ISSN 0026-2617, Microbiology, 2009, Vol. 78, No. 5, pp. 535–546. © Pleiades Publishing, Ltd., 2009.
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REVIEW

Mechanisms of Oxygen Regulation in Microorganisms

V. K. Plakunov[1] and O. V. Shelemekh

Winogradsky Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia

Received October 20, 2008

Abstract—The review considers the main mechanisms of metabolism regulation that operate in pro- and eukaryotic microorganisms upon changes in the partial pressure of oxygen in the medium, i.e., upon transition from normoxia via hypoxia to anoxia or upon the reverse transition. The involvement in these processes of hemes, the Hap transcription factors, the Rox1 repressor protein, sterols, and other regulatory factors is discussed.

Key words: oxygen regulation, normoxia, hypoxia, anoxia, osmotic shock, global regulation.

DOI: 10.1134/S0026261709050026

Molecular oxygen is a potent regulator of metabolism of all organisms on our planet. The amount of the relevant data published is very large, and in this review we will focus on the mechanisms of oxygen regulation that are most typical of unicellular pro- and eukaryotic microorganisms.

GENERAL CHARACTERIZATION OF THE PHENOMENON OF OXYGEN REGULATION OF METABOLISM

Any impact causes changes in expression of many genes in the cell, whether this impact is adverse (e.g., anaerobiosis for an aerobe) or beneficial (e.g., emergence of a preferential carbon source in the medium). This effect is a constituent part of the global regulation of metabolism, and is mediated by signaling (or sensing) systems. The controlling mechanisms may be comparatively simple, as it is in the case of induction or repression, where changes in intracellular metabolic processes are a direct reaction to the change in the concentration of the actuating factor. In more complicated cases, these mechanisms involve transduction of the external signal across the cytoplasmic membrane and the subsequent transmission to a particular site of the genome or to an organelle (e.g., flagellum). Such multistage mechanisms suggest the presence of a special apparatus (signal transduction system), which includes membrane receptors of signals and proteins transmitting the signal (signaling proteins). The process of signal transduction involves, as a rule, covalent modification of proteins (methylation, phosphorylation, homodimerization). Regulation of this type is commonly termed global regulation. It involves operons and regulons (groups of operons), controlled by a common regulatory protein, as well as modulons, which include several (up to 50) operons and employ several regulatory proteins; among these regulatory proteins there is usually a major one.

The operons and regulons constituting a modulon may control independent processes, but the combined effect lies in a quick response to an external signal. An example of this type of regulation is chemotaxis and other tactic responses as a result of which the organism (or its part in the case of multicellular organisms) moves to the zone most favorable for it. An important role in the mechanisms of global regulation is played by cyclic and polyphosphorylated nucleotides (cAMP, guanosine-tetraphosphate, etc.).

Change in the partial pressure of oxygen in the medium is a typical stress impact, and it triggers protection systems of global regulation in the cell. The genes whose expression depends on the oxygen level may be conventionally subdivided into two groups: aerobic genes (their expression is maximal under aerobic conditions) and hypoxic genes (their expression is maximal under microaerobic and anaerobic conditions). Oxygen-dependent genes constitute a considerable portion of the genome of unicellular organisms.

Most components of the respiratory chain, such as cytochromes, and other proteins involved in strictly aerobic metabolism, e.g., the enzymes protecting the cell from reactive oxygen species (ROS), are encoded by aerobic genes. A large number of genes whose induction (or derepression) occurs upon transition to hypoxia and anoxia also encode proteins involved in metabolic pathways that utilize oxygen, e.g., alternative oxidases of the electron transport chain or reductases and desaturases involved in the biosynthesis of heme, sterols, and unsaturated fatty acids. At first glance, the products of these genes should not function under conditions of hypoxia; however, it is under hypoxia that their expression is maximal (this is true for, e.g.,

[1] Corresponding author; e-mail: plakunov@inmi.host.ru

enzymes involved in heme and sterol biosynthesis, which use oxygen as a substrate). Evidently, induction/derepression of these genes in needed to increase the efficiency of the corresponding metabolic pathways under conditions of oxygen deficiency.

The increase in the rate of oxygen-dependent processes in hypoxia may result either from the increase in the rate of oxygen utilization (due to an increase in enzyme quantities) or from an increase in the affinity of the enzymes to oxygen. Some of the hypoxic genes encode isoenzymes that are more efficient in hypoxia than their aerobic analogues.

The *cox5a–cox5b* functional gene pair in yeast may serve as an example. The former gene belongs to aerobic genes and encodes the Va subunit of cytochrome *c* oxidase, and the latter one belongs to hypoxic genes and encodes the cytochrome *c* oxidase Vb subunit. In hypoxia, the cytochrome *c* oxidase with the Vb subunit has a higher rate of turnover and heme oxidation than the aerobic analogue [1].

Recently, data have been obtained showing that, among hypoxic genes, there are genes whose expression depends not merely on the presence/absence of oxygen but on its concentration, or may be maximal not in complete anaerobiosis, but in hypoxia (microaerobiosis) [2, 3]. An example of the second gene type is the hypoxic *ole1* gene, which encodes the key desaturase of the biosynthesis of unsaturated fatty acids in *Saccharomyces cerevisiae*: its expression is maximal (increases about fourfold compared to normoxia) at an oxygen concentration of 0.5 μM and starts decreasing upon transition to strict anaerobiosis [2].

The aerobic genes, as well as hypoxic genes, do not constitute a common regulon or operon; they are scattered over the genome and in most cases are regulated independently from each other. Several common factors/mechanisms regulate quick and efficient switch from the aerobic to microaerobic and then to anaerobic metabolism. Apart from this, each of the genes under discussion may also be regulated by complementary factors that are not part of a single regulation system.

OXYGEN REGULATION IN YEAST

Regulation of gene expression by oxygen is best studied in *Saccharomyces cerevisiae.* Many researchers use this eukaryotic microorganism as a model, since it is capable of growth under anaerobic conditions. In combination with the regulation of gene expression by carbon substrates, oxygen regulation makes it possible to efficiently utilize carbon sources, gaining energy both under aerobic (respiration + fermentation) and anaerobic (fermentation only) conditions.

In yeast, the effect of oxygen on gene expression is mediated by several signaling systems. Best studied is the system with heme playing the central role [3]. Widely known is the system in which the roles of the signaling element and common regulator of transcription factors are played by ergosterol [4–7]. Other mechanisms of oxygen regulation also exist in the cell. All of them are interdependent and tightly interrelated.

The role of hemes in oxygen regulation. Hemes are prosthetic groups of cytochromes and some oxygen-binding proteins, e.g., catalase. Hemes play multiple regulatory roles in microbial cells; in particular, they are part of the oxygen signaling system in yeast. Oxygen is required for heme synthesis. The enzyme coproporphyrinogen-III oxidase uses oxygen in the formation of protoporphyrinogen-IX, and protoporphyrinogen-IX oxidase uses oxygen in the formation of protoporphyrin. The step of the synthesis that involves coproporphyrinogen-III oxidase is rate-limiting. Thus, the heme concentration in the cell is proportional to the concentration of oxygen if the latter is above 0.1 mM. All enzymes required for heme biosynthesis are produced in the cell constitutively, even during anaerobiosis, and synthesis of heme begins immediately upon the transition from anaerobic to aerobic conditions [3].

In *S. cerevisiae*, heme activates nuclear genes encoding constituents of the aerobic respiratory chain, including the *cox5a, cox6, cox7, cox9*, and *cox8* genes (encoding subunits of cytochrome *c* oxidase), *cyc1* (aerobic isoform of cytochrome *c*), *cor1, cor2*, and *cor5* (ubiquinol–cytochrome *c* reductase), *cyb2* (cytochrome *b2*), *cyt1* (cytochrome *c1*), *aac1* and *aac2* (aerobic isoforms of mitochondrial adenine translocase), and mitochondrial genes *coxI* and *coxII*. The group of heme-dependent aerobic genes of *S. cerevisiae* also includes the genes involved in oxidative stress response: *ctt1/cta1* (cytosolic and peroxisomal forms of catalase) and *sod1/sod2* (Cu,Zn-superoxide dismutase, Mn-superoxide dismutase). Transcription of the *cta1* gene is also regulated (repressed) by glucose. Heme also activates the *tif51a* gene (aerobic isoform of the translation initiation factor eIF5A), *rox1* (repressor of hypoxic genes), and other genes [2, 3, 8–11].

Heme suppresses the transcription of the *cox5b* gene (encoding the Vb subunit of cytochrome *c* oxidase) and the *cyc7* gene (encoding the anaerobic isoform of mitochondrial adenine translocase). Heme-dependent hypoxic genes include *erg11*, *cpr1, hmg2* (sterol biosynthesis), *ole1* (fatty acid biosynthesis), *hem13* (encoding coproporphyrinogen-III oxidase, which is the enzyme of the rate-limiting step of heme biosynthesis), and *anb1* (anaerobic isoform of the translation initiation factor eIF5A) [2, 3, 8–11]. The hypoxic genes also include the genes that are involved in pseudohyphal growth of *S. cerevisiae* and are activated by the transcription regulation factors Ste12 and Tec1 and negatively regulated by factor Dig1. Under anaerobic conditions, it is typical of *S. cerevisiae* to form elongated cells regardless of the carbon source and other factors [12].

In *S. cerevisiae*, there are pairs of duplicate genes that are not interrelated genetically but fulfill the same function. One of the genes in the pair encodes an aero-

bic form of the protein, and the other gene, a hypoxic form; examples are the already mentioned *cox5a/cox5b* system (the Va/Vb subunit of cytochrome *c* oxidase), *cyc1/cyc7* (isoforms of cytochrome *c*), *aac2/aac3* (isoforms of mitochondrial adenine translocase), and *tif51a/anb1* (aerobic/anaerobic forms of the eIF-5 translation initiation factor). In all cases, the aerobic and hypoxic isoforms are interchangeable. Oxygen/heme activate transcription of the aerobic isoform of the pair and suppress the transcription of the hypoxic one. The genes in the pairs show a high degree of homology (e.g., 66% for *cox5a/cox5b* and 79% for *cyc1/cyc7*) [3]. This suggests that such gene pairs arose as a result of DNA duplication and subsequently underwent evolutionary changes to function at different concentrations of oxygen. For example, it was shown that in hypoxia isoform 2 of cytochrome *c* operates more efficiently than isoform 1, which is most efficient in normoxia [13].

The effect of oxygen on the expression of aerobic/hypoxic genes may manifest itself in different ways. First, for each heme-dependent aerobic/hypoxic gene there exists the so-called expression threshold in the oxygen concentration range of 0.5 to 1 μM (or lower, for some hypoxic genes). The expression threshold is the concentration of oxygen above or below which an abrupt increase or decrease in the expression of the given gene is observed. The expression thresholds are different for different genes but their values are constant. For example, the expression threshold for the aerobic genes *cox4*, *cox6, cox7, cox8*, and *cox9* are approximately equal to 1 μM O_2. At oxygen concentrations above 1 μM, a sharp increase in the expression is observed, and below 1 μM, an abrupt decrease is observed [2, 3].

In addition, many aerobic and hypoxic genes respond to different oxygen concentrations in a graded fashion. For example, in the O_2 concentration range from 200 μM (the equilibrium concentration with air) to the expression threshold, the expression level of the aerobic genes *cox4, cox7, cox8*, and *cox9* decreases so as to be proportional to the oxygen concentration [2]. Although the expression of most of these genes is maximal at 200 μM (air), they are also expressed in hypoxia/anaerobiosis, and the level of their expression under anaerobic conditions varies from 3 (*cyc1*) to 40% (*cox9*) of the maximal expression level [2, 3].

The expression threshold of the hypoxic genes is usually much lower than that of the aerobic genes. For the *cpr1, hem13, hmg1, hmg2, erg11*, and *ole1* genes (synthesis of sterols, heme, unsaturated fatty acids), the expression threshold is below 0.5 μM. Their mRNA can be detected in cells under aerobic conditions as well; the expression level under aerobic conditions is about 30% of the maximal expression level in hypoxia [2, 3].

For the functional pairs *cox5a/cox5b* (the Va/Vb aerobic and hypoxic subunits of cytochrome *c* oxidase), *cyc1/cyc7* (aerobic/hypoxic isoforms of cytochrome *c*), *tif51a/anb1* (aerobic/hypoxic isoforms of the translation factor eIF-5a), *aac2/aac3* (aerobic/hypoxic isoforms of mitochondrial adenine translocase), the dose-dependent effect is usually not observed, but there exists the so-called threshold of switch from one isoform to the other, and this threshold is very low (between 1 and 0.5 μM O_2). The expression of the hypoxic genes *cox5b* and *cyc7* is observed only under anaerobic conditions [2, 3].

On certain hypoxic genes (*cyc7*, *hem13, hmg1, erg11*), heme exerts a dual effect: activation (mediated by the transcription factor Hap1) and repression (mediated by the repressor Rox1). This may be needed for fine-tune regulation of the transcription level at various oxygen concentrations, especially those close to the threshold ones (when the expression of these genes sharply increases). However, the combined effect of oxygen on the transcription of these genes in normoxia consists in its suppression.

As a rule, activation of aerobic genes proceeds much faster than activation of hypoxic genes. For some aerobic genes, activation is observed within 15 minutes after transition to aerobic conditions [2]. There are several reasons for this phenomenon. First, most of aerobic genes are expressed constitutively. Signal molecules for oxygen presence are heme and ROS. The cell, even if it was grown anaerobically, always contains the set of enzymes necessary for heme synthesis, and this process begins immediately upon the appearance of oxygen. ROS are also generated very quickly. On the other hand, upon transition to hypoxia and then to anaerobiosis, the synthesis of heme and sterols (see below) stops, but their reserves in the cell are depleted gradually, via dilution in the course of culture growth.

There exist several heme-dependent transcription regulation factors: Hap1 (*h*eme *a*ctivated *p*rotein), which activates transcription of many aerobic genes and some hypoxic genes; Hap2/3/4/5, which activates transcription of many aerobic genes (its function is also regulated by the carbon substrate); Rox1 (*r*egulation by *ox*ygen) and Mot3, which are repressors of hypoxic genes under aerobic conditions.

In a simplified way, the functioning of the oxygen sensor that involves heme can be presented as follows. Under aerobic conditions, heme concentration is sufficient for activation of Hap1 and Hap2/3/4/5. These factors activate expression of aerobic genes. One of the genes activated by factor Hap1, *rox1*, encodes the repressor of hypoxic genes. This repressor, Rox1, also controls the expression of the *upc2* gene, encoding the activator of hypoxic genes. When oxygen concentration falls below submicromolar, heme synthesis is suppressed, and the intracellular heme concentration begins to decrease due to dilution in the course of culture growth. This leads to deactivation of factors Hap1 and Hap2/3/4/5, which causes suppression of the expression of aerobic genes under their control, includ-

ing the *rox1* gene. As a result, derepression and activation of hypoxic genes is observed [3, 10].

This is a simplified model, since there exist additional regulation mechanisms, but it adequately describes the principles of the expression of oxygen-dependent genes.

The mechanisms of regulation of transcription factors by hemes are poorly studied. Hemes may act as cofactors or ligands which bind with proteins that regulate transcription and thereby control their activity (the activity of the transcription factor Hap1 is regulated according to this principle). In this case, first, the activity of transcription regulators depends on the heme concentration; at very low oxygen concentrations, close to K_m of coproporphyrinogen-III oxidase, heme provides for the effect of switching on/off or switching over. Second, heme may be a redox-sensitive prosthetic group of transcription factors (a redox-sensitive effector regulating the activity of transcription factors), such as Fe-S centers of the bacterial transcription factors Fnr and SoxR. This model can explain the existence of the expression threshold but not the dose-dependent effect. The redox status of iron in the heme molecule may also influence the activity of transcription factors. An example of such a sensor is the FixL system of the bacterium *Rhizobium meliloti*, in which the kinase activity of FixL depends of the spin state of iron in the heme molecule [2, 3].

Transcription factor Hap1 is synthesized constitutively (irrespective of the oxygen concentration in the medium). This protein consists of 1483 amino acid residues and has at least three functional domains. The first one (residues 1–148) is a DNA-binding domain and is involved in the formation of a homodimer, which is the transcriptionally active form. The second domain (residues 1309–1483) is involved in Hap1 activation. The third (residues 244–444) and fourth (1192–1197) domains are involved in binding to repressor proteins and heme. In the absence of heme, protein Hap1 is weakly bound to DNA and is transcriptionally inactive because the domain between residues 244 and 444 is bound to the repressor protein. In the presence of heme, a smaller and transcriptionally active complex is formed because heme binds to the third domain and prevents formation of the complex with the repressor protein. Heme also plays a role in Hap1 activation by binding to the second domain [3].

For Hap1-mediated activation of certain genes, e.g., *cyc1* (encoding the anaerobic isoform of cytochrome *c*), presence of the molecular chaperons Hsp70 and Hsp90 is required. These chaperons form complexes with Hap1 [14–16].

The activity of Hap1 not only depends on the presence of O_2; it is also proportional to O_2concentration, as is the expression level of Hap1-regulated genes. The oxygen concentration of 1 μM is the threshold: below it, an abrupt decrease in Hap1 activity is observed [17]. This may explain the dose-dependent effect of oxygen on the expression of Hap1-regulated aerobic genes.

In *S. cerevisiae*, Hap1 activates the transcription of many aerobic genes involved in aerobic respiration and protection against ROS, such as *cyc1*, *cyc7* (isoforms of cytochrome *c*), *cyb2* (cytochrome *b2*), *cor2* (ubiquinol–cytochrome *c* reductase), sod_2 (superoxide dismutase), *hmg1* (sterol synthesis), *tif51a* (factor eIF-5a), and *hem13* (heme synthesis). Hap1 also activates the transcription of *rox1* (encoding repressor of hypoxic genes) [3].

There are data showing that Hap1 may play a role not only of a transcription activator but also of a transcription repressor [18]. Hap1 suppresses the transcription of its own gene, irrespective of the heme effect [19]. In the absence of heme (under anaerobic conditions), Hap1 may be a repressor of other genes. In *S. cerevisiae*, Hap1 under anaerobic conditions is a direct repressor of such genes as *erg2, erg5, erg11, hmg1, cyb5, erg5, erg8*, and *are2* (ergosterol metabolism). For the Hap1-mediated repression of these genes, the Tup1/Ssn6 complex is required, which, in hypoxia, acts together with Hap1 as a specific corepressor [18]. It is the presence or absence of heme that determines whether Hap1 acts as an activator or as a repressor. In the presence of heme, Hap1 acts as an activator, and in the absence of heme, as a repressor; this makes possible differential regulation of the expression of target genes at intermediate concentrations of oxygen.

The transcription factor Hap2/3/4/5. The heteromer Hap2/3/4/5 activates transcription of genes primarily in response to the availability of oxygen and/or during growth on nonfermentable substrates (lactate, glycerol, etc.) It consists of four polypeptides: Hap2, a protein of 265 amino acid residues which contains a DNA-binding domain; Hap3, a protein of 144 amino acid residues which is also necessary for binding to DNA; Hap4, a protein of 554 amino acid residues which is involved in activation of the complex; and Hap5, a protein of 216 amino acid residues which is necessary for the assembly of the complex and its DNA-binding activity. The heterotrimer Hap2/3/5 is involved in binding to DNA, whereas Hap4 is a regulatory subunit necessary for activation of the complex [3].

The function of Hap2/3/4/5 is modulated by the Ssn6/Tup1 complex of general factors/modulators of transcription with the involvement of protein kinase Snf1 in response to the presence of glucose (fermentable substrate). In an overwhelming majority of microorganisms, mass changes in gene expression are observed in the presence of glucose, including suppression of respiration. On nonfermentable substrates (lactate etc.), induction of Hap2 and Hap4 is observed, accompanied by enhancement of the expression of genes regulated by the Hap2/3/4/5 complex [3, 10, 20]. Thus, in the presence of glucose and absence of heme, the expression of genes regulated by Hap2/3/4/5 is very low; in the presence of heme and glucose, it increases by an order of magnitude, and in the presence of heme and absence of glucose, by one more order of magnitude. Probably because of this, certain genes regulated

by the Hap2/3/4/5 complex are also regulated by factor Hap1. This allows their expression to be maintained at a relatively high level both in the presence and absence of glucose [3].

The Hap2/3/4/5 complex binds to DNA independently of heme, and the role of heme in the activation of this complex is unknown. However, it was established that activation of the transcription of certain genes by Hap2/3/4/5 is independent of heme but depends on the nature of the carbon substrate (e.g., the *aco1, cit1, kgd1, kgd2*, and *lpd1* genes encoding the enzymes of the tricarboxylic acid cycle). In the case of the genes *hem1* and *hem3*, encoding enzymes of heme biosynthesis, activation depends neither on heme nor on carbon substrate [3].

Hap2/3/4/5 also activates the transcription of such genes of *S. cerevisiae* as *cyc1* (aerobic isoform of cytochrome *c*), *cor2* (ubiquinol–cytochrome *c* reductase), *cox4, cox5a, cox5b, cox6* (subunits of cytochrome *c* oxidase), and *rox1* (repressor of hypoxic genes) [3].

Repressor Rox1. The protein Rox1 represses the transcription of nearly all hypoxic genes. It consists of 368 amino acid residues. The N-terminal region is homologous to other Hmg (high-mobility group) proteins and contains a DNA-binding domain. The C-terminal region is necessary for the repression of transcription and interaction with the Ssn6/Tup1 complex. The effect of Rox1 is independent of oxygen, but the transcription of its gene, *rox1*, is regulated (activated) by the heme-dependent factor Hap1, and heme presence is necessary for *rox1* transcription. In addition, the Ssn6/Tup1 complex is required for Rox1-mediated repression [11, 20–23]. In the absence of one of these factors, the expression of Rox1-regulated genes becomes constitutive. The factors Ssn6 and Tup1 are required for activation of many other repressors. Ssn6 is known to directly interact with Rox1 and to be involved in the stabilization of the Rox1–DNA complex.

In the presence of heme (i.e., under aerobic conditions), the *rox1* gene is transcribed and translated, which is partly regulated by Hap1. The Rox1 level in the cell is regulated via autorepression (Rox1 represses expression of its own gene). In the cell nucleus, Rox1 binds to a coordinately regulated sequence of hypoxic genes and forms a complex with Ssn6/Tup1, which results in the repression of the transcription of hypoxic genes [11, 22, 23]. Data have been published showing that the affinity of Rox1 to different hypoxic genes is different, and this explains the differences in the transcription level of these genes at the same concentration of oxygen and heme [11].

In the absence of heme (under anaerobic conditions), *rox1* transcription is repressed, and the Rox1 level gradually decreases. This results in derepression of hypoxic genes. In particular, in *S. cerevisiae*, Rox1 suppresses the expression of the genes *cox5b* (anaerobic form of the Vb subunit of cytochrome *c* oxidase), *aac3* (anaerobic isoform of mitochondrial adenine translocase), *hem13* (coproporphyrinogen-III oxidase, the enzyme catalyzing the rate-limiting step of heme synthesis), *erg11, cpr1* (sterol synthesis), *anb1* (anaerobic form of the translation initiation factor eIF-5a) [3, 9, 10, 24]. In addition, Rox1 plays an important role in the regulation of genes encoding late stages of glycolysis (*gpm2, gpm3, cdc19*), ethanol formation (*adh1, adh5*), and glycogen synthesis (*csy1, gip2*) and decomposition (*gph1*) [9].

Data of several research teams [1, 3, 9–11, 13, 24] allow an assumption to be made that Rox1 (at least in *S. cerevisiae*) plays a key role in the regulation of almost all systems whose function is oxygen-dependant (structural proteins of the cell wall, various enzymatic systems, such as those of lipid and sterol metabolism, mitochondrial functions, aerobic redox balance, carbohydrate metabolism, etc.).

Other heme-dependent regulators of transcription. It has been shown that the repressors Mox1, Mox2, and Mot3 take part in oxygen regulation [9, 25]; Mot3 can be not only a repressor but also an activator of transcription [26]. Repressor Rox3 (Ssn7), which is involved in the regulation of genes involved in general stress response, regulates the expression of the hypoxic gene *cyc7* (encoding an isoform of cytochrome *c*) [27].

Certain hypoxic genes, including *anb1* and *dan/tir/erg*, the repressor of which under aerobic conditions is Rox1, are also controlled by another repressor, Mot3 [21, 25, 28]. This repressor belongs to general transcription regulation factors and is heme-dependent. In hypoxia, the expression of the *mot3* gene is suppressed by Hap1 together with the Tup1/Ssn6 complex, which acts as a corepressor, and a fourth unidentified factor. Rox1 and Mot3 function in parallel and independently of each other [21]. Data exist that Hap1 is an activator of *mot3* transcription under aerobic conditions [17].

THE ROLE OF STEROLS IN OXYGEN REGULATION

The synthesis of ergosterol, like the synthesis of hemes, requires the presence of oxygen. Ergosterol-mediated oxygen regulation is far less studied than the heme-mediated regulation. However, it is known that ergosterol also plays a significant role in oxygen regulation in yeast. For example, about one third of the hypoxic genes are presumed to be regulated by ergosterol (by the negative feedback principle) in *Schizosaccharomyces pombe* [6] and *S. cerevisiae* [9].

In yeast, including *S. cerevisiae* and *Candida albicans*, the expression of *erg* genes encoding ergosterol biosynthesis enzymes is regulated by the transcription factors Upc2 and Ecm22 [4, 7].

It was established that factor Upc2 also regulates the expression of hypoxic genes [4, 7]. Upon transition from normoxia to hypoxia, the ergosterol concentration decreases steadily (as the heme concentration does) due to dilution in the course of culture growth. The activity

and concentration of the transcription factor Upc2 under hypoxia are regulated by the ergosterol level. With the decrease in the ergosterol level, accumulation and activation of the transcription factor Upc2 is observed.

Factor Upc2 activates transcription of hypoxic genes, e.g., those encoding proteins transporting sterols into the cell, including *sut1*, *aus1, hes1, pdr11* in *S. cerevisiae, snq2*, *cdr1, cdr2, cdr11, cdr4* in *C. albicans*, and the *dan/tir* genes of mannoprotein synthesis [4, 7]. In *S. cerevisiae*, the mannoproteins Dan1, Dan2, Dan3, Dan4, Tir1, Tir2, Tir3, and Tir4 are synthesized in hypoxia as well as in response to other stress factors; they replace the normal mannoproteins Gwp1 and Gwp2 (accordingly, in hypoxia, repression of the *gwp1* and *gwp2* genes is observed) [29]. Upc2 also activates the expression of genes of ergosterol synthesis (*erg1, erg2, erg3, erg4, erg5, erg7, erg10, erg11, erg25, erg26, erg27, cpr1*), which belong to hypoxic genes: their transcription is considerably enhanced upon a decrease in O_2 concentration, when ergosterol deficiency arises [4, 7]. It was established that Upc2 is involved in the regulation of expression of the genes *hem14* (heme synthesis), *scc3* (protein synthesis), and *scs2* (sphingolipid synthesis) [9]. In addition, Upc2 activates the expression of its own gene *upc2* [4, 7, 9].

The expression of some of the above-listed genes is also regulated by other factors. In particular, for basal expression of the *erg2* gene (and, presumably, other *erg* genes) and *hmg1* gene, the heme-dependent factor Hap1 is required (which is active under aerobic conditions) [4, 9]. The heme-dependent repressor Rox1 was shown to be involved in the regulation of the *erg3*, *erg4, erg9, erg11, erg25*, and *hes1* genes. The *upc2* gene itself is regulated by Rox1. Nevertheless, upon transition to hypoxia, accumulation and activation of Upc2 in the cell is observed exactly in response to a decrease in sterol concentration, and the Upc2 function becomes independent of Hap1. Activation of the transcription of *erg2, hmg1*, and other above-listed genes is mediated by factor Upc2 in response to the decrease in the ergosterol level and is independent of Hap1, Rox1, and the level or presence of heme [4, 7].

The expression of the *dan1* and *tir1* genes (encoding mannoproteins of the cell wall induced by various stress factors) is regulated both by the sterol-dependent transcription activation factor Upc2 and by the heme-dependent repressors Rox1 and Mot3 [4, 5, 29]. Accordingly, the induction of Dan1 and Tir1 occurs in response both to decrease in sterol concentration and to decrease in the concentration of heme [4, 7].

As mentioned earlier, under anaerobic conditions Hap1 acts as a direct repressor of the *erg2*, *erg5, erg11*, *hmg1*, and *erg8* genes. It was shown that Hap1 does not suppress transcription of the *erg* genes completely; the import of sterols from the medium into the cell is blocked while oxygen is present in even trace amounts [18]. Most probably, concomitant activation by Upc2 and repression by Hap1 are necessary for the control of sterol metabolism under various oxygen levels. Sterol synthesis continues in the cell until anaerobiosis is complete, and Upc2-mediated activation of the corresponding genes enables more efficient utilization of the limited amount of oxygen [18].

Certain *erg* genes, including *erg2, erg6*, and *erg9*, are also regulated by the dose-dependent transcription repressor/activator Mot3 [4, 30]. For example, the expression of the *dan1* gene is activated by Upc2 and suppressed by Mot3; activation by Upc2 requires the Rpd3 complex, which binds to the promoter of *dan1* and prevents Mot3-mediated repression [30].

Activation of some *erg* genes requires the nuclear protein Yer064C with an unknown function [4]. This demonstrates how closely various regulation systems are interrelated in the cell.

It should be mentioned that virtually all *S. cerevisiae* genes regulated by Upc2 are also regulated by factor Ecm22 (*erg, hmg1*). In turn, the level of Ecm22 also depends on the ergosterol concentration. The DNA-binding and regulatory/activation domains of factors Upc2 and Ecm22 are very similar (70–80% identity); however, these factors are active at different ergosterol concentrations (at least when they act as inducers of *erg* genes) [4]. In normoxia, and when the ergosterol level only starts to decrease, it is Ecm2 that mainly functions; at later stages, upon a considerable decrease in the ergosterol level (prolonged anaerobiosis), Upc2 functions. In the course of prolonged anaerobiosis, the concentration and activity of Emc22 decrease, in contrast to those of Upc2. In addition, the functioning of Emc22, unlike that of Upc2, is dependent on factor Hap1 (i.e., heme) under any conditions [4].

Like Hap1, Upc2 can act not only as an activator but also as a repressor. The dual effect of Upc2 was demonstrated for the *mdr1* gene of *C. albicans*, which encodes a drug transporter. In hypoxia, Upc2 acts as an activator of the *mdr1* gene, and under anaerobic conditions it acts as a repressor [7].

In *Schizosaccharomyces pombe*, no transcription factors analogous to Hap1 and Rox1 (heme-dependent) or to Upc2 have been found; the main activator of the expression of hypoxic genes is the Sre1 protein. This regulator of transcription is sterol-dependent. It is activated proteolytically upon decrease in the ergosterol concentration in the cell (in hypoxia), with the involvement of protein Scp1. Sre1p directly binds to the promoter of the genes that it activates. The Sre1p factor controls expression of 68% of all genes that are induced at least 2-fold under hypoxic conditions. These are mainly genes the products of which are involved in the syntheses of ergosterol (e.g., *erg2*, *erg5, erg6, erg11*, *erg24, erg25, erg27*), heme (*hem12*, *hem13, hem14, hem15*), ubiquinone (*abc1, coq3, coq4, coq5, coq6*), sphingolipids, and ceramide (*sur2, scs7*), and use oxygen as a substrate [6]. Factor Sre1p also activates the expression of many genes with an unknown function, including the *SPBC6B1.08c*

and *SPAP8A3.02c* genes, which encode proteins belonging to the group of oxoglutarate-dependent dioxygenases. Factor HIF-1, activator of the expression of hypoxic genes in animal cells, also belongs to this group. Products of the *SPBC6B1.08c* and *SPAP8A3.02c* genes may also function, along with Sre1, as activators of hypoxic genes in *S. pombe*. In addition, Sre1 activates the transcription of its own gene [5].

In *S. pombe* (as in other yeasts), hypoxia induces genes that encode enzymes of the glycolytic pathway and suppresses genes that encode components of the aerobic respiratory chain, enzymes protecting cells against ROS, and enzymes of ATP synthesis. The expression of these genes is independent of Sre1 and is regulated by other factors [6].

It was shown that *S. pombe* cells actively accumulate glycerol and trehalose in hypoxia as a result of induction of many genes of carbohydrate metabolism [5, 6]. These compounds are also accumulated in response to osmotic stress as compatible solutes. Consequently, under conditions of hypoxia, *S. pombe* cells are more tolerant to osmotic shock.

One more significant difference between the systems of oxygen regulation in *S. pombe* and *S. cerevisiae* is that *S. pombe* lacks analogues of the *S. cerevisiae dan/pau* genes (Upc2-regulated genes of mannoprotein synthesis), as well as the *aus1* and *pdr11* genes (they are also Upc2-regulated and encode proteins transporting sterols into the cell). Under anaerobic conditions, *S. cerevisiae* cells begin to take up sterols and unsaturated fatty acids from the medium, whereas anaerobic growth of *S. pombe* is restricted, among other factors, by the reserve of ergosterol in the cell [5].

OTHER MECHANISMS OF OXYGEN REGULATION

One of the possible ways of the regulation of cellular processes consists in changing the cellular redox potential at the expense of the reduction/oxidation of various molecules. In yeast, glutathione, NAD, and cysteine residues in proteins play a significant role in this process [9, 31–33]. In *S. cerevisiae*, the transition to anaerobic conditions results in a significant activation of the expression of the genes involved in the maintenance of the redox potential: *gpd2* (encoding the anaerobic form of glycerol-3-phosphate dehydrogenase) [33] and *rhr2* (encoding enzymes of glycerol synthesis) [9], as well as of the genes of trehalose synthesis: *tps1, tps2*, and *tps3*. These genes belong to redox-regulated genes [9]. The induction of trehalose synthesis is required for the protection of membrane proteins against the increase in the ethanol concentration under anaerobic conditions, and the synthesis of glycerol is related to maintenance of the redox potential. Trehalose and glycerol are osmoprotectants; therefore, *S. cerevisiae*, as well as *S. pombe*, is more tolerant to osmotic stress under anaerobic conditions [33].

It was also shown that, during growth on nonfermentable substrates, the first adaptation stage upon transition of *S. cerevisiae* to anaerobic conditions is controlled by the Msn2/Msn4 factors of general stress response [10].

On the contrary, upon transition from anaerobic to aerobic conditions, a considerable role in signal recognition is played by ROS, and the regulation of oxygen-dependent genes is in part fulfilled by factors Yap1, Yap7, Skn7 (the system of oxidative stress response) [2, 3, 10, 34]. The Mga2 factor, which activates the *ole1* hypoxia gene, was also identified [35, 36].

In *S. cerevisiae*, the mitochondrial translation factor PET494 (regulating the expression of the aerobic *coxI, coxII*, and *coxIII* genes (cytochrome *c* oxidase subunits)), factor CBS2 (regulating the expression of the aerobic *cob* gene (a subunit of cytochrome *bc1* complex)), and the hypoxic genes *anb2, anb5, anb13, anb15* are oxygen-dependent; however, neither heme nor heme-dependent activators and repressors take part in their regulation [3].

INTERACTION OF VARIOUS SYSTEMS OF OXYGEN REGULATION IN YEAST

Upon transition from aerobiosis to hypoxia, the microbial culture passes several stages of adaptation, and the above-described specific systems (involving hemes and sterols) play a key role in the last stages, i.e., during prolonged anaerobiosis. At the initial stage (within two cell generations) a significant role is played by other expression regulation factors. The earliest changes in the gene expression are mediated by the system of general stress response, with the involvement of factors Msn2/4, as well as MCB, SCB, PAC, and RRPE [10]. Factors Msn2/4 control the expression of almost all genes of general stress response. Similar changes occur in cells undergoing different stress impacts (e.g., starvation, oxidative stress, osmotic stress). About 60% of the genes of the general stress response found in yeasts are oxygen-dependent. In particular, in the process of functioning of the Msn2/4 system, changes occur in the expression of genes involved in the metabolism of energy reserves (glycogen, trehalose) and in transport and metabolism of alternative sources of energy and carbon; rRNA synthesis and transcription are suppressed. This allows the metabolism to be fully adapted to new conditions. Interestingly, this transient stage of response was not observed in *S. cerevisiae* grown on a fermentable substrate [10].

After about one fourth of a generation, heme begins to play a significant role in the regulation of expression. Repression of the transcription activation factors Hap1 and Hap2/3/4/5 and the systems they regulate (respiration, mitochondrial functions, pyrimidine metabolism, metal ion homeostasis) is observed, as well as derepression of systems regulated by repressor Rox1 (transport of various compounds into the cell, utilization of car-

bon sources (carbohydrates), glycolysis, gluconeogenesis, maintenance of redox potential, etc.). The last to be switched on (approximately after two cell generations) are ergosterol-dependent systems involving factor Upc2 and systems regulated by factor Mot3 (homeostasis of sterols and unsaturated fatty acids, maintenance of the cell wall functions) [10]. The heme-dependent and sterol-dependent systems switch on later, as after the transition to anaerobic conditions, the concentrations of heme and sterols decrease not abruptly, but gradually, via their dilution in the process of culture growth.

Conversely, upon reoxygenation, the heme- and ergosterol-regulated systems and systems independent of heme and sterols (those activated by changes in the ROS concentrations, systems of general stress response, etc.) begin to function immediately and simultaneously. Simultaneous activation of the oxidative stress response system (with the involvement of factors Yap1 and Msn2/4) and of other aerobic genes (with the involvement of factor Hap1) takes place. Many aerobic genes the products of which are involved in the protection against oxidative stress and in other oxygen-utilizing metabolic pathways are activated by several of these factors simultaneously. For example, the genes of lipid and fatty acid metabolism are activated by factors Hap1 and Yap1 simultaneously; as a result, their activation occurs very rapidly [10]. The repression of hypoxic genes is also abrupt, especially of those regulated by the heme-dependent repressor Rox1. This effect is explained by the permanent (even under anaerobic conditions) presence in the cell of the enzyme system for heme synthesis, which provides for the immediate onset of heme synthesis upon the appearance of available oxygen. Interestingly, the repression of some hypoxic genes regulated by the sterol-dependent factor Upc2 is observed not immediately upon transition to aerobic conditions but with a lag; for some of these genes, there is even added activation by factors Yap1 and/or Hap2/3/4/5 [10].

OXYGEN REGULATION IN PROKARYOTES

In bacteria, like in yeast, heme plays a significant role in oxygen regulation. A great diversity of heme-containing sensor proteins is observed in prokaryotes. As a rule, in these proteins the heme-binding sensor domain regulates the activity of the second domain, which is most often a kinase. In some cases, oxygen binding to heme results in conformational changes in the protein and activates the kinase cascade; in other cases, oxidation of the heme iron plays a significant role; sometimes, binding to oxygen results in the breakage of the heme–protein bond, etc. [37].

One of the best studied systems of oxygen regulation in bacteria is the two-component FixL–FixJ system, where protein FixL is a sensor and a kinase, and protein FixJ is a transcription regulator activated by FixL. This system is involved in activation of hypoxic genes in many prokaryotes. Protein FixL contains a kinase domain and a heme-binding sensor domain (belonging to a large group of PAS domains, which function as sensors in diverse regulation systems in bacteria and eukaryotes). Heme plays the role of a cofactor. The heme-binding domain regulates the kinase activity of FixL. Under aerobic conditions, heme binds to oxygen, and, as a result of conformational changes, the kinase activity of FixL is inhibited. In hypoxia, when heme is not bound to oxygen, FixL is autophosphorylated and phosphorylates the transcription factor FixJ, which leads to its dimerization and activation. In the active state, FixJ activates the expression of hypoxic genes [38–40]. Particularly, in nitrogen-fixing bacteria, which experience hypoxia upon formation of symbiotic nodules, FixJ induces the expression of nitrogen fixation genes (*nif, fix*), as well as genes encoding alternative terminal oxidases, which are more efficient under conditions of hypoxia [38–40].

In the obligatory aerobic pathogen *Mycobacterium tuberculosis*, the system of oxygen regulation works in an analogous way. With the progression of the infection process, the oxygen content in injured lung tissues becomes limited, and *M. tuberculosis* cells experience hypoxia. In *M. tuberculosis*, the main factor regulating the transcription of hypoxic genes is the DosR (DevR) factor. Its activity is regulated by two sensor kinases, DosT and DosS (DevS), which contain heme as a cofactor. When bound to oxygen, these kinases are inactive; upon oxygen removal from the environment, their activation takes place. Activation of DosT and DosS is observed at different oxygen concentrations: the activation threshold is 26 mM for DosT and 3 mM for DosS. These kinases presumably function at different physiological statuses of the host macroorganism [41].

One more example of a heme-containing sensor is the AppA–PpsR system in *Rhodobacter sphaeroides.* This bacterium is a facultative phototroph which in the presence of oxygen gains energy from aerobic respiration, and in the absence of oxygen in the light, from photosynthesis. The triggering of the photosynthetic apparatus is regulated by the AppA–PpsR system. Protein PpsR functions as a repressor, and AppA is a sensor–antirepressor. Noteworthy is that AppA is not only an oxygen sensor but also a light sensor. For the fulfillment of the first function, heme is used, and chromophore is used for the second. In this case, activation of AppA is achieved via dyscoordination of the oxygen-bound heme in the protein molecule [37].

One more example of a heme-containing sensor is the Pdea1 (AxPdea1) protein in *Acetobacter xylinum.* It also contains a heme-binding sensor domain belonging to the PAS domain group; however, it is linked to phosphodiesterase, found only in bacterial signaling systems. Phosphodiesterase signaling systems are widely spread among bacteria, especially pathogens, such as *Vibrio cholerae.* The oxygen-bound form is inactive, and the hypoxic form is active. In the active state,

AxPdea1 catalyzes the conversion of the cyclic form of the dinucleotide diguanylic acid into a linear form. The cyclic form of diguanylic acid allosterically activates (about 200-fold) cellulose synthase. In hypoxia, AxPdea1 prevents activation of cellulose synthase, and the production of cellulose virtually stops. Therefore, production of cellulose is maximal in biofilms, and it is much lower in submerged culture without forced aeration [39]. Whereas the FixL–FixJ system promotes long-term adaptation by the de novo induction of gene expression, the AxPdea1 protein inactivates the cellulose synthase already existing in the cell, which is an example of a short-term adaptation.

The ArcAB system controlling the expression of hypoxic genes in *Escherichia coli* is also well studied. It includes the membrane-bound protein kinase ArcB and the cytoplasmic protein ArcA with a DNA-binding domain. In hypoxia, the oxygen-sensitive kinase ArcB is autophosphorylated in response to the decrease in the electron flow through the respiratory chain and then phosphorylates protein ArcA, conferring on it the ability to bind to DNA and to regulate a large number of operons, including the operons that control carbon catabolism and the redox state in the cell. Kinase ArcB does not interact with oxygen directly; its autophosphorylation is observed in response to the decrease in the electron flow through the respiratory chain. Under aerobic conditions, the kinase activity of ArcB is inhibited due to formation of intramolecular disulfide bonds upon specific oxidation by quinones of the two redox-active cysteine residues in the sensor. Protein ArcB also belongs to the group of proteins that contain PAS domains [32, 42, 43].

On the whole, the expression of more than one third of the *E. coli* genes is oxygen-regulated; the ArcAB system is involved in the regulation of 85% of these genes. For example, this system is involved in the repression of aerobic genes of tricarboxylic acid cycle (*sdhCDAB*, *icd, fumA, mdh, gltA, acnA, acnB*), genes of the aerobic metabolism of fatty acids (*fad* regulon), and genes encoding aerobic cytochrome oxidase complexes (e.g., the *cyoA* gene, encoding cytochrome *o*–ubiquinol oxidoreductase). Via another mechanism, ArcAB activates the expression of genes the products of which are involved in the utilization of pyruvate and fermentation of sugars, and of the *cydA–cydB* genes encoding all subunits of cytochrome *bd* oxidase, thus activating electron flow via cytochrome *d*, which has a high affinity to oxygen [42–45].

In addition to the ArcAB system, the expression of hypoxic gene in *E. coli* and other prokaryotes is also controlled by the cytoplasmic protein Fnr. It consists of a sensor domain and a DNA-binding domain. Under anaerobic conditions, the signal from the sensor domain to the DNA-binding domain is transmitted as a result of conformational changes leading to the formation of homodimers. In this state, Fnr binds to DNA, repressing the transcription of aerobic genes and activating the expression of anaerobic genes. In the active (anaerobic) state, the sensor domain contains a [4Fe–4S] cluster. In the presence of oxygen (even at a concentration as low as 1 μM), inactivation of Fnr is observed as a result of direct interaction of this cluster with oxygen and its transition to the [2Fe–2S] state. Fnr looses it capacity for dimerization and becomes inactive [32, 43, 46]. Initially, Fnr was identified as a regulation factor of nitrate reduction genes (*nar, nir*) and fumarate reduction genes (*frd*); subsequently, it was found that Fnr regulates many hypoxic genes. In *E. coli*, Fnr plays a global role in the regulation of anaerobic growth and induces at least 103 operons [43, 46]. In *Salmonella enterica* serovar *typhimurium*, at least 7% of the genome is regulated by Fnr, including genes that are also present in *E. coli* but not regulated in it by Fnr. These are genes of ethanolamine utilization, genes encoding some proteins of the general stress response, and the gene encoding phosphotransacetylase. Fnr also regulates the recently identified genes of flagellum biosynthesis (*mcpAC, cheV*), chemotaxis, anaerobic carbon utilization, and virulence (*srfABC*) [47]. On the contrary, in *Neisseria gonorrhoeae*, Fnr plays a less major role. It was established that in *Neisseria gonorrhoeae* Fnr activates the expression of the *ccp* (cytochrome *c* peroxidase) and *aniA* (nitrite reductase) genes [48].

In *E. coli*, many of hypoxic genes are subject to joint regulation by the *ArcAB* system and the Fnr protein. In particular, these genes include the *cydA–cydB* genes (cytochrome *bd* oxidase). Under aerobic conditions, the expression of *cydAB* is minimal; upon transition to microaerobic conditions the expression is activated by the ArcAB system and reaches its maximum; and upon further decrease in the oxygen concentration and transition to anaerobic conditions, Fnr suppresses the *cydAB* expression [43]. Similarly, Fnr and ArcAB jointly regulate the hypoxic genes *focA–pflB* (pyruvate utilization), *sdh, suc* (succinate dehydrogenase, succinyl-CoA synthetase), *narY* (nitrate reductase subunit), *glnE* (glutamine synthase), *tynA* (tyramine oxidase), *yabM* (glucose/lactose transporter), *nanT* (sialic acid transporter), *uraA* (uracil transport), *pnuC* (nicotinamide transport), *glgA* (glycogen synthesis), *mrcA* (penicillin-binding protein 1A), *rarD* (resistance to chloramphenicol), and others [43, 44, 46]. It was found that, upon transition to hypoxia, the Fnr-mediated response develops much faster but is transitory to a large extent, whereas the ArcAB-mediated response develops more slowly. It is thought that the combination of quickly and slowly reacting components in regulatory systems is characteristic of systems that involve parallel regulation of the same genes by several factors. This, on the one hand, allows metabolism to be quickly adapted to short-term effect of stress factors (the quickly reacting components), and, on the other hand, provides for full response if the stress impact persists (the slowly reacting components) [43].

In addition to the above-described systems, other mechanisms of oxygen regulation exist, which are still

to be studied. Thus, in *E. coli*, aerobic and hypoxic genes were identified that are not regulated by either ArcAB or Fnr. Among aerobic genes, these are genes the products of which are involved in the metabolism of small molecules (*fabG, rfbX, katE*), synthesis or degradation of macromolecules (*rplB, rplC, rplO, hflC, rplF, rplQ, rplI, rpsE, rho, prfB, rplD, rpsH, tsf, rplE, nfi, tig, lysS*), and others. Among hypoxic genes, this is e.g., the *htpG* gene (encoding a heat shock protein), which is presumably regulated by the system of general stress response, and others [44, 45].

OXYGEN REGULATION AT CONCOMITANT IMPACT OF ADDITIONAL STRESS FACTORS

In most of studies, researchers confine themselves to consideration of the regulating role of oxygen as a single stress factor affecting the microbial population. However, in natural environments, microorganisms, as a rule, are subject to several environmental stress factors simultaneously. For example, in addition to hypoxia, this may be high concentration of salts and unfavorable values of pH and/or temperature.

In a series of investigations that we carried out with prokaryotic (*Rhodococcus, Shewanella*, and *Halobacterium*) and eukaryotic (*Candida, Rhodotorula*, and *Debaryomyces*) microorganisms isolated from saline ecotopes (including stratal waters of oil fields), we showed that, when hypoxia and high salt concentrations are combined, these stress factors are in antagonistic relations. This leads to an increase in the degree of microorganism halophily (halotolerance): weakly halophilic organisms become moderately halophilic, and some halotolerant organisms become halophiles [49]. It turned out that a high salt concentration (hyperosmotic shock) causes, in addition to osmotic impairments, suppression of respiration and impairment in the synthesis of proteins that protect the cell against ROS [50, 51]. Microaerobic conditions (hypoxia, or decreased partial pressure of oxygen) cause induction (activation) of hypoxic genes that encode respiratory chain components and protection enzymes that are less sensitive to the impact of salt, and also an increase in the content of osmoprotectants in the cell [52]. As a result, hypoxia decreases the adverse effects of hyperosmotic shock and increases the degree of halophily (halotolerance) of microorganisms.

Thus, in this case, hypoxia acts as an antistress factor. Interaction of the regulatory mechanisms triggered by hypoxia provides for the adaptation of cells to other stress factors, and this may explain the frequent isolation of weakly halophilic aerobic microorganisms from ecotopes with a low partial pressure of oxygen and increased content of salts (in particular, from stratal waters of oil fields) [53].

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