Limnology and Freshwater Biology 2019 (5): 297-301 DOI:10.31951/2658-3518-2019-A-5-297

Original Article

ISSN 2658-3518
LIMNOLOGY FRESHWATER BIOLOGY
www.limnolfwbiol.com

Valve morphogenesis and silicon dynamics in the synchronized culture of *Ulnaria danica*

Bedoshvili Ye.D., Volokitina N.A., Marchenkov A.M.*

Limnological Institute, Siberian Branch of the Russian Academy of Sciences, Ulan-Batorskaya Str., 3, Irkutsk, 664033, Russia

ABSTRACT. Formation of diatom siliceous cell walls occurs inside the cells and depends on the availability of silicon from the environment. In the current work we have studied the valve morphogenesis of a freshwater pennate diatom *Ulnaria danica* in a laboratory culture synchronized by silica starvation and absence of light. Approximate timelines were established for the initiation of valve synthesis and formation of its major components. It was shown that the silicon surge uptake takes place, e.g. silicon concentration in the medium reduces during the first 30 minutes after the addition of silicon to a synchronized culture. During the formation of main valve elements, intracellular silicon pool is lower than it is after the end of synchronization, returning to the original level only 90 minutes after the addition of silica and beginning of the morphogenesis.

Keywords: diatoms, synchronized culture, morphogenesis, *Ulnaria danica*

1. Introduction

Diatom algae are unicellular autotrophic organisms that create species-specific siliceous structures at micro- and nanoscale. Mechanisms that regulate a process of silicon absorption from the environment and its transport to the silica deposition vesicle are related to the subsequent valve formation processes, but currently they are poorly understood. Diatom cell wall consists of two structures called valves, each connected to a system of ring-shaped girdle bands. All cell wall components synthesized sequentially inside the cell in a specialized organelle called silica deposition vesicle (SDV) (Reimann, 1964; Drum and Pankratz, 1964). Silicon is mostly available to diatoms as undissociated silicic acid (Martin-Jézéquel et al., 2000) which is imported to the cell by a SIT protein (Hildebrand et al., 1997; Hildebrand et al., 1998; Petrova et al., 2007; Sapriel et al., 2009). When silicic acid is abundant in the environment, diatom cells use it to build valves and girdle bands immediately after it is imported. Under the culturing conditions, though, the medium contains a limited amount of silicic acid. When cells are starved for silicon, they start "preparing" for a potential future silicon addition, and a small amount of currently available silicon forms an intracellular pool, which is probably stored in silica-containing inclusions (Grachev et al., 2017). After silicon is added to the environment, the cells respond by impulsively consuming silicic acid and immediately spending it on cell wall formation (Thamatrakoln and Hildebrand, 2008).

The majority of valve morphogenesis studies are performed on marine diatom species. Two most common model objects are *Thalassiosira pseudonana* (Thamatrakoln et al., 2012) and *Phaeodactylum tricornutum* (Armbrust et al., 2004), a centric and a pennate diatoms, both marine. Large-scale studies of a freshwater diatom frustule morphogenesis are being performed on the pennate species *Fragilaria radians* (Kütz.) D.M. Williams & Round for the last twenty years by Limnological Institute SB RAS (Grachev et al., 2002; Kaluzhnaya and Likhoshway, 2007; Safonova et al., 2007; Kharitonenko et al., 2015; etc.).

Diatoms are quiet diverse group of unicellular eukaryotes. Despite their common ability to synthesize silica exoskeletons by silicic acid extraction from water environment the phylogenetic diversities between different diatom groups are comparable with ones of humans and fishes. For this reason, to detect unknown participants of valve morphogenesis it is necessary to compare both species with a very different valve structure and more similar ones. For example, *Fragilaria radians* and *Ulnaria danica* (Kütz.) Compère & Bukhtiyarova are two closely related species. After a recent revision of genus *Synedra*, its species were reassigned to different genera: *S. acus* subsp. *radians* is considered synonymous to *Fragillaria radians* (Kütz.) D.M. Williams & Round (Williams and Round, 1987), and *S. ulna* subsp. *danica* is synonymous to *Ulnaria danica* (Kütz.) Compère & Bukhtiyarova (Bukhtiyarova and Compère, 2006). A comparison of the processes of valve morphogenesis and silicon uptake of such morphologically similar species has not been carried

*Corresponding author.
E-mail address: marchenkov.am@bk.ru (A.M. Marchenkov)

© Author(s) 2019. This work is distributed under the Creative Commons Attribution 4.0 License.

out previously.

The aim of this work was to establish the connection between cell wall morphogenesis and silicon dynamics (inside and outside the cell) in *Ulnaria danica*, as well as compare its valve morphogenesis stages to those of *Fragilaria radians*.

2. Materials and methods

The *Ulnaria danica* culture was grown in glass bottles with volume at 15 L during 16 days in DM medium for freshwater diatoms (Thompson et al., 1988) under natural illumination. Then cells were synchronized according to the protocol published earlier (Kharitonenko et al., 2015). Briefly, the cell biomass was concentrated on the polycarbonate filters with pore diameter 5 μm (Reatrack, Russia) and passed to DM medium without silicon. The cells were exposed during three days in the darkness at 16 ˚C in polycarbonate bottle with volume 16 L and then concentrated again. The part of these cells was used in the further experiment for SEM and silicon concentration determination. The main part of cell biomass were passed into the DM medium with silicon in the form of sodium metasilicate (Na_2SiO_3*$9H_2O$, 57 mg/L). After this five samples were taken after 30, 60, 90 and 120 minutes. The cells for determination of intracellular silicon pool were frozen with liquid nitrogen and then stored at -20 ˚C.

For further electron microscopy, the cell culture was concentrated by sequential centrifuging, washed in three changes of 6 % SDS for 30 minutes in a water bath (95 ˚C), washed five times in distilled water, placed in concentrated 68.4% nitric acid and incubated for 1 hour in a water bath (95 ˚C). After an hour, nitric acid was removed, and cells were washed thrice with ethanol, treated for 24 hours with 36 % hydrochloric acid and washed in water at least five times. Suspensions of cleaned valves were placed on SEM stubs, gold-coated in an SCD 004 sputter coater (Balzers) and examined on the Quanta 200 FEI Company (USA) scanning electron microscope. Valves were counted among 200 randomly encountered.

Silicon concentration in the medium and inside the cell was measured according to the published protocols (Guideline 52.24.433-95; Hervé et al., 2012). For determination intracellular silicon pool the frozen cells were resuspended in 1 ml of distilled water, incubated at 95 ˚C for 10 min, cooled and concentrated by centrifugation (1450 g) with centrifuge Allegra X-12R, Beckman Coulter (USA). The intracellular silicon in samples of supernatant as well as samples of the medium DM was measured using the silicomolybdate assay (Guideline 52.24.433-95) with standard sample of silicon solution (EKO-analitika, Russia) in a range of 0 to 12 mg/L. All measurements were taken in triplicate.

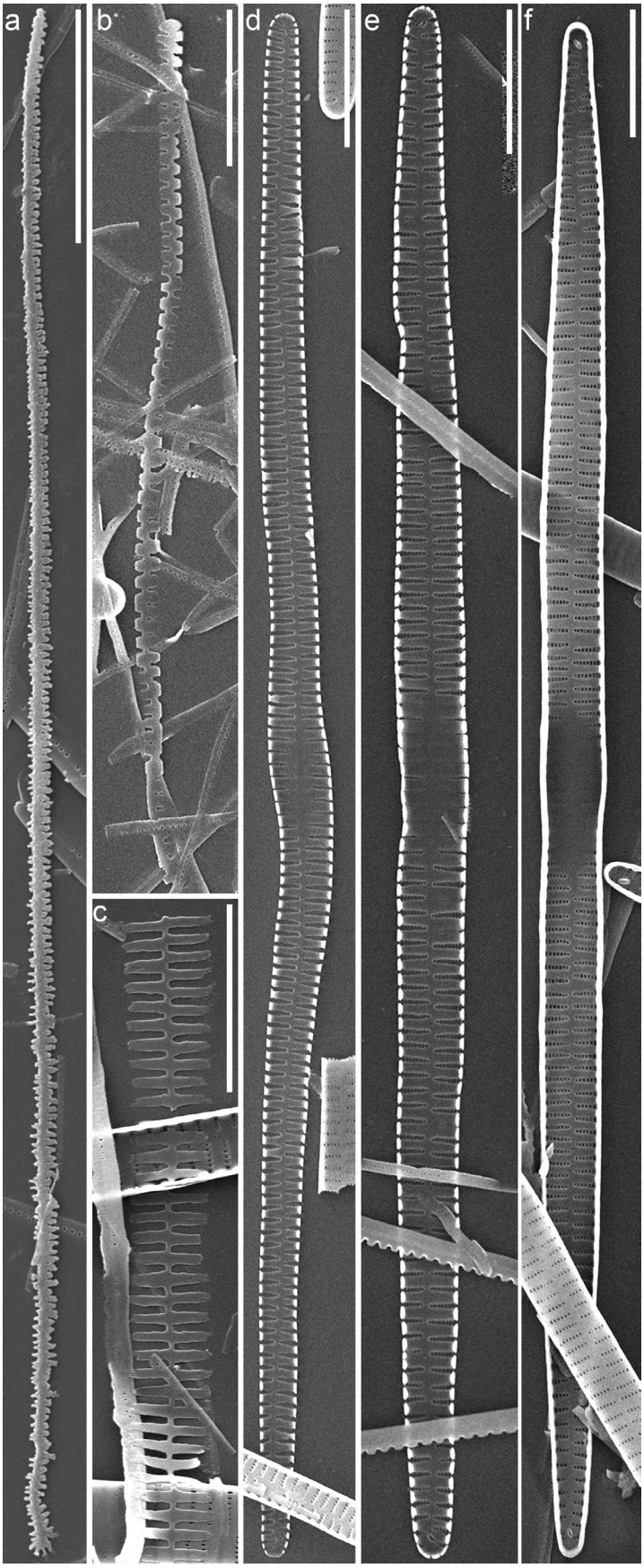

Fig. 1. The sequence of morphogenesis stages in *U. danica* (SEM). a — stage I, thread-like filament; b — stage II, virga formation; c — stage III, virga growth; d — formation of valve mantle; e — vimin formation; f — mature valve. The scale is 10 μm.

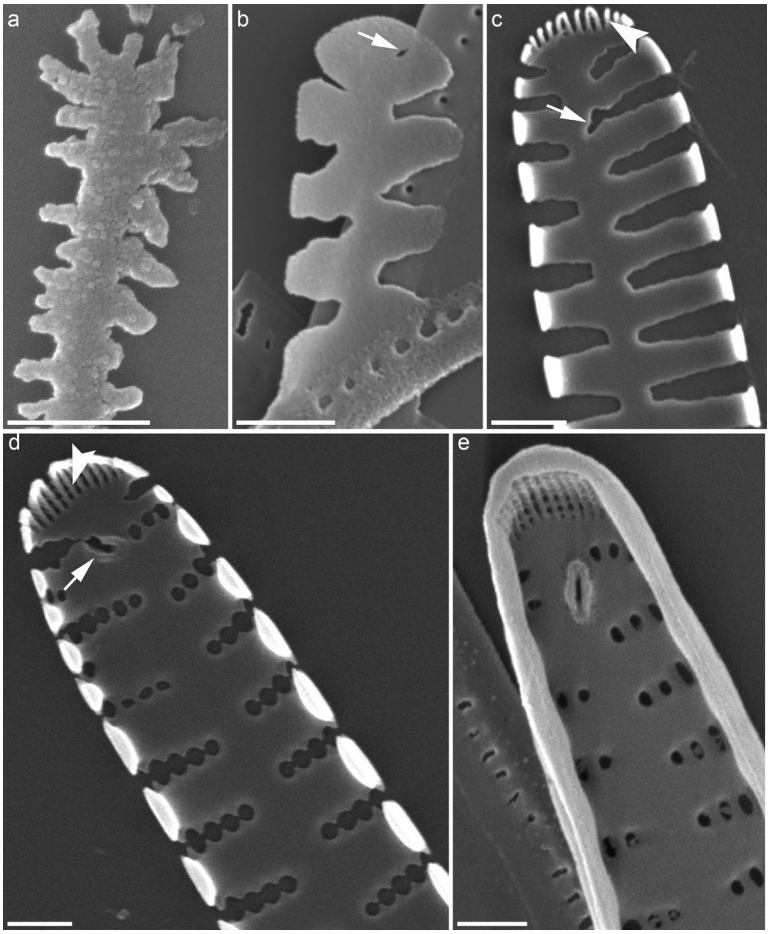

Fig. 2. The apex of *U. danica* valves at various morphogenesis stages. a — stage I; b — stage II; c — stage IV; d — stage V; e — mature valve. White arrows point to rimoportula, short ones point to apical pore area. The scale is 1 μm.

3. Results

Valves of a synchronized culture taken during 120 minutes after silicon addition were studied with SEM. Valve morphogenesis stages were identified by markers described for a related species *F. radians* (Kaluzhnaya and Likhoshway, 2007; Kharitonenko et al., 2015). The earliest stage that we have managed to register (**stage I**) is a sternum, long siliceous filament (Fig. 1a) with forming first-order branchings (virgae) that later merge and form mature virgae. At this stage the position of future rimoportula is yet unmarked (Fig. 2a) and the entire structure is rigid; unlike later stages, when valve can even bend in half, sternum always appeared straight.

The first signs of rimoportula appear on **stage II** (Fig. 1b, Fig. 2b) when virgae have already formed and are growing (**stage III**, Fig. 1c). Axial pore area starts developing from separate thin filaments at **stage IV**; by this time, valve mantle has started forming (Fig. 1d, Fig. 2c). Creation of second-order branchings (viminae) and axial pore area happens during **stage V** (Fig. 1e, Fig. 2d). Growth of velums on areolae apparently continues even after valve mantle is finished and valves appear to be fully formed (Fig. 1f, Fig. 2e).

Valves on different stages were found in all SEM preparations. However, in the sample taken 120 minutes after silica addition, all valves were on morphogenesis stages IV-V. The presence of the valves on different morphogenesis stages is shown in Table 1.

Measurements of silicon concentration in the medium have shown that it drops to less than half of the original level during 30 minutes after finishing three days of synchronization and introducing silicon to diatom culture (Fig. 3). For the next 2 hours the silicon concentration was either decreasing insignificantly or not decreasing at all. Silicon concentration in the intracellular silicon pool of synchronized culture (Fig. 4) was also decreasing during the first 60 minutes and returning to the original level after 90 minutes. The measured silicon concentration in the medium was above 57 mg/L, ranging from 62 mg/L to 83 mg/L. We suspect that this is caused by the DM preparation protocol, which involves using sodium metasilicate to reach the required pH.

4. Discussion

The stages of *U. danica* morphogenesis are similar to those in a previously described related species *F. radians* (Kharitonenko et al., 2015), although the earlier stages of valve forms happen quicker for *U. danica* than for *F. radians*, despite its larger size. Most of the primary valve morphogenesis (horizontal differentiation—Schmid and Volcani, 1983) took place during the first two hours after the end of synchronization. The order in which various morphological structures have

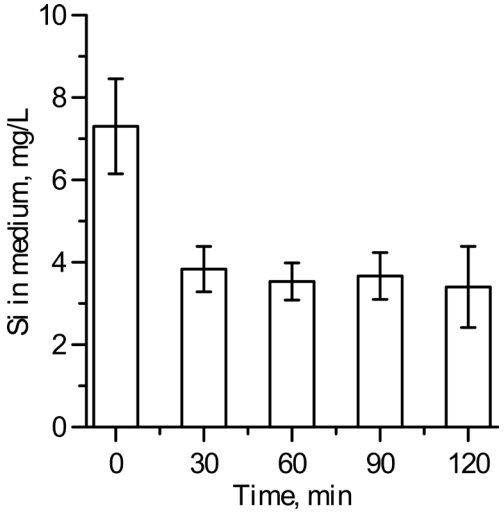

Fig. 3. The dynamics of silicon concentration in the medium.

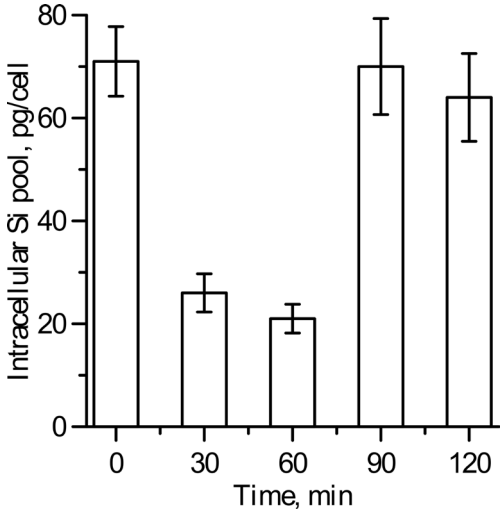

Fig. 4. The dynamics of silicon concentration in the cells.

appeared is the same as in *F. radians*; silica deposition itself first happens horizontally (like in other species — Schmid and Volcani, 1983), then the valve starts vertical differentiation and getting thicker.

It is interesting that forming sternum seems to have no flexibility, unlike valves on the later stages of development. This fact can demonstrate the variance in the state of silicic acid or general chemical composition of SDV contents between earlier and later morphogenesis stages. It is visible that at earlier stages virgae are more numerous and narrow; they are likely to merge at later stages similarly to *F. radians* (Kaluzhnaya and Likhoshway, 2007), but scanning electron microscopy cannot visualize this process in *U. danica*. Our data also show that even if rimoportula does indeed start forming at the earliest morphogenesis stages, it is not detectable at the sternum stage. The loop that later becomes rimoportula (Kaluzhnaya and Likhoshway, 2007, Fig. 26) doesn't appear until at least Stage II, during virga formation. Thus, the details described in this work extend the previously available information about valve morphogenesis in pennate diatoms (Cox and Kennaway, 2004).

It is known that assimilation of silicon by diatoms occurs according to Michaelis–Menten kinetics, and that silicon-starved cultures tend to quickly consume silicon from the environment when it becomes available (Conway and Harrison, 1977). The chemical analysis of silicon concentration has shown that it is consumed immediately, but for the first 60 minutes (during which the valve is built) its concentration inside the cell does not increase. *T. pseudonana* has slightly different kinetics of silicon use under similar conditions (Thamatrakoln and Hildebrand, 2008). Unlike *U. danica, T. pseudonana* does not store a significant amount of silicon during starvation, but does increase the intracellular silicon concentration after the cells are placed into a silicon-rich medium. The species we have studied, on the other hand, can accumulate silicon during starvation or keep its level constantly low; during silicon starvation, morphogenesis does not start, because otherwise the cell may not have enough material to finish the valve, which would probably be lethal. The intracellular silicon levels drop at the earlier stages of morphogenesis, when silica is actively deposited to the forming valve. Later, though, silica deposition speed decreases and intracellular silicon pool replenishes (this happens 90 minutes after providing silicon to synchronized cultures), because at later stages silica deposits slower and therefore is not consumed as quickly. That is why there is no significant silica concentration decrease in medium after the initial surge uptake (Fig. 3). The difference in silicon acquisition in spending between *T. pseudonana* and *U. danica* can be caused by the scale valve morphogenesis, since valve thickness and division time is several times higher in the latter. *T. pseudonana*,

Table. 1. The distribution of valves on different morphogenesis stages along sampling times.

	Time, min				
Stages of morphogenesis	0	30	60	90	120
I, Fig. 1a, Fig. 2a	-	+	-	-	-
II, Fig. 1b, Fig. 2b	-	+	+	-	-
III, Fig. 1c	-	+	+	+	-
IV, Fig. 1d, Fig. 2c	Sporadically	Sporadically	+	+	+
V, Fig. 1e, Fig. 2d	Sporadically	Sporadically	+	+	+

which has smaller cells and less thick walls, can afford to spend silicon on building valve and girdle bands as it is being absorbed from medium. The creation of intracellular silicon pool or maintenance of certain silicon level during its shortage in the environment has been documented in other diatoms (Azam, 1974; Sullivan, 1976; 1977).

Thus, in *U. danica* silicon accumulation is linked to the valve morphogenesis. This species can accumulate silicon inside the cell, although not during horizontal valve differentiation (at that time all incoming silicon is immediately consumed); but when there is no active silica deposition, its intracellular level remains constant.

Acknowledgments

The reported study was funded by RFBR according to the research project № 18-34-00438. The work was carried out in the Collective Instrumental Center 'Ultramicroanalysis' at the Limnological Institute of the Siberian Branch of RAS.

References

Armbrust E.V., Berges J.A., Bowler C. et al. 2004. The genome of the diatom *Thalassiosira pseudonana*: Ecology, evolution, and metabolism. Science 306: 1-33. DOI: 10.1126/science.1101156

Azam F. 1974. Silicic acid uptake in diatoms studied with [^{68}Ge] germanic acid as a tracer. Planta 121: 205-212. DOI: 10.1007/BF00389321

Bukhtiyarova L.N., Compère P. 2006. New taxonomical combinations in some genera of Bacillariophyta. Algology 16: 280-283.

Conway H.L., Harrison P.J. 1977. Marine diatoms grown in chemostats under silicate or ammonium limitations IV. Transient response of *Chaetoceros debilis*, *Skeletonema costatum*, and *Thalassiosira gravida* to a single addition of the limiting nutrient. Marine Biology 43: 33-43. DOI: 10.1007/BF00392568

Cox E., Kennaway G. 2004. Studies of valve morphogenesis in pennate diatoms: investigating aspects of cell biology in a systematic context. In: 17th International Diatom Symposium, pp. 35-48.

Drum R.W., Pankratz S. 1964. Pyrenoids, raphes, and other fine structure in diatoms. American Journal of Botany 51: 401-418. DOI: 10.2307/2439832

Grachev M.A., Denikina N.N., Belikov S.I. et al. 2002. Elements of the active center of silicon transporters in diatoms. Molecular Biology 36: 534-536. DOI: 10.1023/A:1019860628910

Grachev M.A., Bedoshvili Y.D., Gerasimov E.Y. et al. 2017. Silica-containing inclusions in the cytoplasm of diatom *Synedra acus*. Doklady Biochemistry and Biophysics 472: 44-48. DOI: 10.1134/S1607672917010124

Guideline 52.24.433-95. 1995. Guidelines for the determination of silicon in natural and treated wastewater. Rostov-on-Don, 8. (In Russian)

Hervé V., Derr J., Douady S. et al. 2012. Multiparametric analyses reveal the pH-dependence of silicon biomineralization in diatoms. PLoS One 7. DOI: 10.1371/journal.pone.0046722

Hildebrand M., Volcani B.E., Gassmann W. et al. 1997. A gene family of silicon transporters. Nature 385: 688-689. DOI: 10.1038/385688b0

Hildebrand M., Dahlin K., Volcani B.E. 1998. Characterization of a silicon transporter gene family in *Cylindrotheca fusiformis*: sequences, expression analysis, and identification of homologs in other diatoms. Molecular Genetics and Genomics 260: 480-486. DOI: 10.1007/s004380050920

Kaluzhnaya Ol.V., Likhoshway Ye.V. 2007. Valve morphogenesis in an araphid diatom *Synedra acus* subsp. *radians*. Diatom Research 22: 81-87. DOI: 10.1080/0269249X.2007.9705696

Kharitonenko K., Bedoshvili Ye., Likhoshway Ye. 2015. Changes in the micro- and nanostructure of siliceous frustule valves in the diatom *Synedra acus* under the effect of colchicine treatment at different stages of the cell cycle. Journal of Structural Biology 190: 73-80. DOI: 10.1016/j.jsb.2014.12.004

Martin-Jézéquel V., Hildebrand M., Brzezinski M.A. 2000. Silicon metabolism in diatoms: implication for growth. Journal of Phycology 36: 821-840. DOI: 10.1046/j.1529-8817.2000.00019.x

Petrova D.P., Bedoshvili Y.D., Shelukhina I.V. et al. 2007. Detection of the silicic acid transport protein in the freshwater diatom *Synedra acus* by immunoblotting and immunoelectron microscopy. Doklady Biochemistry and Biophysics 417: 295-298. DOI: 10.1134/S1607672907060014

Reimann B.E.F. 1964. Deposition of silica inside a diatom cell. Experimental Cell Research 34: 605-608. DOI: 10.1016/0014-4827(64)90248-4

Safonova T.A., Annenkov V.V., Chebykin E.P. et al. 2007. Aberration of morphogenesis of siliceous frustule elements of the diatom *Synedra acus* in the presence of germanic acid. Biochemistry (Moscow) 72: 1261-1269. DOI: 10.1134/s0006297907110132

Sapriel G., Quinet M., Heijde M. et al. 2009. Genome-wide transcriptome analyses of silicon metabolism in *Phaeodactylum tricornutum* reveal the multilevel regulation of silicic acid transporters. PLoS One 4. DOI: 10.1371/journal.pone.0007458

Schmid A.-M., Volcani B. 1983. Wall morphogenesis in *Coscinodiscus wailesii*. I. Valve morphology and development of its architecture. Journal of Phycology 19: 387-402. DOI: 10.1146/annurev-physiol-012110-142145

Sullivan C.W. 1976. Diatom mineralization of silicic-acid. I. $Si(OH)_4$ transport characteristics in *Navicula pelliculosa*. Journal of Phycology 12: 390-396. DOI: 10.1111/j.1529-8817.1976.tb02862.x

Sullivan C.W. 1977. Diatom mineralization of silicic acid. II. Regulation of $Si(OH)_4$ transport rates during the cell cycle of *Navicula pelliculosa*. Journal of Phycology 13: 86-91. DOI: 10.1111/j.1529-8817.1977.tb02892.x

Thamatrakoln K., Hildebrand M. 2008. Silicon uptake in diatoms revisited: A model for saturable and nonsaturable uptake kinetics and the role of silicon transporters. Plant Physiology 146: 1397-1407. DOI: 10.1104/pp.107.107094

Thamatrakoln K., Korenovska O., Niheu A.K. et al. 2012. Whole-genome expression analysis reveals a role for death-related genes in stress acclimation of the diatom *Thalassiosira pseudonana*. Environmental Microbiology 14: 67-81. DOI: 10.1111/j.1462-2920.2011.02468.x

Thompson A.S., Rhodes J.C., Pettman I. 1988. Culture collection of algae and protozoa: catalogue of strains. Kendal: Natural Environmental Research Council Press.

Williams D.M., Round F.E. 1987. Revision of the genus *Fragilaria*. Diatom Research 2: 267-288. DOI: 10.1080/0269249X.1987.9705004