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REVIEWS

Mixotrophy in Microorganisms: Ecological and Cytophysiological Aspects

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Abstract—Mixotrophy is the ability to combine autotrophic and heterotrophic modes of nutrition. It is widely spread in a variety of microorganisms including such important plankton groups as dinoflagellates and cyanobacteria. In marine ecosystems, mixotrophy complicates our concept of the flow of materials and energy and therefore has been thoroughly studied for recent decades. Nevertheless, the exact data on the auto/heterotrophy balance during mixotrophic growth are still lacking, mainly due to insufficient knowledge of physiological and molecular grounds of this phenomenon. In this review, we address the ecological and cytophysiological aspects of the problem of mixotrophy in microorganisms as well as discuss possible causes of the relatively slow progress in this field.

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Key words: autotrophy, heterotrophy, mixotrophy, differential gene expression, physiology of microorganisms.

INTRODUCTION

Historically, ecology and cell physiology were developing by their own, more or less independent, ways. Traditionally, ecologists collected field data, construct mathematical models of ecosystem functioning, perform environmental expertise and prognosis. Cell physiologists, in turn, by using a wide set of laboratory methods, studied structure and function of cells, mostly cells of the higher animals and plants. It seemed that these two branches of biology have different aims and little points of contact. However, in the epoch of growing interest in unicellular organisms whose biomass exceeds that of all other living creatures on the Earth, all has changed [1–4].

As long as the cells of multicellular organisms are concerned, the relationship between ecology and cell physiology is not so obvious, as it is primarily the individual complex tissues and organs that become responsible for the organisms' interactions with environment and that are directly outside the competence of cell physiology. The situation is different when the objects of studies are cells of microorganisms. In this case, responsible for interaction with environment as well as with other representatives of the ecosystem become the cell itself performing functions of the integral organism. As a result, the borderline between ecology in its classical understanding and cell physiology disappears and it is reasonable to consider the novel synthetic discipline—ecological cytophysiology. Besides, connection of ecology and cytophysiology are also realized in that the fine physiological and biochemical processes in cells of microorganisms play the unsurpassed role in the biosphere by providing functioning of biogeochemical cycles of elements [5].

Mixotrophic nutrition in protists is a prominent example of cellular mechanisms providing inter-

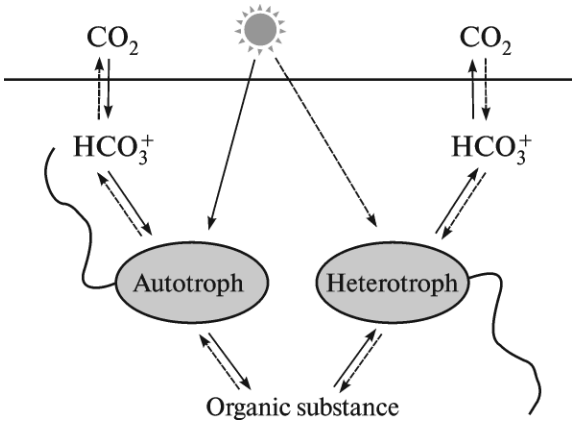

Fig. 1. Role of mixotrophy in carbon flows in trophic networks at the level of planktonic microorganisms. *Solid arrows* show direction of carbon flows in the "classical" point of view on trophic networks with the type II mixotrophs regarded as autotrophs, while the type III mixotrophs—exclusively as heterotrophs. *Dashed arrows* show direction of carbon flows at use by mixotrophs of alternative metabolism strategies.

action of unicellular organisms with their environment and has a great ecological importance [6–8]. Studies on mixotrophy have long been undertaken, but success in this area so far is still limited [9]. The major reason for this is that mixotrophy has been mostly studied by traditional methods of field and laboratory ecology. However, as time has shown, it was not sufficient despite the undoubted importance of these works. Key questions about mixotrophy in microorganisms can only be answered if the efforts of ecologists, cell physiologists, and cell biochemists are combined. In this review, we outline how ecological and cytophysiological studies on mixotrophy are interconnected and what further developments can be expected in this promising area.

MIXOTROPHY FROM THE POINT OF VIEW OF ECOLOGY

Mixotrophy is a metabolic strategy of some organisms, which combines features of both autotrophy and heterotrophy, i.e., use of different sources of carbon and energy [6, 10]. Most commonly present is the combination of phototrophy (photosynthesis) and aerobic heterotrophy, the latter achieved either by phagocytosis of solid particles and entire organisms or by osmotrophic absorption of dissolved organic compounds [11]. It is to be noted that apart from the above-mentioned types of nutrition, some microorganisms capable for mixotrophic growth can obtain nutrients autotrophically through chemosynthesis and heterotrophically through liquid phase endocytosis (i.e., pinocytosis). However, both chemosynthesis and pinocytosis have been rarely found in mixotrophs and poorly studied. These phenomena are therefore left beyond the scope of this review.

Various organisms may have different extents of their capability for mixotrophy. Based on this, all mixotrophs can be divided into three groups [12]:

(1) Type I mixotrophs have their own plastids. They are able to grow with equal success both autotrophically and heterotrophically. Each of these two types of metabolism can provide independently the organism's viability under the corresponding conditions.

(2) Type II mixotrophs also contain their own plastids, but are capable predominantly for the autotrophic growth. These organisms consume organic matter only as an additional source of nutrition and energy when, for whatever reason, autotrophy becomes insufficiently efficient.

(3) Type III mixotrophs are primarily heterotrophic, but occasionally acquire the capability for the autotrophic mode of life, usually owing to the autotrophic symbionts or the so called kleptochloroplasts, i.e., chloroplasts "borrowed" from other organisms.

In this review, we will concentrate on mixotrophs of the second type, although other types of mixotrophy also are of essential interest.

The discovery of mixotrophy was first regarded as a curious fact, as yet another example of nature's inventiveness. It soon became clear, however, that mixotrophy in the microcosm is a rule rather than an exception. It turned out to be widely spread among prokaryotes and protists, including such large groups of unicellular plankton organisms as dinoflagellates, cryptophytes, and golden algae [7]. This circumstance attracted special attention, for ecologists had regarded the majority of the representatives of these groups as phototrophic and had therefore placed them at the base of ecological pyramids as primary producers. Up to now, more than a half of the described dinoflagellates still have been considered by researchers as one of major phytoplankton groups providing the

ocean with the primary production [13]. On the other hand, such organisms as ciliates had been always thought to be classic heterotrophs; however, many of them turned out to use in full measure the advantages provided by photosynthesis [8, 14, 15]. The unexpectedly wide spread of mixotrophy in nature and a real splashing of reports on newly discovered mixotrophs have pushed away the traditional views on organization of food networks in the world ocean at the level of microorganisms [16–21] (Fig. 1). However, it appeared to be a difficult task to unravel their actual organization. For this, first of all, two questions had to be answered: what is the balance between autotrophy and heterotrophy during the mixotrophic growth and what are the factors controlling this balance?

THE BALANCE OF AUTO- AND HETEROTROPHY DURING THE MIXOTROPHIC GROWTH

Microscopy was and remains the primary method for approximate evaluation of the number of cells with heterotrophic nutrition in a mixotrophic population. The principle is that feeding on other organisms by a mixotroph results in formation of digestive vacuoles in its cell. The vacuoles are easily visible under microscopic observation of the cell culture. The presence of phagocytized organisms in digestive vacuoles of mixotrophs can be detected by the use of epifluorescent microscopy: certain pigmented organisms, e.g. cryptomonads, show autofluorescence under excitation with light of appropriate wavelength [22, 23]. In other cases, preliminary fluorescent labeling of prey may be used [24, 25] as well as fluorescent DNA staining (e.g., with DAPI) or CARD-FISH technique [26]. The two latter methods also make digestive vacuoles containing stained DNA of the phagocyted prey clearly visible; hence, the quantitative assessment of the mixotrophic ability is possible.

Unfortunately, the method described above is only useful for estimation of the proportion of heterotrophs in a mixotrophic population, but it does not allow answering question of contributions of autotrophic and heterotrophic modes of nutrition to mixotrophic growth. The point is that thereby the researcher can only evaluate the proportion of the population using the heterotrophic nutrition, but these phagocytizing cells simultaneously with heterotrophic nutrition can also use photosynthesis. Besides, the absence of digestive vacuoles in cells from the studied culture does not guarantee the absence of heterotrophy. There is some evidence that organisms that have recently phagocytized cells can show no food vacuoles under microscopic investigation due to quite rapid digestion of prey and alternation of cell cycle phases [27].

Another essential drawback of the microscopic method is that it can be used only for estimation of the proportion of phagocytizing mixotrophs, i.e., those consuming other organisms. However, mixotrophy can also occur as a combination of autotrophy with osmotrophic absorption of dissolved organic compounds, e.g. glucose and other monosaccharides, urea, and amino acids [11]. Evidently, it is impossible to detect osmotrophic heterotrophy by means of microscopy. For this purpose, as well as for more accurate estimation of a contribution of phagotrophic heterotrophy, alternative approaches are needed.

One of such approaches is evaluation of photosynthetic activity and the population growth rate in the studied organisms. For instance, in this way it has been shown that photosynthetic activity in the population of dinoflagellate *Dinophysis norvegica* in the sub-euphotic zone of the Baltic Sea was insufficient for maintenance of the observed growth rate. Hence, population should have been maintained additionally at the expense of heterotrophic nutrition [28].

The most effective method for estimation of a heterotrophic contribution to mixotrophic population growth is use of C14-labeled substrates. This approach was tried out in 2006 by Adolf et al. [27]. Its essence consists in that in several experiments, one of sources of carbon for the studied culture—inorganic bicarbonate HCO_3^- or cells of alimentary organisms—contains the C14 radioisotope. By activity of C14 present in the biomass it is possible to judge about intensity of incorporation of some particular substrate into cells of the studied organisms. The same principle can be used in experiments with application of substrates labeled with the stable isotopes of carbon C13 [29]. It is to be emphasized that such methods allow judging only about the activity of incorporation of the used substrates into the biomass, rather than about

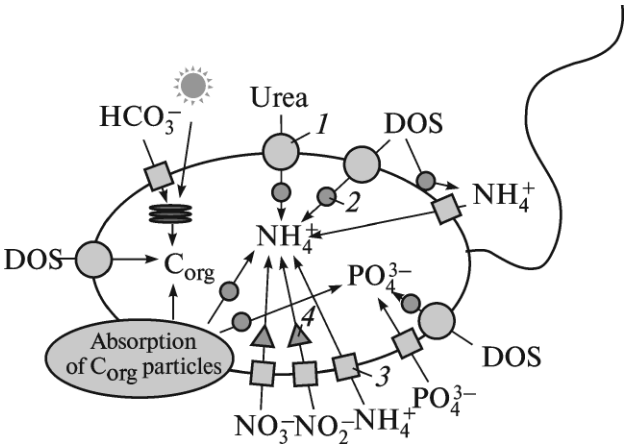

Fig. 2. Possible pathways of delivery of carbon, nitrogen, and phosphorus into the cell of the mixotrophic organism. DOS—Dissolved organic substance; C_{org}—carbon of organic substances; (*1*) transporters of organic substances; (*2*) enzymes performing hydrolysis of organic substances with release of inorganic ions; (*3*) transporters of inorganic ions; (*4*) enzymes performing reduction of inorganic ions.

the auto/heterotrophy balance. Therefore, at the present stage of the isotope labeling methods of studies of mixotrophy, they are to be used in combination with other approaches, as it has been performed in the paper of Adolf et al. [27].

Taking into the account the disadvantages of the existing methods, all of them do not provide accurate quantitative estimations of the auto/heterotrophy balance in mixotrophs. Development of reliable and at the same time available methods of evaluation of this balance, including *in situ*, is a work for the future.

ENVIRONMENTAL FACTORS CONTROLLING MIXOTROPHIC GROWTH

The next question extremely important for ecologists is: how mixotrophy is regulated? At which moment do autotrophs begin assimilating the dissolved organic substance or even hunting on other representatives of the microworld? It have seemed natural to suggest that autotrophic nutrition is used at good illumination and sufficient concentration of inorganic sources of nitrogen and phosphorus necessary for photosynthesis and growth, whereas the presence of available organic substrates and/or food organisms in the environment should have promoted heterotrophic nutrition [30, 31]. However, the later experiments have shown that regulation of mixotrophy is based on complicated mechanisms. Thus, it was found out that good illumination in protists can induce not only photosynthesis, but also phagocytosis, while the presence of organic substrates are able to accelerate the inorganic carbon fixation, thus supplying the organism with necessary biogenic elements [23, 32, 33]. Many studies suggest that other, sometimes unexpected factors (temperature, turbulence, media, life cycle stage) also can play the role of trigger.

Currently we do not understand all principles of regulation of mixotrophy in microorganisms and can merely state that most likely there do not exist universal laws for all mixotrophic organisms. Also it should not be forgotten that all the existing methods of estimation of the above-mentioned mixotrophy balance above are not perfect, so the researchers can overlook important facts during experiments on the mixotrophy control. One thing is undisputable: it will be impossible to understand the pathways of mixotrophy regulation and to use successfully this knowledge in ecology and biogeochemistry until the phenomenon of mixotrophy has not been studied from the point of view of cell biology.

MIXOTROPHY FROM THE POINT OF VIEW OF CYTOPHYSIOLOGY

Mixotrophy is a complex type of metabolism that combines several strategies (Fig. 2). On one hand, a cell has to maintain its photosynthetic molecular machinery and the entire set of enzymes involved in the Calvin cycle, which are necessary for photosynthesis and inorganic carbon fixation. On the other hand, heterotrophic growth requires the presence of membrane transporters of simple organic compounds, often ecto- and exoenzymes for extracellular hydrolysis of organic polymers or phagocytic activity with subsequent digestion by a set of lytic enzymes in the case of phagotrophic nutrition. Thereby the mixotrophic growth seems rather power-consuming and the organism should always maintain the optimal balance between physiological expenditures and benefits. Molecular mechanisms providing fine regulation of this balance, e.g. mixotrophy-inducing signaling pathways, differential gene expression, activation of phagocytosis, and recognition of food organisms

are studied by cell biologists. Quite a few resources used by a cell in the process of mixotrophic growth should be justified by advantages of such nutrition.

PHYSIOLOGICAL ROLE OF MIXOTROPHY

The first hypothesis that did not lost its actuality was that the capability for mixotrophic growth was the possibility of obtaining carbon when photosynthesis is impossible, for instance, when illumination is insufficient. In the same situation, organic molecules can also serve as electron donors for energy production in the respiratory chain and for reduction processes in the organism. Indeed, in many studies, phagocytosis has been shown to promote growth at low illumination [30, 34, 35]. However, there also have been found species incapable for phagocytosis in darkness and even at the relatively low illumination. At the same time, in these organisms, for instance, in the dinoflagellate *Prorocentrum minimum*, with rising illumination the phagocytic activity level also increased [32].

The next suggestion about physiological role of mixotrophy was that in waters deficient in these inorganic sources of nitrogen and phosphorus, such as nitrate, ammonium, and phosphate, these most important biogenic elements can be obtained from organic compounds. This suggestion was indirectly confirmed by experiments, in which deficit of inorganic substrates induced phagocytosis or osmotrophic absorption organic substances. Thus, at ammonium and nitrate ion deficiency in medium, many microorganisms are known to absorb urea [36, 37]. In cell, urea can be decomposed into inorganic carbon and ammonium—the classic source of nitrogen for the majority of plankton organisms, which can be used in protein syntheses and a potential precursor for protein biosyntheses [38]. The urea decomposition can be performed by ureases and urea:carbon-dioxide ligase (EC 6.3.4.6) that are widely spread among microorganisms [39, 40]. Besides, the existence in many plankton protists and bacteria of the intra- and extracellular hydrolytic enzymes that decompose peptides with release of ammonium indicates that nitrogen can also be delivered into a cell at the expense of the phagotrophic mixotrophy [41, 42].

However, other authors have shown that some organisms feed mixotrophically even in the presence of sufficient amounts of inorganic nitrogen and phosphorus. This fact allowed suggesting that a unicellular organism might have used the mixotrophic nutrition to obtain various microelements, vitamins, and growth factors, although it is not yet clear which precisely they are [43–45].

The physiological role of mixotrophy is likely to differ depending on the available substrate and the studied organism. But the final conclusions require cytological experiments that allow tracing destiny of organic substances consumed by mixotrophs, for instance, with use of substrates labeled by stable or radioactive isotopes [46, 47].

GENE EXPRESSION DURING MIXOTROPHIC GROWTH

As noted above, the microorganisms growing under different conditions and using autotrophic and mixotrophic modes of nutrition should have different sets of genes for economy of cell resources. There may be differential expression of genes encoding enzymes responsible for hydrolysis and for metabolism of simple organic substances (glucose, amino acids, urea, etc.), genes of photosynthetic machinery as well as genes of membrane transporters of organic substances. For instance, in unicellular eukaryotes, the urea delivery into the cell from environment is provided by the high-affinity transporters DUR3, aquaporins, and amide channels [48, 49].

As early as in 1998, it was shown that marine cyanobacteria Prochlorococcus sp. decreased expression of gene of one of proteins of the photosystem II *pbs*A on addition of glucose into the culture kept in darkness as compared with the culture kept in darkness, but without addition of glucose [50]. Based on this, there was suggested a possibility of mixotrophic growth of cyanobacteria Prochlorococcus sp.; this suggestion was confirmed only 10 years later [51]. In this work the authors not only showed that cyanobacteria could have uptaken glucose from environment even at its very low concentration comparable with the mean glucose concentration in the World ocean, but also have studied effect of glucose on expression of several genes by using PCR with reverse transcription in the real-time regime. They showed that addition of glucose (1 μmol/l) induced a sharp rise in

expression of genes involved in glucose metabolism: the *zwf* and *gnd* genes encoding enzymes of the pentose phosphate pathway as well as the *dld* gene encoding D-lactate:NAD^+ oxidoreductase (EC 1.1.1.28) or D-lactate dehydrogenase. The expression of the *mel*B gene encoding a putative membrane transporter of sugars increased to the lesser degree, but still more than threefold as compared with the autotrophic control.

The Chinese researchers have also used real-time PCR to investigate expression of three genes of microalgae *Chlorella sorokiniana* under autotrophic and mixotrophic conditions [52]. Among the studied genes there was present the *rbc*L gene encoding 3-phospho-D-glycerate carboxylase (EC 4.1.1.39)—a large catalytic subunit of ribulose-1,5-bisphosphate carboxylase. This subunit (RuBisCO) plays a crucial role in the Calvin cycle, a metabolic pathway of inorganic carbon fixation. The authors cultivated algae under autotrophical conditions as well as in the medium containing glucose as substrate. During the autotrophic growth in the absence of glucose, the *rbc*L expression level was expectedly high at the culture logarithmic growth phase and decreased more than twice at the stationary phase, but remained high. In the presence of glucose in the medium, the studied gene was practically not expressed at any phase of the culture growth. This indicated a fall in the photosynthetic activity in *C. sorokiniana* in the presence of glucose in the medium. Interestingly, several years earlier, other researchers showed that the level of photosynthetic activity in the Nannochloropsis sp. algae did not depend on whether the culture was kept under the autotrophical or mixotrophical conditions, unlike the intensity of respiration, which increased markedly during the mixotrophic growth [53]. Nevertheless, the fact that in the presence of appropriate organic substrates some autotrophic organisms can switch to mixotrophy by decreasing or even ceasing their photosynthetic activity, indicates that mixotrophic organisms are able to regulate expression of necessary genes. This conclusion is very important, as until rather recently many authors believed that photosynthesis and heterotrophic nutrition during mixotrophic growth occurred simultaneously and independently [54, 55].

Apart from studying expression of individual genes, it is possible study the set of all proteins of organisms at cultivation under different conditions. Such approach was recently used for a comparative analysis of proteomes in a dinoflagellate *Prorocentrum micans* grown autotrophically and mixotrophically [56]. These flagellates were cultivated under the autotrophic and mixotrophic conditions, the total protein fraction was isolated from cell lysates, and proteins were separated by two-dimensional electrophoresis. As a result, some proteins were found to be present in the proteome exclusively during the autotrophic growth, while others—only during the mixotrophic growth. Besides, the quantitative analysis by the MALDI-TOF mass spectrometry showed the amount of many proteins to depend on the type of nutrition. This indicates that, in the case of *P. micans*, expression of the genes responsible for photosynthesis and heterotrophic nutrition can be regulated not only in the on/off turning manner, but also possible is the fine regulation of their expression in correspondence of external conditions and the used nutrition type.

Remarkably, out of 1200 proteins in the proteome, only 27 (2.3%) were expressed differently under the two experimental regimens, including 12 proteins that were present only under the mixotrophic conditions. Obviously the differences even in the slightest part of the expressed genes can affect significantly morphology and physiology of microorganisms. Unfortunately, we have managed to identify only 5 out of 27 studied proteins by leveling of their amino acid sequences obtained by the MALDI-TOF method against the proteins recorded in database. Meanwhile it is impossible to establish the precise function of these proteins due to the absence of genetic information related directly to dinoflagellates. The plankton protists on the whole are very poorly represented in the genome and proteome databases, which is a serious problem at their study [57]. Recently, much more attention has become to pay to this problem [1], so it is hoped that soon the situation will change for the better.

MECHANISMS OF REGULATION OF GENE EXPRESSION UNDER MIXOTROPHIC CONDITIONS

Gene expression regulation under mixotrophic conditions is now studied much better in bacteria

than in eukaryotes. Most works have been carried out on cyanobacteria—important primary producers able to utilize small organic substances. Thus, cyanobacteria are known to absorb and catabolize glucose. Besides, they are able to use urea under conditions of nitrogen deficiency.

One of the basic mechanisms of regulations of gene transcription in prokaryotes is the existence of alternative σ-subunits of bacterial RNA polymerase realizing transcription. This enzyme transcribes various groups of genes depending on the type of σ-subunit in its composition [58]. One of the cyanobacterial σ-subunits is the SigE protein. On cyanobacteria Synechocystis sp. PCC 6803 it was shown that mutants for the *sigE* gene, as compared with wild type, contained much smaller amounts of transcripts of genes of important pathways of glucose catabolism: the glycolytic and the oxidative pentose phosphate pathways. Besides, in these mutants the activity of enzymes associated with the pentose phosphate oxidative pathway—D-glucose 6-phosphate:NAD^+ oxidoreductase (EC 1.1.1.49) and 6-phospho-D-gluconate:NAD+ oxidoreductase (EC 1.1.1.44) was very low. In darkness, activities of these enzymes did not increase, unlike the wild type, and the glucose transport level into the cell was decreased. As a result, mutant cells were unable to grow under mixotrophic conditions [59]. Apart from regulation of sugar catabolism, the SigE subunit, alongside with the SigB and SigC subunits, is involved in regulation of during nitrogen deficiency [60].

It is to note that most papers on regulation of gene expression in mixotrophs deal with genes responsible for nitrogen metabolism. The point is that nitrogen is a most important biogenic element limiting the phytoplankton growth in the ocean. Concentration of bioavailable nitrogen determines the fixation rate of inorganic carbon and biomass production during photosynthesis; therefore, it eventually determines the success of ecosystem [61, 62]. Autotrophs acquire nitrogen primarily from nitrate and ammonium but seawater typically has very low concentrations of these ions and thus cannot maintain growth of the high number of photosynthesizers. In this case, alternative sources of nitrogen come into play, i.e., such nitrogen-containing organic compounds as urea and amino acids [63]. Problems of regulation of nitrogen metabolism, including organic nitrogen uptake by photosynthetic organisms, therefore paid considerable attention, including the unique role of this element in ecosystem functioning.

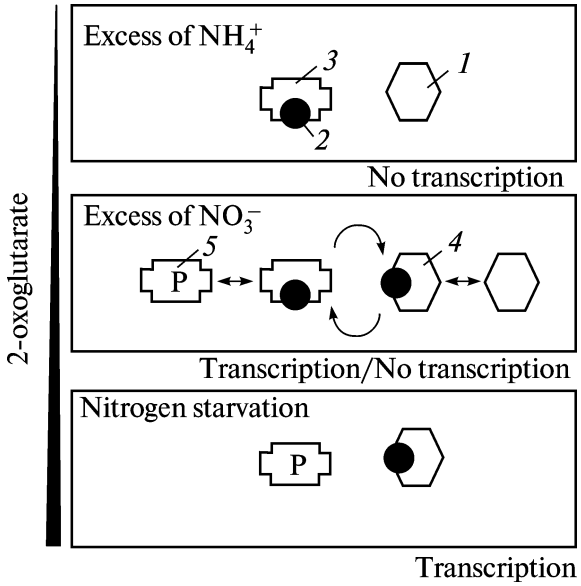

Fig. 3. Interaction of the NtcA, PII, and PipX proteins at regulation of the cyanobacterial nitrogen metabolism (from: Espinosa et al. [68], modified). (*1*) inactive NtcA protein; (*2*) PipX protein; (*3*) PII protein; (*4*) activated NtcA protein; (*5*) phosphorylated PII protein. *Arrows on the left* show direction of decrease of 2-oxoglutarate concentration in the cell. In the presence of ammonium in the medium and at a low 2-oxoglutarate concentration in the cell, the PipX protein is bound to PII. At intermediate 2-oxoglutarate concentration, the PipX protein is bound sometimes to PII, sometimes to NtcA by activating the latter. The activated NtcA initiates expression of genes responsible for utilization of alternative nitrogen sources, but cases of initiation of expression of transcription of these genes are also rare due to competition for binding of PipX protein to protein PII. The nitrogen starvation and a high intracellular 2-oxoglutarate concentration in the cell favor binding of PipX protein to NtcA protein, its activation, and initiation of its controlled genes. Meanwhile, the PII protein is phosphorylated and is in the inactive state.

The best studied to date is regulation of nitrogen metabolism in cyanobacteria. In the absence of the most available nitrogen source—ammonium, these unicellulars begin to use nitrogen from alternative compounds including urea. Cyanobacteria perceive concentration of the available ammonium through concentration of intracellular 2-oxoglutarate—the substance necessary for glutamic acid synthesis, in the course of which one

ammonium ion binds to two molecules of 2-oxoglutarate. Synthesis of 2-oxoglutarate is the terminal reaction of the Krebs cycle in cyanobacteria, so the amount of this product in the cell directly depends on ammonium assimilation [64].

A transition of cell from use of ammonium to use of other nitrogen sources is provided by the transcription regulatory protein NtcA belonging to the CAP protein family—activators of genes of catabolism [65]. If the ammonium concentration in the medium is low, the NtcA protein directly activates transcription of genes involved in uptake and assimilation of nitrogen from other compounds. Apart from the NtcA protein, control of nitrogen metabolism is provided by the regulatory protein PII widely spread among various groups of living organisms from bacteria to plants [66]. Its activity also is controlled by 2-oxoglutarate but, unlike the NtcA protein, the high concentration of 2-oxoglutarate in the absence of ammonium in the medium inhibits the PII activity. There are fine regulatory relations between the two proteins, i.e., NtcA affects activity of PII, and vice versa. Molecular grounds of these relationships had been unknown until the discovery of a small regulator protein PipX [67] (Fig. 3). Subsequent experiments showed that PipX worked as a mediator between NtcA and PII during regulation of nitrogen metabolism [68]. According to the interaction model proposed by the authors of the cited paper, the high concentration of ammonium and the low concentration of intracellular 2-oxoglutarate promote the PipX binding to PII, the NtcA protein remaining inactive. In turn, the high 2-oxoglutarate concentration in cell leads to formation of the PipX-NtcA complex. NtcA is thereby activated, which triggers transcription of genes responsible for degradation of alternative nitrogen sources. At intermediate concentrations of 2-oxoglutarate, for instance, during growth of the culture on a nitrate-containing medium, NtcA and PII compete for binding to PipX by regulating thereby activity of each other.

It is quite that we will be able to understand regulation of mixotrophy at the ecosystem level only when the molecular regulatory mechanisms of cellular response under conditions of mixotrophic growth are deciphered. Hence, cytological studies have acquired the ecological importance.

CONCLUSION

The mixotrophic nutrition includes the whole specter of molecular mechanisms aiming at utilization of organic substances, maintenance of the photosynthetic machinery, synthesis of hydrolytic enzymes, and chemical recognition of food organisms. In this review, we have addressed only a part of possible directions of investigation of mixotrophy at the boundary of several biological disciplines. It is remarkable that the majority of works of ecological direction deal with plankton protists, while most works on molecular mechanisms and biochemical grounds of mixotrophy are performed on bacteria. This has its explanation.

Protists are important primary producers in the ocean. Depended on their activity is the uninterrupted turnover of the carbon cycle that supports the existence of all other living organisms [61, 69]. The ability for mixotrophy found in many protists is naturally of interest to ecologists. On one hand, the mixotrophic growth may result in decreased fixation rates of atmospheric CO_2 during photosynthesis. On the other hand, in waters poor in inorganic substrates, mixotrophy may have the opposite effect by providing nitrogen and phosphorus for biosynthesis of organic compounds. Most attention has been paid to a separate group of protists—Dinoflagellata [12, 70]. Concentrated in this group is the huge number of mixotrophs, many of them producing various kinds of toxins and causing water bloom harmful for humans [71]. It seems that it is the ability of dinoflagellates to grow mixotrophically that accounts for more frequent cases of blooms in eutrophic waters rich in nutrients [33, 72].

Why then practically all data on gene expression and its regulation during mixotrophic growth have been obtained in studies on cyanobacteria—despite the obvious necessity of detailed study of physiology of mixotrophic protists and dinoflagellates in particular? This seems to be due to the above-mentioned absence of genomic data on various protist groups, which complicates extremely such cytological studies. This is especially actual for dinoflagellates known by their huge genomes [73, 74]. Due to the very large size, not a single dinoflagellate genome has been completed sequenced by 2011, although the impetous devel-

opment and a fall of cost of sequencing technologies give hope that this hurdle will be cleared in the next five years [75]. At any case, investigations on mixotrophy continue and the studies on physiology of mixotrophic protists at the cellular level are currently among the preferred directions. Hopefully, we will soon get the answers to the questions put forward more than twenty years ago.

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