
REVIEWS

Filamentous Cyanobacteria as a Prototype of Multicellular Organisms

T. V. Potapova^a and O. A. Koksharova^{a, b, *}

^aBelozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia

^bInstitute of Molecular Genetics, Russian Academy of Sciences, Moscow, Russia

*e-mail: koksharova@genebee.msu.ru

Received March 13, 2019; revised March 21, 2019; accepted March 21, 2019

Abstract—Filamentous cyanobacteria belong to the oldest organisms on our planet. Many cyanobacteria exist in the form of trichomes, i.e., cell chains comprising hundreds of cells connected by intercellular interactions. Under deficiency of environmental nitrogen, the cells in trichomes of some cyanobacteria undergo specialization to perform separate functions of oxygenic photosynthesis and nitrogen fixation. Thus, the trichome transforms into a complex organism (complex system), in which vegetative cells and the heterocysts exchange with photosynthetic and nitrogen fixation products. The transmission of metabolites may proceed via the periplasmic space or through the special contact structures called microplasmodesmata, septosomes, septal contacts, or nanopores. In filamentous cyanobacteria, the storage and transmission of energy at the cellular level is accompanied by electrical processes occurring in cell membranes. Theoretical and model analysis of extracellular currents induced by the local illumination in trichomes of *Phormidium uncinatum* showed that the trichomes are cell associations organized into unified cables capable of transferring energy along the trichome. From the viewpoint of modern molecular genetics, filamentous cyanobacteria showing the distribution of functions between neighboring cells are the prototype of a multicellular organism and a convenient model for elucidating the regulatory mechanisms of multicellularity, which, apparently, appeared more than once during the evolution in different phylogenetic groups, including bacteria, fungi, algae, and plants.

Keywords: cyanobacteria, intercellular interactions, membrane transport, membrane bioenergetics, electric currents

DOI: 10.1134/S102144372001015X

INTRODUCTION

In living systems at different levels of organization, many processes rely on similar basic principles that are manifested in only slightly different molecular mechanisms that developed in the evolution along with the complication of genetic programs [1]. The idea of basic similarity for natural processes in various living systems allows researchers to avoid study the millions of different species that live on the planet but rather to focus on model objects, such as *Drosophila*, mouse, *Arabidopsis*, wheat, rice, yeast, *Chlamydomonas*, ciliated protozoa, *Escherichia coli*, and some bacterial viruses. Filamentous cyanobacteria take an important place in this row. By November 2018, the genomes of approximately 328 cyanobacterial species and strains had been decoded (<https://www.ncbi.nlm.nih.gov/genome/browse#!/overview/cyanobacteria>). The *Anabaena* sp. strains ATCC 29413, PCC 7120 and the *Nostoc* sp. strain ATCC 29133 are the most popular model species of cyanobacteria [2].

Cyanobacteria are the oldest and ubiquitous photoautotrophic organisms of our planet. The appearance of oxygenic photosynthesis in ancient cyanobacteria approximately 2.4–2.7 billion years ago was one of the main evolutionary factors in the history of the Earth, which gave rise to the evolution of eukaryotes [3]. Due to the activity of cyanobacteria, the atmosphere and surface waters accumulated enough oxygen for the appearance of oxygen-consuming life forms, including animals featuring high respiration rates [4]. Ancient cyanobacteria laid the foundation for the symbiosis of plastids with algae and higher plants [5–7]. Currently, the plant plastids and cyanobacteria occurring in the majority of natural environments account for a large part of the primary production that underlies the food chains of the biosphere. By performing the photosynthetic oxygen evolution, they play a key role in the carbon cycle in the biosphere. In addition, cyanobacteria are the main nitrogen-fixing organisms in the oceans and terrestrial ecosystems that supply bound nitrogen to the biosphere.

Many cyanobacteria exist in the form of trichomes, i.e., the filaments containing hundreds of cells connected by intercellular interactions. Judging from the

Abbreviations: MP—membrane potential; PIC—permeable intercellular contacts; PSI and PSII—photosystems I and II.

fossil remnants of cyanobacteria, multicellular organization appeared 2.45–2.22 billion years ago. Comparative phylogenetic analysis of 16S rRNA sequences showed that filamentous cyanobacteria appeared earlier than the Great Oxidation Event and apparently played a key role in this event [8]. A comparative phylogenetic analysis of 16S rRNA gene sequences of morphologically and genetically different cyanobacterial taxa led to an interesting conclusion that the majority of modern cyanobacteria originated from a common multicellular ancestor and returned to unicellularity at least five times during their evolution [9].

The fundamental universal natural technologies of biological systems [1] include the coupling of membrane electrogenesis with cellular bioenergetics. The first law of bioenergetics states: “*A living cell avoids direct utilization of energy from external sources for the completion of useful work. First, it transforms this energy into a convertible form of ATP, $\Delta\mu\text{H}^+$, or $\Delta\mu\text{Na}^+$ and then uses it in various energy-demanding processes*” [10, pp. 12–14]. The transport of substances across the plasma membrane against the concentration gradient requires energy costs. The primary membrane transporters are membrane-embedded proteins that directly use the energy of light, redox reactions, or ATP hydrolysis for their work. Secondary membrane transporters are integral membrane proteins that perform the cotransport of molecules by coupling their transfer with the downhill movement of particular ion species. In this case, the energy is stored at a preliminary stage in the form of ionic gradients. Stebbeg et al. [2] reviewed the current knowledge on transport systems for organic substances in cyanobacteria, which is a very important issue in view of the great environmental and industrial importance of these microorganisms.

In eukaryotic cells, the primary transformation of energy from external sources into convertible forms takes place in the membranes of mitochondria and chloroplasts. In cyanobacteria, these processes proceed in thylakoid membranes and partly in cytoplasmic membranes [11, 12]. Presently, the structure and functions of main bioenergetic complexes have been studied quite thoroughly on the molecular-genetic and biophysical levels. A wealth of information refers to electron transfer in photosynthetic complexes that capture light energy and transmit it to proton ATPases. A question arises of how the coordinated functioning of individual bioenergetic complexes is regulated in membranes of different cell types and how the cooperative interactions of neighboring cells are implemented. Many aspects of this problem are considered in a special issue of BBA–Bioenergetics “*Organization and Dynamics of Bioenergetic Systems in Bacteria*” [13]. This issue covers such topics as electron transport in the plasma-membrane respiratory chains of heterotrophic bacteria and in the thylakoid membranes of cyanobacteria. A great deal of attention is paid to the spatial distribution of electron transport and the proton-motive force in different membrane

regions. Other topics considered include the energy-transducing membranes as dynamic systems, the membrane biogenesis, and regulation and development of membrane structures and functions.

Analysis of the photoelectric activity of filamentous cyanobacteria is an interesting and promising approach to elucidating the functional role of electric events in cell membranes of the trichome. In the second half of the 20th century, a group of researchers in Russia focused on the functional role of intercellular interactions mediated by ion-permeable intercellular contacts [14]. For this purpose, they conducted a theoretical and model analysis of extracellular currents induced by local illumination of trichomes of the filamentous cyanobacterium *Phormidium uncinatum* [15–18]. Analysis showed that the trichomes are cell associations organized into continuous cable structures capable of transmitting energy over a considerable distance in the manner similar to that in mycelial fungi and the cultured monolayers of animal cells [19–21]. Sawa et al. [22] described a fuel cell, in which live cyanobacteria (*Synechocystis* sp. PCC 6803) were used as electric current generators. This approach meets the goal of green electricity production [23, 24].

This review aims at the analysis of advances and problems in understanding the distribution of functions between the cells in filamentous cyanobacteria. Particular attention is paid to the anticipated bioenergetic cooperation between photosynthesizing and nitrogen-fixing cells, which is based on electrical (electrogenic) processes in cell membranes and electrical communications between the trichome cells.

PHOTOSYNTHESIS AND ELECTROGENESIS IN CYANOBACTERIA

Cyanobacteria are capable of photosynthetic conversion of light energy into the forms accessible for cellular metabolism, e.g., the transmembrane electric potential difference (membrane potential, MP), which, in turn, provides energy to other intramembrane proteins that have no direct contacts with light-harvesting proteins [25; 26, pp. 84–85]. One of such proteins is an enzyme that synthesizes ATP from ADP and phosphate: ATP synthase (F_0F_1 -ATPase). This evolutionarily conserved enzyme is present in the plasma membrane and thylakoid membranes of bacteria, in the thylakoid membranes of plants, in algal chloroplasts, and in the inner mitochondrial membranes [27]. The energy for ATP synthesis is provided by the movement of protons through the biomembrane along the gradient of the electrochemical potential. The transmembrane potential difference arises due to the electron transfer across the membrane by the enzyme complexes of the respiratory or photosynthetic chains and requires the absorption of light energy.

Cyanobacteria possess membranes of three types: the outer membrane, the cytoplasmic membrane,

and the thylakoid membrane. Photosynthesis of cyanobacteria was found to proceed in thylakoid membranes [28]. The lack of thylakoids was noted only in the unicellular cyanobacterium *Gloeobacter violaceus* that separated early in evolution from other cyanobacteria; photosynthesis in this species proceeds at specialized regions of the cytoplasmic membrane [29]. Some authors do not exclude that the plasmalemma and thylakoid membranes in filamentous cyanobacteria are interconnected into a continuous network [30–32] or, at least, have the contact sites where thylakoid membranes adjoin the plasma membranes [28]. Direct contacts between the cytoplasmic and thylakoid membranes were observed in the cells of *Synechocystis* sp. PCC 6803 [33–35]. Such membrane contacts possibly facilitate the diffusion of water-soluble and lipid-soluble molecules along the membrane system [35].

A group of researchers developed a procedure for the separation of plasmalemmal and thylakoid membranes of the unicellular cyanobacterium *Synechocystis* sp. PCC 6803 [36]. Immunoblotting of isolated purified membranes revealed that the plasma membranes contain many protein components tightly associated with the reaction centers of both photosystems. Furthermore, these proteins exist in the membranes in the form of chlorophyll-containing multiprotein complexes. The biogenesis of thylakoid membranes correlates with the development and processing of the cytoplasmic membrane. The authors assumed that many early reactions in biogenesis of photosynthetic reaction-center complexes proceed in the plasma membrane rather than in thylakoid membranes of these cyanobacteria [36].

In studies of the functional activity of cyanobacterial photosystems, the most advanced technologies are applied. The technique of hyperspectral confocal fluorescence microscopy revealed the physical segregation of photosynthetic complexes in *Synechocystis* sp. PCC 6803: photosystem I (PSI) was mostly located in the inner thylakoid regions, whereas phycobilisomes and photosystem II (PSII) were predominant on the outer thylakoid surface [37, 38]. At the same time, the examination of *Synechococcus* sp. PCC 7942 with the combination of electron microscopy and immunochemistry demonstrated that ATPases and PSI complexes are mainly localized on the outer surface of thylakoid membranes, whereas the PSII and cytochrome b_6f complexes are distributed randomly between the inner and the outer membrane surfaces of thylakoids [39].

Recently, cryoelectron tomography has appeared in the arsenal of techniques for the study of cyanobacteria. This method is based on measuring the intensity of a neutron beam scattered by the sample at small scattering angles (small angle neutron scattering). Other modern methods include confocal microscopy and atomic force microscopy [40]. Studies by means of small angle neutron scattering showed that illumina-

tion produces a reversible reorganization in the thylakoid membrane of cyanobacteria [41, 42]. A variable distance between the thylakoid membranes is considered as a regulatory factor that correlates with many *in vivo* photosynthetic processes.

A series of photo-induced electron transfer steps occurring in the PSII reaction center converts light energy into the energy with an electrochemical potential and accounts for the splitting of water. Electron transfer reactions are associated with the formation of an electrochemical gradient across the thylakoid membrane, which is required for the ATP synthase activity. In addition, NADPH is synthesized [43]. The produced ATP and NADPH are consumed for CO₂ fixation in the Calvin cycle and in other anabolic processes occurring in cyanobacterial cells. The functions of genes and proteins providing cyanobacteria with the unique ability of carbon dioxide uptake from the atmosphere for its further use in photosynthetic dark reactions were the subject of detailed studies [44].

Technological improvements in structural biology over recent decades have brought abundant information on molecular details of the structure and function of electron-transport complexes in the thylakoid membranes of cyanobacteria. The atomic structures of individual protein complexes were deciphered, and their spectroscopic analysis was performed [45, 46]. However, a number of problems remain unresolved. It is still unknown how the individual components of electron transport are synthesized and degraded, how their functions are regulated, and how they interact with each other within the same membrane or in different cell membranes. The understanding of how each component works and is finely regulated in a living cell is still a matter of further studies.

MEMBRANE TRANSPORT IN CYANOBACTERIAL CELLS

Cyanobacterial cells are surrounded by the plasma membrane and, in addition, by an outer membrane and a layer of peptidoglycan. The internal volume of most cyanobacteria contains the thylakoid membranes accommodating photosynthetic systems. Cyanobacterial ion transport systems—their properties, kinetics, and energy supply—were investigated using the preparations of intact cells, isolated membrane vesicles, and the proteoliposomes with reconstituted carrier proteins. Unfortunately, the features of transport processes in different types of cyanobacterial membranes are poorly characterized to date.

The structural features of cyanobacterial trichomes permit the cell-to-cell transmission of certain ions and molecules via the periplasmic space. For this purpose, the cytoplasmic membranes of neighboring cells must contain specific proteins capable of transporting certain substances across the cell membrane in the required direction. A recent review [47] summarized data on many transporters in the cytoplasmic mem-

branes of filamentous heterocyst-forming cyanobacteria, including the transporters of amino acids, oxoacids, and sucrose, as well as transporters of some amino acids needed for optimal diazotrophic growth. However, the possibility of diffusional exchange between vegetative cells and heterocysts through the periplasm is still a debated issue and is far from being commonly accepted.

In the model cyanobacterium *Synechocystis* sp. PCC 6803, representatives of all main types of membrane channels and carriers have been detected in the thylakoid membrane [48]. (1) K^+ channels are involved in regulating the electric component of proton-motive force. (2) The primary transporters are represented by Ca^{2+} -transporting ATPase that provides copper ions required for the functioning of thylakoid membranes. (3) Among secondary transporters, the Na^+/H^+ exchanger was identified that balances the proportions of sodium and potassium ions and alleviates the toxic effect of excess Na^+ content.

Stebegg et al. [2] reviewed recent data on the transport of various organic substances through the plasma membranes of model cyanobacterial species. In particular, the transport of monosaccharides (glucose, xylose, arabinose, etc.), disaccharides (sucrose, maltose, lactose, etc.), and polysaccharides was described in detail. The transport of amino acids and peptides, carboxylic acids, alcohols, organic dyes, DNA, urea, herbicides, antibiotics, and other compounds was thoroughly analyzed.

The so-called ABC transporters represent a group of transport proteins that are very important for cyanobacteria. These transporters are intramembrane proteins that use ATP energy to transfer substances across the membrane against the concentration gradients [49]. All such transporters contain a transmembrane domain and two attached ATPases located in the cytoplasm. The quaternary structure of ABC transporters appears conserved in evolution. The transport mechanism is based on conformational changes in the transmembrane domain, which are induced by ATP hydrolysis in the corresponding ATPase domain.

According to Davidson et al. [50], ABC transporters can be regarded as universal technologies of biological systems, since they are common in all kingdoms of life and, apparently, have a common predecessor. In terms of their functional role, ABC transporters can be divided into three groups: (1) importers of peptides, amino acids, mono- and oligosaccharides, ions, metals, and vitamins; (2) exporters of substances, such as lipids, polysaccharides, and proteins, including toxins and virulence factors; and (3) members that do not transport any substance but participate in DNA repair and mRNA translocation [50].

According to Shvarev and Maldener [49], the genome of a model heterocyst-forming filamentous cyanobacterium *Anabaena* sp. PCC 7120 contains 187

open reading frames encoding ABC transporter domains. This number accounts for approximately two-thirds of the 313 genes encoding transport proteins together with accessory proteins and nearly 3% of the total genome. This includes 91 ATP-binding domains, 51 permease domains (transmembrane domains), and 38 domains binding the periplasmic proteins. The authors of the review [49] consider in detail the role of *Anabaena* sp. PCC 7120 specific ABC transporters in adaptation to diazotrophic growth.

The functioning of ABC transporters is an indispensable condition for the survival of cyanobacteria in changeable environments; cyanobacteria were the first organisms where these transporters were discovered [50–52]. Unfortunately, the dynamics and functioning of the most famous primary transport system, i.e., H^+ -transporting ATPase in photosynthetic membranes of cyanobacteria is still incompletely characterized [12]. At the same time, this knowledge is basic for understanding of how the functions are distributed in the trichome between vegetative cells and heterocysts.

Recently, electrical phenomena in bacteria have attracted growing interest. Prindle et al. [53] assessed the membrane potential (MP) in bacterial films using an MP-sensitive fluorescent probe, thioflavin T (ThT), and observed metabolic oscillations correlated with the MP waves. The mathematical modeling of these events led to the conclusion that the potassium channels change their permeability under stress and that the bacteria use the resulting MP oscillations as electrical signaling to neighboring cells within the biofilm. The authors believe that a depolarization wave can restrict the accumulation of glutamate or the release of ammonia by peripheral cells, thus creating favorable conditions for cell functioning in the middle of the bacterial layer. The idea is put forward that the regulated conductance of potassium channels in the plasma membrane provides the basis for electrical interactions among the cells in bacterial biofilms. The analysis of available data suggests that the plasma membranes of bacteria accommodate the principal ion channels (K^+ -, Na^+ -, and Cl^- -channels) known also for other organisms. A hypothesis is proposed that the long-range electrical interactions in bacterial biofilms involve the regulation of K^+ -channel conductance in the plasma membrane. The transport systems for glutamate (anion) and ammonium (cation) are tentative players in electrical interactions because the transport of charged metabolites depends on the transmembrane electric potential difference and the proton-motive force.

“DIVISION OF TASKS” AND INTERCELLULAR EXCHANGE IN TRICHOMES OF CYANOBACTERIA

In the absence of nitrogen, the trichomes of some cyanobacteria differentiate specialized cells that fulfill various functional tasks: vegetative cells perform pho-

tosynthesis and release oxygen, while heterocysts fix atmospheric nitrogen. At the same time, the corresponding metabolites and regulatory substances are the subject of intercellular exchange. Owing to the intercellular exchange in diazotrophic filaments of cyanobacteria, the reduced carbon is transported to heterocysts, while the fixed nitrogen is conveyed to vegetative cells [47]. Statistical analysis of fluctuations in gene expression by individual cells along the trichome revealed the correlation of gene expression in neighboring cells [54], which implies the existence of intercellular communications along the cyanobacterial trichomes.

The cell-to-cell transfer of ions and molecules can occur via the periplasm [55–57] or through special contact structures called microplasmodesmata, septosomes, septal contacts, or nanopores [57–60]. The septa between heterocysts and vegetative cells were shown to possess channels with the diameter of 12 nm and the length of 20 nm [61]. Each septum between vegetative cells comprises 100–250 microplasmodesmata [62], while the septa between vegetative cells and heterocysts contain approximately 50 microplasmodesmata [58].

After a local injection of fluorescein, a marker of permeable contacts, this dye was found to distribute between the cells along the trichome [16]. Other fluorescence probes, such as 5-carboxyfluorescein (mol wt 376 D) and calcein (622 D) were also used in combination with FRAP analysis (fluorescence recovery after photobleaching) for studying the intercellular transport. The FRAP experiments with the model cyanobacteria *Anabaena* sp. PCC 7120 revealed that calcein diffuses between vegetative cells as well as between vegetative cells and heterocysts. Remarkably, the amount of diffused calcein increased tenfold in trichomes growing in a nitrogen-deficient medium compared to the trichomes grown in the presence of nitrate [63]. Intercellular calcein exchange was absent in *Oscillatoria*, a filamentous cyanobacterium that does not form heterocysts [64]. Calcein and 5-carboxyfluorescein are not involved in cell metabolism, unlike such fluorescent indicator as esculin (glycosylated coumarin, mol wt 340 D), which is a sucrose analogue and was recently introduced into the practice for analysis of intercellular exchange [65]. The interconnections between the cells allow small molecules, such as sucrose, to move from vegetative cells to heterocysts [65–69] and allow the transfer of the dipeptide beta-aspartyl-arginine from the heterocysts to vegetative cells [70, 71].

Judging from measurements with fluorescent probes, the intercellular exchange of metabolites was impaired in mutants defective in SepJ, FraC, and/or FraD proteins [63–65, 72–74]). At the same time, different mutants showed the disturbed exchange of different molecular probes.

The cell-to-cell passage of substances may principally involve their leakage into the periplasm and the subsequent entry into neighboring cells by means of specific transporters. For example, it is reasonable to assume that the cytoplasmic membranes of vegetative cells contain the exporters of sugars, while the heterocysts contain sugar importers and, conversely, the heterocysts possess the exporters of amino acids, while vegetative cells have the amino acid importers [55]. Inactivation of the two amino acid transporters specific for vegetative cells was found to disturb the N₂-dependent growth of *Anabaena* sp. PCC 7120, thus proving the role of these transporters in diazotrophic physiology [75, 76]. The importance of periplasm as a pathway for metabolic exchange implies a low permeability of the outer membrane for certain metabolites; recent data confirmed the low permeability of the outer membrane to sucrose and glutamate [77]. Many transporters of the cytoplasmic membranes were characterized for the model cyanobacterium species *Anabaena* [78]. The examined components include the transporters of amino acids, oxoacids, and sucrose [79, 80] as well as those of some amino acids required for optimal diazotrophic growth [75, 76].

The ABC exporter HetG is reportedly involved in the transmission of the PatS protein. It also transmits the HetN protein-related signal from the heterocysts to vegetative cells. The transmission of these regulating agents, at least between vegetative cells, was noticed to involve the septal protein SepJ. Frain et al. [81] outlined the main pathways for translocation of proteins related to intercellular exchange: general secretory pathway (Sec), twin arginine translocation (Tat), and the signal recognition particle (SRP). They also emphasized the importance of membrane organization and the relationships between translocation, integration, and targeting of proteins within the various membranes of cyanobacteria. Motility of trichomes depends on the coordinated activity of molecular motors in different cells, which also requires cell-to-cell communication [82].

Flores and Herrero [83] discussed particular mechanisms allowing cyanobacteria (1) to produce two types of cells with different metabolic activities, i.e., photosynthesizing vegetative cells and nitrogen-fixing heterocysts, (2) to keep these cells together, and (3) to ensure the intercellular exchange of substances required for growth. The intercellular septa in filamentous cyanobacteria contain a protein SepJ that plays a special role in trichomes' functioning. The protein SepJ also interacts with the protein ZipN [84] that was previously identified as an essential component of the protein complex responsible for the division of cyanobacterial cells. The development of heterocysts from vegetative cells depends on the activity of a number of proteins that regulate the genetic differentiation program. This program runs as a response to nitrogen deprivation and implicates a protein NtcA, a global nitrogen-controlled transcription factor in cyanobacteria. The fulfillment of the program requires specific

regulators, such as HetR, which are needed for differentiation and are synthesized in cells within the first hours of nitrogen starvation. The knowledge of how the functional interactions between NtcA and HetR are implemented and how the conversion of too many vegetative cells into the heterocysts is prevented by other regulating factors is crucial for understanding the whole process that leads to the formation of a true multicellular bacterium [85]. The number of heterocysts in the trichome is also under strict genetic control. Differentiation of excess heterocysts is prevented by diffusing inhibitors formed in proheterocysts or heterocysts [47]. The products of the *patS* and *hetN* genes are involved in the inhibition [86–88].

A group of authors [89] investigated the diazotrophic growth and the mechanisms of intercellular transport in trichomes of *Cylindrospermopsis raciborskii* CS-505 consisting of 100 vegetative cells and only two terminal heterocysts. The rate of fluorescent probe passage between fast-growing terminal vegetative cells and heterocysts was higher than that between slow-growing vegetative cells in the middle of the trichome.

The role of intercellular septa and the periplasm in the cell-to-cell transmission of molecules is of great interest. Analysis of mutants incapable of fixing nitrogen and characterized by filament fragmentation in liquid media revealed the role for products of several genes (*fraC*, *fraD*, and *fraG* (also known as *sepJ*)) in the intercellular transfer of molecules [72–74]. The review on morphology, physiology, and genetics of intercellular interactions in filamentous cyanobacteria [74] emphasizes that cyanobacterium *Anabaena* sp. PCC 7120 contains not only the obligatory components of septal contacts (the proteins SepJ, FraC, and FraD) but also the proteins required for the formation and functioning of nanopores. Some components were identified, such as amidases, peptidoglycan-binding proteins, and several membrane transporters. Analysis of intercellular exchange disorders in *Anabaena* mutants revealed the functional similarity of intercellular septa of cyanobacteria with gap junctions of animal cells [74].

Thus, it is commonly accepted that, in trichomes with the “division of labor,” vegetative cells obtain nitrogen-containing organic substances from heterocysts by means of diffusional transfer across the intercellular septa [71, 90], whereas heterocysts receive the products of carbon dioxide fixation from vegetative cells [65, 90]. At the same time, the study of electrical cable properties of trichomes suggests that heterocysts may contain the membrane transport systems, primarily the membrane ATPases that directly employ the energy comprised in the membrane potential [14–21]. Heterocysts need ATP for the activities of nitrate reductase and ABC transporters [49]. A rather regular distribution of heterocysts in cyanobacterial filaments (one heterocyst per 10–15 vegetative cells [47]) seems not accidental, because this arrangement is suited for the passage of electric currents through intercellular

contacts, which allows the effective use of the membrane potential energy generated by vegetative cells.

ELECTRICAL CONNECTIONS IN TRICHOMES AND FEASIBILITY OF INTERCELLULAR ENERGY TRANSMISSION

In 1946, Hodgkin and Rushton [91] experimentally showed that single fibers, such as the squid giant axon, behave like an electric cable. The distribution of electric potential in such cables was entirely consistent with the theory developed earlier by Lord Thomson to describe the distribution of electricity in a telegraph cable laid at the bottom of the Pacific Ocean. The researchers introduced the microelectrode into the axon and passed electric current, thus changing the membrane potential at the point of electrode insertion. Using the second microelectrode, they measured the membrane electric potential at different distances from the first electrode. The electric potential was found to decrease exponentially with distance. The plot of the potential decline yielded direct estimates of the characteristic cable length. By measuring the membrane voltage directly near the current electrode at a given strength of the passing current, the so-called input resistance (R_{in}) of the fiber can be determined. This resistance is exactly half of the characteristic resistance, because the current entering the fiber spreads in two directions. Knowing the fiber diameter, the researchers calculated the axon membrane resistance, which turned out to be very small compared to the resistance of insulation in technical cables; they also calculated specific resistance of the axoplasm, which, on the contrary, was approximately 25 million times higher than that of copper.

The work of Hodgkin, Huxley, and Rushton was awarded the Nobel Prize, and the concept of cable theory promoted rapid progress in practical electrophysiology. In particular, these ideas were successfully used in analysis of electrical interactions in various systems where living cells are interconnected by permeable cell-to-cell contacts: multicellular microorganisms, developing embryos, cardiac and muscle tissues, etc. [14]. The notions of long or short distances for biological cables are defined by the length constant and are measured in meters, centimeters, etc. The terms of fast or slow responses are specified by the so-called time constant, which depends on specific resistance and electric capacity of the membrane. The “time constant” of biological cables is expressed in seconds.

Electrophysiological experiments performed in the 1980s by means of standard methods designed for studying the generation and propagation of electric potentials in nerve and muscle fibers revealed that illumination of cyanobacterium *Phormidium uncinatum* leads to the formation of electric potential difference across the cell membranes (negative potential inside

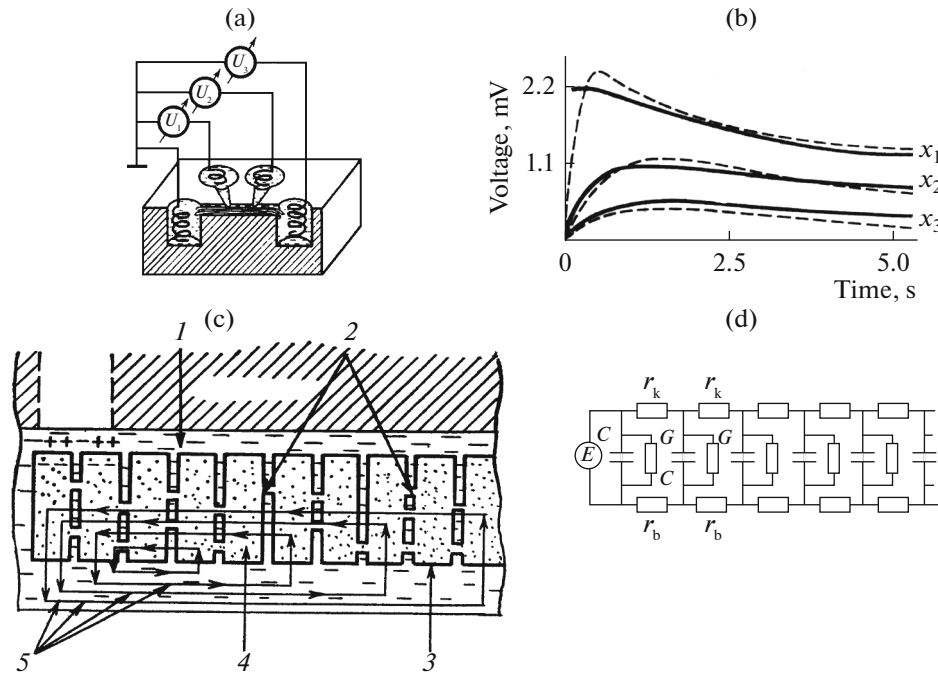


Fig. 1. Propagation of electricity along the trichome bundle of a filamentous cyanobacterium *Phormidium uncinatum* [14]. (a) Diagram of the setup for extracellular measurements of photoelectric responses: a bundle of parallelly aligned trichomes was placed into a groove at the bottom of a Plexiglas chamber filled with distilled water. U_1 , U_2 , and U_3 are silver electrodes connected to a reference electrode; (b) the electrical responses of a trichome bundle to local beam illumination ($d = 20 \mu\text{m}$) with photosynthetically active light. Solid lines are theoretical curves calculated for an equivalent electric cable at various distances between the light spot and the recording electrode: $x_1 = 0$, $x_2 = 400 \mu\text{m}$, and $x_3 = 800 \mu\text{m}$; dashed lines are the experimental responses recorded at the same separation distances x_1 , x_2 , and x_3 ; (c) schematic view of the trichome segment structure: (1) extracellular medium; (2) permeable intercellular channels; (3) cell membrane; (4) intracellular medium; (5) lines showing pathways of electric currents induced by local illumination of the trichome; (d) equivalent electrical circuit of the trichome segment: r_k —resistance of intercellular channels; r_b —resistance of bathing medium; G —leakage conductance of the cell membrane; C —electric capacity of the cell membrane; E —electromotive force of a generator.

the cell) [14–17]. Theoretical and model analysis of extracellular currents induced by local illumination of the *Ph. uncinatum* trichome bundle showed that trichomes are cell associations organized into continuous cable structures (Fig. 1).

Extracellular electrical measurements carried out with the trichome bundles [14–17] or individual trichomes [18, 92] of *Ph. uncinatum* revealed a significant hyperpolarization of the shaded parts of the sample in response to local illumination of a small region of the trichome with photosynthetically active light. Within the framework of cable theory, the decreasing magnitude of the electric currents under prolonged local illumination of the trichome can be ascribed to the hyperpolarization of plasma membranes in the trichome darkened regions due to the presence of electrical communication through the intercellular channels [14–17]. The properties of electric currents recorded under local illumination of a trichome bundle were found to be similar to the currents arising in an artificial cable circuit in response to local connection of a voltage generator. The currents induced in trichomes by local illumination were also simulated by means of a mathematical model describing the equivalent electric

cable. All these data confirmed the assumed cable structure of the trichome and quantified the cable properties of trichomes in filamentous cyanobacteria. In our experiments, the hyperpolarization propagated from illuminated to darkened cells, in apparent similarity with the potential propagation in an electric cable having the length constant of approximately $400 \mu\text{m}$.

In the 1980s, a unique experimental setup was assembled at the Institute of Biophysics (Academy of Sciences of the Soviet Union) for simultaneous recording of photoelectric responses and spectral characteristics in elongated microobjects [92] (Fig. 2).

For observations in transmitted light, the light from a xenon incandescent lamp (1) passes through a condenser (2) to a mirror (3) of the microscope turret positioned under the microscope stage, and then it is directed to a microscope condenser (4). The image of an object (5) is collected by a microscope objective lens (6) and directed through a beam splitter plate with a long-pass filter (7) to a photoelectrometric unit. The latter is equipped with a spherical mirror (9) having a probing aperture (8); this mirror directs light to an eyepiece (10). In this case, the image plane of the examined object coincides with the mirror plane (8).

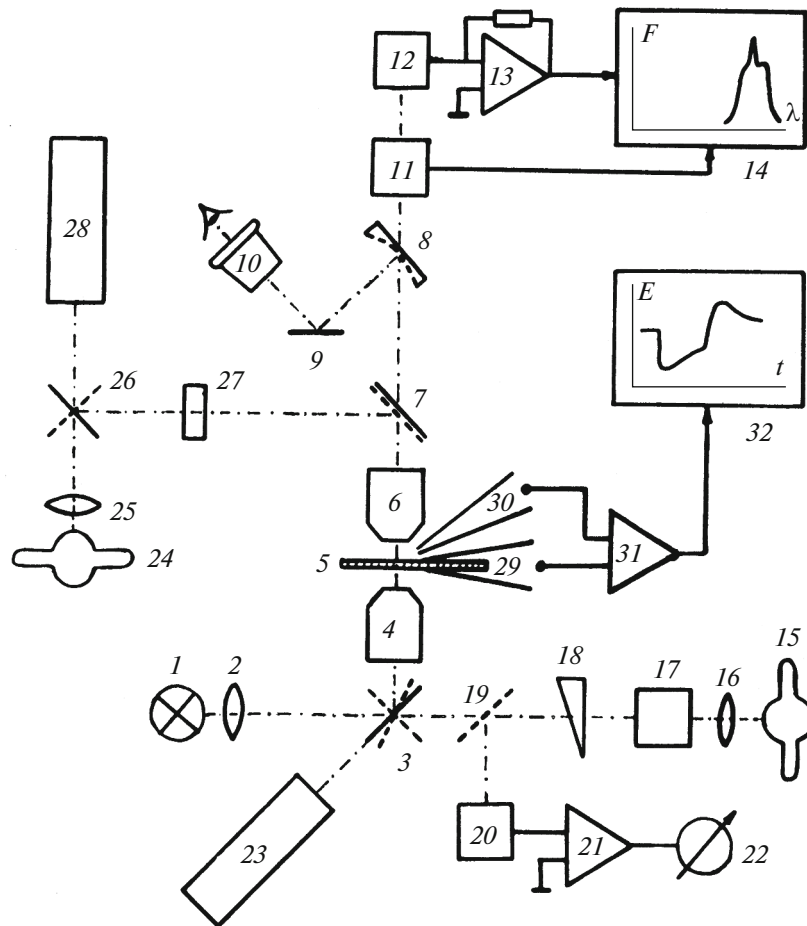


Fig. 2. Schematic diagram of the setup used to record the photoelectric responses and spectral characteristics of individual trichomes [96]. (1) xenon incandescent lamp; (2) condenser; (3) mirror of the turret under a microscope stage; (4) microscope condenser; (5) sample; (6) microscope objective lens; (7) interchangeable beam-splitting plate with a long-pass filter; (8) probing aperture in a spherical mirror; (9) mirror; (10) eyepiece; (11) monochromator; (12) photoamplifier; (13) amplifier of a photocurrent; (14) two-coordinate chart recorder; (15) mercury lamp with discrete spectrum; (16) condenser; (17) monochromator; (18) optical wedge; (19) mirror; (20) thermocouple; (21) preamplifier; (22) recorder; (23) laser; (24) mercury lamp with discrete spectrum; (25) condenser; (26) rotary mirror; (27) a set of filters; (28) gas laser; (29, 30) microelectrodes; (31) preamplifier; (32) chart recorder.

Absorption spectra were measured with light from the same source (1). For records in the UV spectral region, the incandescent lamp was replaced with a mercury lamp with discrete spectrum. A 59×0.65 UV objective lens was used as a condenser (4). A nonluminescent Millipore membrane filter served as a scattering plate; it was introduced in front of the objective lens (5), when the beam-splitting plate (7) was removed. The criterion for the quality of scattering compensation was the absence of light attenuation by the object in the far red region ($\lambda > 730$ nm). The optical density (D) was defined by the formula:

$$D = -\log I/I_0,$$

where I is light intensity at a given wavelength that passed through the object and I_0 is a nonattenuated background intensity. During measurements, a part of the object image (5) incident on the probing aperture

in the mirror (8) was projected onto the entrance slit of a monochromator (11) mounted over the photoelectric unit. Depending on the spectral range, the monochromatic radiation was then directed to photoelectroamplifiers (12). The amplifier of a photocurrent (13), based on a microcircuit and assembled directly in a shielded case of the photoamplifier, fed the signal to a two-coordinate chart recorder (14) whose horizontal coordinate was calibrated in wavelengths and controlled from a monochromator (11).

For local monochromatic photostimulation of individual cells, the image of the arc of a mercury lamp with discrete spectrum (15) was focused with a condenser (16) on the entrance slit of a monochromator (17). Next, monochromatic light was directed through an optical wedge (18) to a mirror (19) and a thermocouple (20). The thermocouple voltage was amplified using a preamplifier (21), assembled on a

microcircuit, and fed to the recorder (22). After adjusting the intensity of monochromatic radiation with an optical wedge (18), the mirror (19) was turned to direct the light flux to the mirror (3) of the turret and a condenser (4). Various UV lenses were used as a condenser, which allowed wide-range variations in the size and intensity of the image of the monochromator output slit (17) in the plane of the object. For local stimulation with a coherent monochromatic light, a suitable laser (23) could be used.

Luminescence of the object was excited with a mercury lamp with discrete spectrum (24) mounted on the microscope base. In this case, the exciting light passed through a condenser (25), a rotary mirror (26), a set of light filters (27), a beam splitter plate (7), and was directed through an objective lens (6) to an object (5). The luminescence spectra were recorded similarly to the registration of transmitted light.

To emphasize high sensitivity of the setup, it should be noted that the variation coefficient of luminescence intensity for the reference uranium glass did not exceed 1%, even when the diameter of the probing aperture was set at its minimum value of approximately 2 μm in the plane of the sample.

For local irradiation of individual cell structures with a wavelength of 337 nm, a gas laser (28) was used in combination with a "blue" beam-splitting plate. The optical design of the experimental system ensured a minimum beam angle corresponding to 1 μm diameter in the sample plane. The system allowed the study of objects by the method of phase contrast as well as by absorption and luminescent photometry. The potential difference between microelectrodes (29, 30) was fed into a preamplifier (31) and recorded on a chart recorder (32).

Simultaneous measurements of microspectral characteristics and extracellular electric currents of *Ph. uncinatum* individual trichomes proved that the hyperpolarization of cell membranes was induced by absorption of light in the photosynthetically active spectral regions (Figs. 3, 4).

An individual trichome of *Ph. uncinatum* was tightly fixed in a glass micropipette filled with distilled water. The tip diameter of a micropipette was 5–6 μm , which is comparable to the trichome diameter. A smaller part of the trichome was placed in a drop of distilled water on a glass slide fixed on a microscope stage, and a large part was located inside the pipette. This method for trichome mounting reduced the leakage currents and increased the voltage drop in the clamped zone. The electrical resistance between the electrode placed in a water drop and the electrode inside the pipette was 100–200 M Ω in the absence of the trichome and was 200–700 M Ω after mounting the trichome (Fig. 3).

The extracellular photoelectric response of the trichome to local illumination reflects a multistep process of cell membrane hyperpolarization. The typical

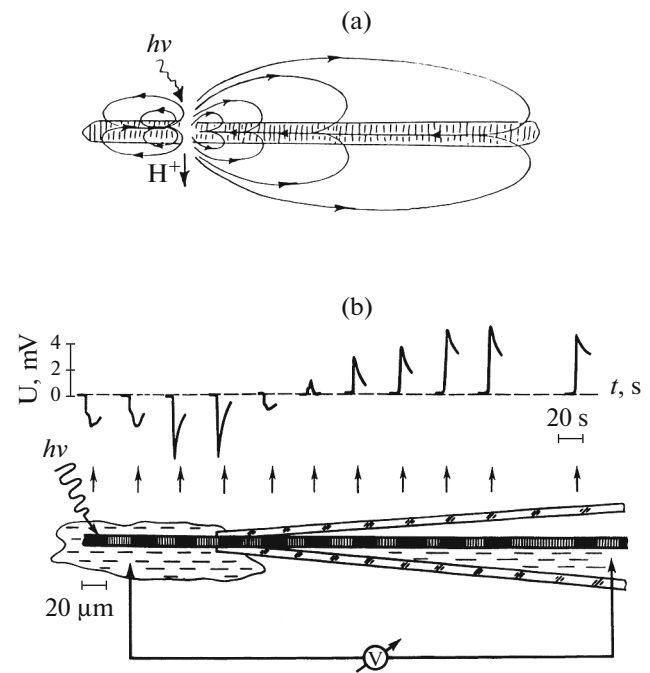


Fig. 3. Electric currents induced by local illumination of the trichome. (a) Streamlines of electric currents caused by local illumination of the trichome with photosynthetically active light [19]. Local illumination of any 2–10 cells induced extracellular electric currents whose magnitude and location depended on the position of illuminated area; (b) scheme of the experiment [18]. U designates the voltage (mV); t is time (s).

response consists in the biphasic increase in extracellular electric current followed by the decline of the current during prolonged light exposures (Fig. 4).

The magnitudes and shapes of individual responses depended on light intensity, the spectral composition of radiation, duration of preliminary dark adaptation, pH of the medium, and cultivation conditions [93]. Comparative analysis of photoelectric responses and the absorption spectra of trichome disclosed the relation of photoelectric activity to the absorption of photosynthetically active radiation by chlorophyll (the peak at 680 nm) and phycocyanin (625 nm), the main photosynthetic pigments of cyanobacteria [18, 93]. Remarkably, the spectral characteristics of light-induced electrical responses were subject to local heterogeneities [18, 93, 94].

The hyperpolarization of cell membranes induced by local illumination and extending to a distance of tens of cells can energize ATP synthases and drive ATP synthesis in membranes of neighbored shaded cells. In our experiments, dicyclohexylcarbodiimide (DCCD), an inhibitor of ATP synthase, increased the amplitude of extracellular electric currents severalfold [15].

Thus, the study of trichome photoelectric activity in a filamentous cyanobacterium *Ph. uncinatum*, combined with the theoretical and model analysis, showed

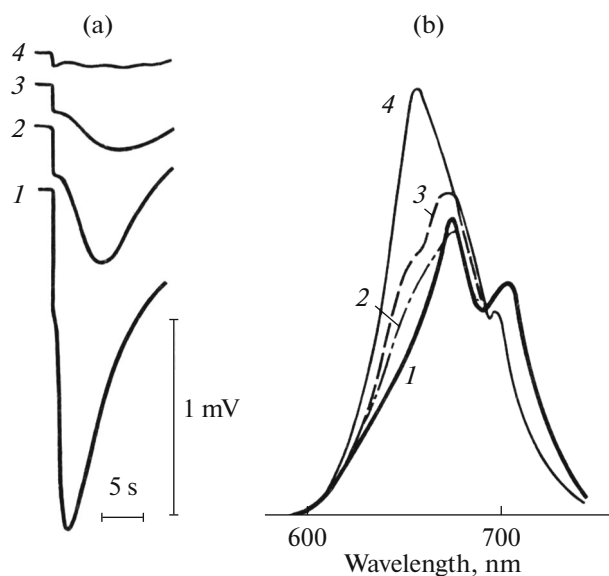


Fig. 4. Spectral characteristics of the electrical responses of the *Phormidium uncynatum* individual trichome. Changes in (a) photoelectric responses and (b) fluorescence spectra of the same region in the trichome of *Ph. uncynatum* under the action of intense radiation from a mercury lamp ($\lambda = 436$ nm). The length of the illuminated trichome area and the diameter of the optical probe were 20 μm . (a) Illumination with a monochromatic light ($\lambda = 620$ nm); (b) excitation of fluorescence at $\lambda = 436$ nm. Irradiation time: (1) 45 s; (2) 3 min; (3) 4 min; (4) 6 min [18]. Data were recorded from an oscilloscope screen. Scale bars for horizontal and vertical axis are shown.

that the trichome is organized into a continuous cable structure by virtue of intercellular electrical communications [14–17]. The electric currents generated by photosystems in the plasma membranes of illuminated cells convey the work-performing capacity from the cells that absorb light energy to darkened cells that cannot be directly energized from an external source. The hyperpolarization of cell membranes generated by the photosynthetic apparatus can perform a number of energy-dependent functions: it promotes the functioning of ATP synthases, transport mechanisms, the motor apparatus, etc., both at the point of illumination and at a distance of tens of cells from the illuminated site. In filamentous cyanobacteria featuring the separation of functions between heterocysts and vegetative cells, the intercellular electrical connections within the trichome ensure the operation of plasma-membrane ATP synthases in heterocysts that are lacking photosynthetic systems of their own. Thus, the heterocysts remain functional and well supplied with ATP resources without the increase in oxygen concentration.

The power transmitted along the trichome via intercellular channels was roughly estimated. Local illumination of 2–10 cells of the trichome induces the change in potential difference V of approximately 20 mV [18, 93]. The micropipette resistance R in these experiments

was 400 M Ω ; thus, the extracellular current can be estimated: $I = V/R = 50$ pA. Since the electric current of 1 pA carries 6×10^6 electric charges per second, and the production of 1 ATP molecule requires the transfer of 4–5 H^+ across the thylakoid membrane of cyanobacteria [95], one may conclude that illuminated cells provide the darkened cells in the trichome with the ability to produce $(1.2\text{--}1.5) \times 10^6$ ATP per second. The ATP turnover in a bacterial cell is $10^7\text{--}7 \times 10^7$ ATP molecules per second on average [96]. It is thus evident that energy transmission along the trichome can significantly contribute to total production of cell energy.

CONCLUSIONS

Multicellularity is a form of organization in living systems, in which a group of cells fulfils more complex functions than any individual cell. Filamentous heterocysts-forming cyanobacteria are the prototype of a multicellular organism, because their cells are not just clustered into agglomerates but represent a functional association unified by exchange of metabolic products and regulatory molecules as well as by electrical communications through highly permeable intercellular contacts (PIC).

In diverse multicellular systems—organs, tissues, developing embryos, cell cultures, and multicellular microorganisms—PIC provide conditions for sharing the intracellular low-molecular-weight components (see reviews [14, 97]). The ability of PIC to pass ionic flows comparable in magnitude to the flows through the plasma membrane allows these structures to participate in the self-organization of multicellular systems. The separation of functions between neighboring cells in any tissue allows some cells to receive an “energy subsidy” from neighbor cells by virtue of ion fluxes through PIC. The energy acquired by this means corresponds to the energy needed for the operation of primary ion pumps (up to one-third of the total cell energy production) [98]. The cell-to-cell transmission of energy by means of intercellular electrical communication through permeable intercellular contacts should apparently be regarded as one of the oldest natural technologies of biological systems [19, 20].

Multicellularity is an evolutionary innovation that represents a new level of organization and is a necessary tool for sophisticated adaptation techniques. From the viewpoint of modern molecular genetics, a group of filamentous cyanobacteria featuring separation of functions between neighboring cells is a prototype of multicellular organization and provides a convenient model for elucidating the mechanisms of regulation of multicellularity, which, apparently, appeared more than once during the evolution in different phylogenetic groups [99, 100]. It is highly important that the ability of bioenergetic cooperation in electrically interconnected cells appeared at the dawn of evolution, billions of years ago, as a structural

and functional basis for the division of labor in trichomes of filamentous cyanobacteria, the first multicellular organisms of our planet.

ACKNOWLEDGMENTS

We are grateful to Professor V.V. Aleshin and to Professor Yu.A. Koksharov for careful reading and discussion of the manuscript and for critical comments.

FUNDING

The study was supported by state program nos. AAAA-A17-117120570011-4 and AAAA-A17-117120820043-7. It was also supported by the Russian Foundation for Basic Research (project no. 17-04-00412).

COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies with human participants or animals performed by any of the authors. The authors declare that they have no conflict of interest.

REFERENCES

- Ugolev, A.M., *Estestvennye tekhnologii biologicheskikh sistem* (Natural Technologies for Biological Systems), Leningrad: Nauka, 1986.
- Stebegg, R., Schmetterer, G., and Rempel, A., Transport of organic substances through the cytoplasmic membrane of cyanobacteria, *Phytochemistry*, 2019, vol. 157, p. 206.
- Ku, C., Nelson-Sathi, S., Roettger, M., Sousa, F.L., Lockhart, P.J., Bryant, D., Hazkani-Covo, E., McInerney, J.O., Landan, G., and Martin, W.F., Endosymbiotic origin and differential loss of eukaryotic genes, *Nature*, 2015, vol. 524, p. 427.
- Lyons, T.W., Reinhard, C.T., and Planavsky, N.J., The rise of oxygen in Earth's early ocean and atmosphere, *Nature*, 2014, vol. 506, p. 307.
- Mereschkowsky, K.S., Über Natur und Ursprung der Chromatophoren im Pflanzenreiche, *Biol. Centralblatt*, 1905, vol. 25, p. 593.
- Famintsyn, A.S., *O roli simbioza v evolyutsii organizmov* (The Role of Symbiosis in the Evolution of Organisms), St. Petersburg: Imper. Akad. Nauk, 1907.
- Sánchez-Baracaldea, P., Raven, J.A., Pisani, D., and Knoll, A.H., Early photosynthetic eukaryotes inhabited low-salinity habitats, *Proc. Natl. Acad. Sci. USA*, 2017, vol. 114: e7737. <https://doi.org/10.1073/pnas.1620089114>
- Schirrmeister, B.E., de Vos, J.M., Antonelli, A., and Bagheri, H.C., Evolution of multicellularity coincided with increased diversification of cyanobacteria and the Great Oxidation Event, *Proc. Natl. Acad. Sci. USA*, 2013, vol. 110, p. 1791.
- Schirrmeister, B.E., Antonelli, A., and Bagheri, H.C., The origin of multicellularity in cyanobacteria, *BMC Evol. Biol.*, 2011, vol. 11, p. 45. <https://doi.org/10.1186/1471-2148-11-45>
- Skulachev, V.P., Bogachev, A.V., and Kasparinsky, F.O., *Membrannaya bioenergetika* (Membrane Bioenergetics), Moscow: Mosk. Gos. Univ., 2010.
- Cardona, T., Battchikova, N., Zhang, P., Stensjo, K., Aro, E.-M., Lindblad, P., and Magnuson, A., Electron transfer protein complexes in the thylakoid membranes of heterocysts from the cyanobacterium *Nostoc punctiforme*, *Biochim. Biophys. Acta*, 2009, vol. 1787, p. 252.
- Liu, L.N., Distribution and dynamics of electron transport complexes in cyanobacterial thylakoid membranes, *Biochim. Biophys. Acta*, 2016, vol. 1857, p. 256.
- Mullineaux, C.W., Organization and dynamics of bioenergetic systems in bacteria, *Biochim. Biophys. Acta*, 2016, vol. 1857. <https://doi.org/10.1016/j.bbabi.2016.01.004>
- Berkinblit, M.B., Bozhkova, V.P., Boitsova, L.Yu., Mittel'man, L.A., Potapova, T.V., Chailakhyan, L.M., and Sharovskaya, Yu. Yu., *Vysokopronitsaemye kontakt-nye membrany* (High Permeability Contact Membranes), Moscow: Nauka, 1981.
- Chailakhyan, L.M., Glagolev, A.N., Glagoleva, T.N., Murvanidze, G.M., Potapova, T.V., and Skulachev, V.P., Intercellular power transmission along trichomes of cyanobacteria, *Biochim. Biophys. Acta*, 1982, vol. 679, p. 60.
- Levin, S.A., Potapova, T.V., Skulachev, V.P., and Chailakhyan, L.M., Propagation of electrical potential changes in filamentous cyanobacteria, *Biofizika*, 1982, vol. 27, p. 280.
- Levin, S.A., Potapova, T.V., Skulachev, V.P., and Chailakhyan, L.M., Analysis of the cable structure of blue-green algae, *Biofizika*, 1982, vol. 27, p. 684.
- Potapova, T.V., Aslanidi, K.B., Shalapenok, A.A., Karnaukhov, V.N., and Chailakhyan, L.M., Photoelectric activity and spectral characteristics of the single trichoma of cyanobacterium *Phormidium uncinatum*, *Dokl. Akad. Nauk SSSR*, 1986, vol. 289, p. 1504.
- Potapova, T.V., Energetic functions of permeable intercellular junctions, in *Intercellular Communication*, Bukauskas, F., Ed., Manchester: Univ. Press, 1991, p. 143.
- Potapova, T.V. and Aslanidi, K.B., Energy coupling of adjacent cells as an universal function of cell-to-cell permeable junctions, *Prog. Cell Res.*, 1995, vol. 4, p. 53.
- Potapova, T.V. and Boitsova, L.Yu., Structure, function, regulation: experimental analysis in groups of non-excitable cells coupled via permeable junctions, *Membr. Cell Biol.*, 1998, vol. 11, p. 817.
- Sawa, M., Fantuzzi, A., Bommelli, P., Howe, C.J., Hellgardt, K., and Nixon, P.J., Electricity generation from digitally printed cyanobacteria, *Nat. Commun.*, 2017, vol. 8, p. 1327. <https://doi.org/10.1038/s41467-017-01084-4>
- Irimia-Vladu, M., "Green" electronics: biodegradable and biocompatible materials and devices for sustainable future, *Chem. Soc. Rev.*, 2014, vol. 43, p. 588.
- Choi, S., Nicroscale microbial fuel cells: advances and challenges, *Biosens. Bioelectron.*, 2015, vol. 69, p. 8.
- Skulachev, V.P., Adenosine triphosphate and the transmembrane hydrogen ion potential—2 convertible and transportable forms of energy in the living cell, *Usp. Sovrem. Biol.*, 1977, vol. 84, p. 165.

26. Harold, F.M., *The Way of the Cell: Molecules, Organisms and the Order of Life*, Oxford: Oxford Univ. Press, 2001.
27. Bald, D., ATP synthase: structure, function and regulation of a complex machine, in *Bioenergetic Processes of Cyanobacteria: From Evolutionary Singularity to Ecological Diversity*, Peschek, G., Obinger, C., and Renger, G., Eds., Dordrecht: Springer, 2011, p. 239. <https://doi.org/10.1007/978-94-007-0388-9>
28. Liberton, M. and Pakrasi, H.B., Membrane systems in cyanobacteria, in *The Cyanobacteria: Molecular Biology, Genomics and Evolution*, Herrero, A. and Flores, E., Eds., Norfolk: Caister Acad. Press, 2008, p. 217.
29. Rexroth, S., Mullineaux, C.W., Ellinger, D., Sendtko, E., Rögner, M., and Koenig, F., The plasma membrane of the cyanobacterium *Gloeobacter violaceus* contains segregated bioenergetic domains, *Plant Cell*, 2011, vol. 23, p. 2379.
30. Vothknecht, U.C. and Westhoff, P., Biogenesis and origin of thylakoid membranes, *Biochim. Biophys. Acta*, 2001, vol. 1541, p. 91.
31. Nickelsen, J., Rengstl, B., Stengel, A., Schottkowski, M., Soll, J., and Ankele, E., Biogenesis of the cyanobacterial thylakoid membrane system—an update, *FEMS Microbiol. Lett.*, 2011, vol. 315, p. 1.
32. Rast, A., Heinz, S., and Nickelsen, J., Biogenesis of thylakoid membranes, *Biochim. Biophys. Acta*, 2015, vol. 1847, p. 821.
33. Van de Meene, A.M., Hofmann-Marriott, M.F., Vermaas, W.F., and Roberson, R.W., The three-dimensional structure of the cyanobacterium *Synechocystis* sp. PCC 6803, *Arch. Microbiol.*, 2006, vol. 184, p. 259.
34. Van de Meene, A.M., Sharp, W.P., McDaniel, J.H., Friedrich, H., Vermaas, W.F., and Roberson, R.W., Gross morphological changes in thylakoid membrane structure are associated with photosystem I deletion in *Synechocystis* sp. PCC 6803, *Biochim. Biophys. Acta*, 2012, vol. 1818, p. 1427.
35. Nevo, R., Charuvi, D., Shimoni, E., Schwarz, R., Kaplan, A., Ohad, I., and Reich, Z., Thylakoid membrane perforations and connectivity enable intracellular traffic in cyanobacteria, *EMBO J.*, 2007, vol. 26, p. 1467.
36. Zak, E., Norling, B., Maitra, R., Huang, F., Anderson, B., and Parkasi, H.B., The initial steps of biogenesis of cyanobacterial photosystems occur in plasma membrane, *Proc. Natl. Acad. Sci. USA*, 2001, vol. 98, p. 13443.
37. Vermaas, W.F., Timlin, J.A., Jones, H.D., Sicclair, M.B., Nieman, L.T., Hamad, S.W., Melgaard, D.K., and Haaland, D.M., In vivo hyperspectral confocal fluorescence imaging to determine pigment localization and distribution in cyanobacterial cells, *Proc. Natl. Acad. Sci. USA*, 2008, vol. 105, p. 4050.
38. Collins, A.M., Liberton, M., Jones, H.D., Garcia, O.F., Pakrasi, H.B., and Timlin, J.A., Photosynthetic pigment localization and thylakoid membrane morphology are altered in *Synechocystis* 6803 phycobilisome mutants, *Plant Physiol.*, 2012, vol. 158, p. 1600.
39. Sherman, D.M., Troyan, T.A., and Sherman, L.A., Localization of membrane proteins in the cyanobacterium *Synechococcus* sp. PCC 7942, *Plant Physiol.*, 1994, vol. 106, p. 251.
40. Liu, L.N. and Scheuring, S., Investigation of photosynthetic membrane structure using atomic force microscopy, *Trends Plant Sci.*, 2013, vol. 18, p. 277.
41. Nagy, G., Posselt, D., Kovacs, L., Holm, J.K., Szabo, M., Ughy, B., Rosta, L., Peters, J., Timmins, P., and Garab, G., Reversible membrane reorganizations during photosynthesis in vivo: revealed by small-angle neutron scattering, *Biochem. J.*, 2011, vol. 436, p. 225.
42. Liberton, M., Page, L.E., O'Dell, W.B., O'Neill, H., Mamontov, E., Urban, W.S., and Pakrasi, H.B., Organization and flexibility of cyanobacterial thylakoid membranes examined by neutron scattering, *J. Biol. Chem.*, 2013, vol. 288, p. 3632.
43. Johnson, G.N., Physiology of PSI cyclic electron transport in higher plants, *Biochim. Biophys. Acta*, 2011, vol. 1807, p. 384.
44. Badger, M.R. and Price, G.D., CO₂ concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution, *J. Exp. Bot.*, 2003, vol. 54, p. 609.
45. Grotjohann, I. and Fromme, P., Structure of cyanobacterial photosystem I, *Photosynth. Res.*, 2005, vol. 85, p. 51.
46. Gabdulkhakov, A.G. and Dontsova, M.V., Structural studies on photosystem II of cyanobacteria, *Biochemistry (Moscow)*, 2013, vol. 78, p. 1524. <https://doi.org/10.1134/S0006297913130105>
47. Herrero, A., Stavans, J., and Flores, E., The multicellular nature of filamentous heterocyst-forming cyanobacteria, *FEMS Microbiol. Rev.*, 2016, vol. 40, p. 831. <https://doi.org/10.1093/femsre/fuw029>
48. Pfeil, B.E., Schoefs, B., and Spetea, C., Function and evolution of channels and transporters in photosynthetic membranes, *Cell. Mol. Life Sci.*, 2014, vol. 71, p. 979. <https://doi.org/10.1007/s00018-013-1412-3>
49. Shvarev, D. and Maldener, I., ATP-binding cassette transporters of the multicellular cyanobacterium *Anabaena* sp. PCC 7120: a wide variety for a complex lifestyle, *FEMS Microbiol. Lett.*, 2018, vol. 365, no. 4. <https://doi.org/10.1093/femsle/fny012>
50. Davidson, A.L., Dassa, E., Orelle, C., and Chen, J., Structure, function and evolution of bacterial ATP-binding cassette systems, *Microbiol. Mol. Biol. Rev.*, 2008, vol. 72, p. 317.
51. Higgins, C.F., ABC transporters: from microorganisms to man, *Annu. Rev. Cell Biol.*, 1992, vol. 8, p. 67.
52. Rees, D.C., Johnson, E., and Lewinson, O., ABC transporters: the power to change, *Nat. Rev. Mol. Cell Biol.*, 2009, vol. 10, p. 218.
53. Prindle, A., Liu, J., Munehiro, A., Ly, S., Garcia-Ojalvo, J., and Sittel, G.M., Ion channels enable electrical communication in bacterial communities, *Nature*, 2015, vol. 527, p. 59. <https://doi.org/10.1038/nature15709>
54. Corrales-Guerrero, L., Tai, A., Arbel-Goren, R., Mariscal, V., Flores, E., Herrero, A., and Stavans, J., Spatial fluctuations in expression of the heterocyst-differentiation regulatory gene *hetR* in *Anabaena*,

- PLoS Genet.*, 2015, vol. 11: e1005031.
<https://doi.org/10.1371/journal.pgen.1005031>
55. Flores, E., Herrero, A., Wolk, C.P., and Maldener, I., Is the periplasm continuous in filamentous multicellular cyanobacteria? *Trends Microbiol.*, 2006, vol. 14, p. 439.
 56. Mariscal, V., Herrero, A., and Flores, E., Continuous periplasm in a filamentous, heterocyst-forming cyanobacterium, *Mol. Microbiol.*, 2007, vol. 65, p. 1139.
 57. Wilk, L., Strauss, M., Rudolf, M., Nicolaisen, K., Flores, E., Kuhlbrandt, W., and Schief, E., Outer membrane continuity and septosome formation between vegetative cells in the filaments of *Anabaena* sp. strain PCC 7120, *Cell. Microbiol.*, 2011, vol. 13, p. 1744.
 58. Giddings, T.H. and Staehelin, L.A., Plasma membrane architecture of *Anabaena cylindrica*: occurrence of microplasmodesmata and changes associated with heterocyst development and the cell cycle, *Eur. J. Cell Biol.*, 1978, vol. 16, p. 235.
 59. Lehner, J., Berendt, S., Dörsam, B., Pérez, R., Forchhammer, K., and Maldener, I., Prokaryotic multicellularity: a nanopore array for bacterial cell communication, *FASEB J.*, 2013, vol. 27, p. 2293.
 60. Mariscal, V., Cell–cell joining proteins in heterocyst-forming cyanobacteria, in *The Cell Biology of Cyanobacteria*, Flores, E. and Herrero, A., Eds., Poole: Caister Acad. Press, 2014, p. 293.
 61. Omari-Nasser, A., Mariscal, V., Austin, J.R., II, and Haselkorn, R., Requirement of Fra proteins for communication channels between cells in the filamentous nitrogen-fixing cyanobacterium *Anabaena* sp. PCC 7120, *Proc. Natl. Acad. Sci. USA*, 2015.
<https://doi.org/10.1073/pnas.1512232112>
 62. Giddings, T.H. and Staehelin, L.A., Observation of microplasmodesmata in both heterocyst forming and non-heterocyst forming filamentous cyanobacteria by freeze–fracture electron microscopy, *Arch. Microbiol.*, 1981, vol. 129, p. 295.
 63. Mullineaux, C.W., Mariscal, V., Nenninger, A., Khanum, H., Herrero, A., Flores, E., and Adams, D.G., Mechanism of intercellular molecular exchange in heterocyst-forming cyanobacteria, *EMBO J.*, 2008, vol. 27, p. 1299.
 64. Mariscal, V., Herrero, A., Nenninger, A., Mullineaux, C.W., and Flores, E., Functional dissection of the three-domain SepJ protein joining the cells in cyanobacterial trichomes, *Mol. Microbiol.*, 2011, vol. 79, p. 1077.
 65. Nurnberg, D.J., Mariscal, V., Bornikoel, J., Nieves-Morion, M., Kraub, N., Herrero, A., Maldener, I., Flores, E., and Mullineaux, C.W., Intercellular diffusion of a fluorescent sucrose analog via the septal junctions in a filamentous cyanobacterium, *mBio*, 2015, vol. 6: e02109.
<https://doi.org/10.1128/mBio.02109-14>
 66. Juttner, R., ¹⁴C-labeled metabolites in heterocysts and vegetative cells of *Anabaena cylindrica* filaments and their presumptive function as transport vehicles of organic carbon and nitrogen, *J. Bacteriol.*, 1983, vol. 155, p. 628.
 67. Cumino, A.C., Marozzi, C., Barreiro, R., and Salerno, G.L., Carbon cycling in *Anabaena* sp. PCC 7120. Sucrose synthesis in the heterocysts and possible role in nitrogen fixation, *Plant Physiol.*, 2007, vol. 143, p. 1385.
 68. Lopez-Igual, R., Flores, E., and Herrero, A., Inactivation of a heterocyst-specific invertase indicates a principal role of sucrose catabolism in heterocysts of *Anabaena* sp., *J. Bacteriol.*, 2010, vol. 192, p. 5526.
 69. Vargas, W.A., Nishi, C.N., Giarrocco, L.E., and Salerno, G.L., Differential roles of alkaline/neutral invertases in *Nostoc* sp. PCC 7120: Inv-B isoform is essential for diazotrophic growth, *Planta*, 2011, vol. 233, p. 153.
 70. Hegazi, M., Piotukh, K., Mattow, J., Deutzmann, R., Volkmer-Engert, R., and Lockau, W., Isoaspartyl dipeptidase activity of plant-type asparaginases, *Biochem. J.*, 2002, vol. 364, p. 129.
 71. Burnat, M., Schleiff, E., and Flores, E., Cell envelope components influencing filament length in the heterocyst-forming cyanobacterium *Anabaena* sp. strain PCC 7120, *J. Bacteriol.*, 2014, vol. 196, p. 4026.
<https://doi.org/10.1128/JB.02128-14>
 72. Merino-Puerto, V., Mariscal, V., Mullineaux, C.W., Herrero, A., and Flores, E., Fra proteins influencing filament integrity, diazotrophy and localization of septal protein SepJ in the heterocyst-forming cyanobacterium *Anabaena* sp., *Mol. Microbiol.*, 2010, vol. 75, p. 1159.
 73. Merino-Puerto, V., Schwarz, H., Maldener, I., Mariscal, V., Mullineaux, C.W., Herrero, A., and Flores, E., FraC/FraD-dependent intercellular molecular exchange in the filaments of heterocyst-forming cyanobacterium *Anabaena* sp., *Mol. Microbiol.*, 2011, vol. 82, p. 87.
 74. Flores, E., Nieves-Morion, M., and Mullineaux, C.W., Cyanobacterial septal junctions: properties and regulation, *Life*, 2019, vol. 9, p. 1.
<https://doi.org/10.3390/life9010001>
 75. Picossi, S., Montesinos, M.L., Pernil, R., Lichtlé, C., Herrero, A., and Flores, E., ABC-type neutral amino acid permease N-I is required for optimal diazotrophic growth and is repressed in the heterocysts of *Anabaena* sp. strain PCC 7120, *Mol. Microbiol.*, 2005, vol. 57, p. 1582.
 76. Pernil, R., Picossi, S., Mariscal, V., Herrero, A., and Flores, E., ABC-type amino acid uptake transporters Bgt and N-II of *Anabaena* sp. strain PCC 7120 share an ATPase subunit and are expressed in vegetative cells and heterocysts, *Mol. Microbiol.*, 2008, vol. 67, p. 1067.
 77. Nicolaisen, K., Mariscal, V., Bredemeier, R., Pernil, R., Moslavac, S., Lopez-Igual, R., Maldener, I., Herrero, A., Schleiff, F., and Flores, E., The outer membrane of a heterocyst-forming cyanobacterium is a permeability barrier for uptake of metabolites that are exchanged between cells, *Mol. Microbiol.*, 2009, vol. 74, p. 58.
 78. Hahn, A. and Schleiff, E., The cell envelope, in *The Cell Biology of Cyanobacteria*, Flores, E. and Herrero, A., Eds., Norfolk: Caister Acad. Press, 2014, p. 29.
 79. Nicolaisen, K., Mariscal, V., Bredemeier, R., Pernil, R., Moslavac, S., López-Igual, R., Maldener, I., Herrero, A., Schleiff, E., and Flores, E., The outer membrane of a heterocyst-forming cyanobacterium is a permeable

- barrier for uptake of metabolites that are exchanged between cells, *Mol. Microbiol.*, 2009, vol. 74, p. 58.
80. Pernil, R., Picossi, S., Herrero, A., Flores, E., and Mariscal, V., Amino acid transporters and release of hydrophobic amino acids in the heterocyst-forming cyanobacterium *Anabaena* sp. strain PCC 7120, *Life*, 2015, vol. 5, p. 1282.
 81. Frain, K.M., Gangl, D., Jones, A., Zedler, J.A.Z., and Robinson, C., Protein translocation and thylakoid biogenesis in cyanobacteria, *Biochim. Biophys. Acta*, 2016, vol. 1857, p. 266.
 82. Khaytan, B., Meeks, J., and Risser, D., Evidence that a modified type IV pilus-like system powers gliding motility and polysaccharide secretion in filamentous cyanobacteria, *Mol. Microbiol.*, 2015, vol. 98, p. 1021.
 83. Flores, E. and Herrero, A., Compartmentalized function through cell differentiation in filamentous cyanobacteria, *Nat. Rev. Microbiol.*, 2010, vol. 8, p. 39.
 84. Camargo, S., Picossi, S., Corrales-Guerrero, L., Valladares, A., Arévalo, S., and Herrero, A., ZipN is an essential FtsZ membrane tether and contributes to the septal localization of SepJ in the filamentous cyanobacterium *Anabaena*, *Sci. Rep.*, 2019, vol. 9: e2744. <https://doi.org/10.1038/s41598-019-39336-615>
 85. Flores, E., Picossi, S., Valladares, A., and Herrero, A., Transcriptional regulation of development in heterocyst forming cyanobacteria, *Biochim. Biophys. Acta—Gene Regulatory Mechanisms*, 2019, vol. 1862, p. 673. <https://doi.org/10.1016/j.bbagr.2018.04.006>
 86. Yoon, H.S. and Golden, J.W., Heterocyst pattern formation controlled by a diffusible peptide, *Science*, 1998, vol. 282, p. 935.
 87. Callahan, S.M. and Buikema, W.J., The role of HetN in maintenance of the heterocyst pattern in *Anabaena* sp. PCC 7120, *Mol. Microbiol.*, 2001, vol. 40, p. 941. <https://doi.org/10.1046/j.1365-2958.2001.02437.x>
 88. Corrales-Guerrero, L., Mariscal, V., Flores, E., and Herrero, A., Functional dissection and evidence for intercellular transfer of the heterocyst-differentiation PatS morphogen, *Mol. Microbiol.*, 2013, vol. 88, p. 1093. <https://doi.org/10.1111/mmi.12244>
 89. Plominsky, A.M., Delherbe, N., Mandakovic, D., Riquelme, B., Gonzales, K., Bergman, B., Mariscal, V., and Vasquez, M., Intercellular transfer along the trichomes of the invasive terminal heterocyst forming cyanobacterium *Cylindrospermopsis raciborskii* CS-505, *FEMS Microbiol. Lett.*, 2015, vol. 362. <https://doi.org/10.1093/femsle/fnu009>
 90. Thomas, J., Meeks, J.C., Wolk, C.P., Shaffer, P.W., and Austin, C.M., Formation of glutamine from [¹³N] ammonia, [¹³N] dinitrogen, and [¹⁴C] glutamate by heterocysts isolated from *Anabaena cylindrica* Lemm., *J. Bacteriol.*, 1977, vol. 129, p. 1545.
 91. Hodgkin, A.L. and Rushton, W.A.H., The electrical constants of a crustacean nerve fibre, *Proc. R. Soc. London, Ser. B*, 1946, vol. 133, p. 144.
 92. Aslanidi, K.B., Potapova, T.V., and Shalapenok, A.A., Method for registration of photoelectric properties of elongated single- and multicellular microorganisms, in *Pribory i laboratornoe oborudovanie dlya nauchnykh issledovaniy po novym napravleniyam biologii i biotekhnologii* (Instruments and Laboratory Equipment for Scientific Research in New Biological and Biotechnological Aspects), Pushchino, 1987, p. 100.
 93. Aslanidi, K.B. and Shalapenok, A.A., Energetics of local cell-to-cell interactions in phototrophic organisms, in *Intercellular Communication: Proceedings in Nonlinear Science*, Bukauskas, F., Ed., Manchester: Univ. Press, 1991, p. 12.
 94. Aslanidi, K.B., Spectral measurements of the functional heterogeneity of cells and their organelles, *Biophysics*, 2015, vol. 60, p. 85.
 95. Magnuson, A., Heterocyst thylakoid bioenergetics, *Life*, 2019, vol. 9, no. 13. <https://doi.org/10.3390/life9010013>
 96. Chapman, A.G. and Atkinson, D.E., Adenine nucleotide concentrations and turnover rates. Their correlation with biological activity in bacteria and yeast, *Adv. Microb. Physiol.*, 1977, vol. 15, p. 253.
 97. Loewenstein, W.R., Cell individuality and connectivity, an evolutionary compromise, in *Individuality and Determinism*, Fox, S.W., Ed., New York: Plenum Publ. Corp., 1984, p. 77.
 98. Aslanidi, K.B., Potapova, T.V., and Chailakhyan, L.M., Energy transport via high permeability contact membranes, *Biol. Membr.*, 1988, vol. 5, p. 613.
 99. Bonner, J.T., The origin of multicellularity, *Integr. Biol.*, 1998, vol. 1, p. 28.
 100. Carroll, S.B., Chance and necessity: the evolution of morphological complexity and diversity, *Nature*, 2011, vol. 409, p. 1102.

Translated by A. Bulychev