows); E – mitochondrion longitudinal section. Legend: Chl – chloroplast; G – Golgi bodies; m – mitochondrion; N – nucleus; P – pyrenoid.
Fig. 7. The cell structure of *Ditylum brightwellii*. TEM. A – cell cross section; B – nucleus cross section (the nucleolus is indicated by the arrow); C – cross section of nucleus and Golgi bodies (the nuclear pores are shown by the arrowheads); D – cross section of G-ER-M unit. Legend: Chl – chloroplast; G – Golgi bodies; m – mitochondrion; N – nucleus.

4. Discussion

4.1 The Golgi bodies number and their arrangement relative to the nucleus

Among the examined species the relationship between the cell size and the number of Golgi bodies is observed. The only Golgi complex is recorded in small cells of *C. muelleri* and *T. proschkinae* from different orders and phylogenetic clades. In this study, *A. baicalensis* and *S. acus* subsp. *radians* have the largest cell size (140 and 290 μm long, respectively). Six Golgi complexes are located in the cells of *S. acus* subsp. *radians*. *Synedra ulna*, a larger cell species of the same genus, possesses a higher number of Golgi complexes (7) located along the filamentous extensions of the nucleus (Schmid, 1989, fig. 17). There are four Golgi bodies in *A. baicalensis* as well as in small-celled (up to 6 μm) centric *A. ussurensis* and pennate *C. ventricosa*. It is attributed to small cytoplasm volume in the *A. baicalensis* cells and to a large vacuole occupying the major part of the cell.
The Cell Ultrastructure of Diatoms - Implications for Phylogeny?

Golgi bodies of all examined species are located perinuclearly, with some exception. For example, we found two types of Golgi arrangement (G-ER-M unit and perinuclear dictyosomes) in the same cell of *D. brightwellii* (a member of clade 2a; fig. 7).

Golgi complexes in *S. acus. subsp. radians* are located along the nucleus according to type 2 contrary to *S. ulna* whose Golgi arrangement is referred to modification of this type (Medlin & Kacmarska 2004). The nucleus shape differs in these two species: long tubular extension is absent in the nucleus of *S. acus. subsp. radians*, whereas in *S. ulna* it is present. Our data support the conclusion of Medlin and Kacmarska (2004) – this is the same perinuclear arrangement of Golgi bodies (type 2) as well as type 3 and it is not worth marking out individual modifications in it.

Golgi bodies in *A. baicalensis* are located only on one side of the nucleus which turns to the cell periphery. The same arrangement of Golgi bodies is described for *Melosira varians* Agardh (Crawford, 1973) from the closely-related order of the same class Coscinodiscophyceae.

4.2 The nucleus shape

According to the literature data, most of diatoms have a pillow-like or spherical nucleus. However, there are some exceptions. For example, the nucleus of *Lauderia annulata* Cleve consists of two lobes situated on different valves and connected in the cell centre with a thin filament (Holmes, 1977). The nucleus in some species of *Surirella* Turpin can be of H-like shape (Drum & Pankratz, 1964). We revealed that the nucleus shape can differ in cells of species of the same genus. Both *S. ulna* and *S. acus subsp. radians* have an oblong nucleus but the latter can form invaginations instead of long extensions. Such nucleus shape increases its surface providing better contact with other cell organelles through the nuclear pores. All examined species have polarization of the nuclear pores which turn to Golgi bodies (figs 1C, 2C, 3A, 4B, D, 5E, 6C). This polarization is clearly expresses in *A. baicalensis* (fig. 2C). The increase of the nucleus surface and the polarization of nuclear pores make it possible to accelerate metabolism of nucleus and cytoplasm. They are not characters of any taxon or phylogenetic clade but reflect physiological state of the cell.

4.3 The number and structure of mitochondrion

Numerous studies of eukaryotes revealed that mitochondrion morphology is complicated and plastic (Hoffmann & Avers, 1973; Bereiter-Hahn & Voth, 1994; Nunnari et al., 1997; Boldogh et al., 2001). According to the literature data, long mitochondria with tubular cristae localized in the peripheral cytoplasm layer are characteristic of diatoms (Round et al., 1990, p. 60). Comparative studies of mitochondrion structure have not been performed before.

The shape of diatom mitochondrion appeared to be extremely changeable even in the cells of the same species. Small-celled *C. muelleri* and *T. proschkiniae*, belonging to clade 2a, possess one large mitochondrion of various shapes in different cells (figs 1D-F, 3D-F). Lager-celled *A. assurensis* from the same clade have many long mitochondria. The longest mitochondria (up to 5 μm) are detected in the cells (approximately 6 μm) of the pennate *C. ventricosa*. Multiple long and often branched mitochondria (figs 2D-E, 5F) are recorded in the cells of two species from different clades having the largest cells – *A. baicalensis* (clade 2a) and *S.
acus subsp. radians (clade 2b). Projections, invaginations and intricate branched shape of mitochondria are similar to complex mitochondrial networks which were detected in other eukaryotes, particularly in yeast (Rizzuto et al., 1998; Mannella, 2000; Rutter & Rizzuto, 2000).

4.4 ER and vesicles

ER is poorly developed in diatoms like in other heterokont algae (Gibbs, 1981; Round et al., 1990; Van den Hoek et al., 1997) (fig. 6D). It is of an intricate shape when it is located near the nucleus (fig. 5B, D). The outer nuclear membrane performs the functions of ER in synthesis and substance transport (fig. 5D). We believe that vesicles near the outer nuclear membrane are budded from it (instead of coalescence). Synthesized nutrients in the vesicles and transported to the Golgi complex (fig. 5D). Perinuclear location of Golgi bodies promotes rapid transport of vesicles and is observed in all cases especially in the cells with oblong nuclei (fig. 5E).

5. Conclusion

Based on the results of analysis of some diatom species, we tried to apply specific features of cell ultrastructure to systematics of diatoms. Golgi arrangement relative to the nucleus is consistent with characters of phylogenetic clades proposed by Medlin & Kacmarska (2004), i.e., perinuclear Golgi arrangement (type 2) is characteristic of the members of phylogenetic clades 2a and 2b, except D. brightwellii with G-ER-M units present in the cell. Both Aulacoseira and Melosira have perinuclear Golgi arrangement.

Other characteristics of cell ultrastructure are not associated with the systematic position of species. The number of Golgi bodies in species from the same clade and order can differ and depends on the cell size and/or cytoplasm volume. The number and size of mitochondria can be similar in species from different orders and also depend on cell size, whereas the shape of mitochondria can differ even in representatives of the same species.

Thus, the application of cell ultrastructure characteristics in phylogenetic reconstructions is limited except pyrenoids: their structure defines certain taxa and phylogenetic clades (Schmid, 2001; Bedoshvili et al., 2009).

6. Acknowledgements

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7. References


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The book "The Transmission Electron Microscope" contains a collection of research articles submitted by engineers and scientists to present an overview of different aspects of TEM from the basic mechanisms and diagnosis to the latest advancements in the field. The book presents descriptions of electron microscopy, models for improved sample sizing and handling, new methods of image projection, and experimental methodologies for nanomaterials studies. The selection of chapters focuses on transmission electron microscopy used in material characterization, with special emphasis on both the theoretical and experimental aspect of modern electron microscopy techniques. I believe that a broad range of readers, such as students, scientists and engineers will benefit from this book.

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