ISSN 0026-2617, Microbiology, 2006, Vol. 75, No. 4, pp. 438–448. © Pleiades Publishing, Inc., 2006.
Original Russian Text © N.P. Zakataeva, E.A. Kutukova, S.V. Gronskiy, P.V. Troshin, V.A. Livshits, V.V. Aleshin, 2006, published in Mikrobiologiya, 2006, Vol. 75, No. 4, pp. 509–520.

CONFERENCE PROCEEDINGS

Export of Metabolites by the Proteins of the DMT and RhtB Families and Its Possible Role in Intercellular Communication

N. P. Zakataeva[a], E. A. Kutukova[a], S. V. Gronskiy[a], P. V. Troshin[b], V. A. Livshits[a, 1], and V. V. Aleshin[c]

[a] *Ajinomoto-Genetika Research Institute, Laboratory no. 2, Pervyi Dorozhnyi proezd 1, Moscow, 117545 Russia*

[b] *CCLRC Daresbury Laboratory, Warrington, WA4 4AD, UK*

[c] *Belozersky Research Institute of Physico-Chemical Biology, Moscow State University, Leninskie Gory, Moscow, 119899 Russia*

Received March 6, 2006

Abstract—The earlier published and new experimental data are summarized on the properties of the genes encoding the membrane proteins of the DMT family (RhtA (YbiF), EamA (YdeD), YijE, YddG, YedA, PecM, eukaryotic nucleotide sugar, triose phosphate/phosphate, and hexose phosphate transporters), the RhtB/LysE family (RhtB, RhtC, LeuE, YahN, EamB (YfiK), ArgO (YggA), CmaU), as well as some other families (YicM, YdhC, YdeAB, YdhE (NorE)). These proteins are involved in the export of amino acids, purines, and other metabolites from the cell. The expression of most of the genes encoding these proteins is not induced by the substrates they transport but is controlled by the global regulation systems, such as the Lrp protein, and activated by the signal compounds involved in the intracellular communication. The level of expression, assessed in experiments on translational fusion of the corresponding bacterial genes with the β-galactosidase gene, depends on the growth phase of the bacterial culture, composition of the medium, and some stress factors, such as pH, osmolarity or decreased aeration. The efflux of normal cell metabolites is assumed to be the natural function of these proteins. This function may play a role in density-dependent behavior of cell populations (quorum sensing). It may have been enhanced in the course of evolution via specialization of these proteins in the efflux of compounds derived from metabolic intermediates and adjusted to the role of transmitters.

DOI: 10.1134/S0026261706040126

Key words: quorum sensing, amino acids, purines, transport, membrane proteins, regulation of gene expression

In the process of growth, bacteria release into the surrounding medium various organic compounds: enzymes, toxins, virulence factors, antibiotics, organic acids, amino acids, purine derivatives, vitamins, signal substances, etc. Only some of these compounds are able to freely diffuse through the cytoplasmic membrane. Most of the compounds are excreted from the cell by transport systems. These transport systems frequently consist of membrane transporters. In recent years, genes have been identified that encode proteins involved in the transport of amino acids and purines from bacterial cells. These proteins belong to the DMT [1] and RhtB/LysE [2, 3] protein families. The proteins of these two families are among the most diverse and widespread membrane proteins. Most prokaryotic genomes contain a significant number—up to a dozen and a half—of different genes responsible for their synthesis. This fact gives evidence of the important physiological role of these proteins, which is also confirmed by the evolutionary conservative nature and ancient origin of some of the subfamilies revealed in the present-day bacteria, archaea, and eukaryotes and, probably, inherited from their common ancestor. All functionally characterized proteins of these families appear to be transporters that use the electrochemical gradient of hydrogen ions (the proton-motive force) for the efflux of different organic compounds from cells. Many of these proteins are characterized by wide substrate specificity, and, in this respect, they resemble multiple drug resistance transporters (MDR transporters). However, despite the fact that the RhtB/LysE and DMT proteins are also able to accomplish efflux of certain xenobiotics, most of them transport the final or intermediate products of normal metabolism, namely, amino acids and their precursors [4–11], nucleosides, and purine bases [12–14]. Moreover, these proteins are capable of exporting from the cells specific compounds synthesized in them, such as the pigment of the *Erwinia chrysanthemi* phytopathogenic strains, which supposedly protects the bacteria from oxidative attack of the host plant [15, 16]. Not surprisingly, the genes of the proteins with substrate specificity to amino acids and

[1] Corresponding author; e-mail: vitaliy_livshits@agri.ru

nucleosides are expressed at a low level. An artificial increase in the expression level of these genes results in a massive release of the corresponding substrates from cells, which negatively affects their viability. However, industrial bacterial strains become in this case more efficient producers. A relevant question arises as to the origin and natural function of these genes. We believe that normal metabolites are natural rather than accidental substrates of the proteins in question and that regulated metabolite excretion may be advantageous under certain conditions.

INTERCELLULAR SIGNALING AND TRANSITION TO THE STATIONARY PHASE

Bacteria are known to synthesize and release signal molecules, which are accumulated in the medium as the cell population grows. When a certain cell concentration (quorum) and, accordingly, the threshold concentration of the signal compound are attained, the latter enters the cells and triggers, through interaction with transcriptional regulators, a coordinated response of the whole cell population, which often includes induction of the synthesis of the signal compound. Such regulatory systems, based on intercellular signaling, are referred to as quorum sensing (QS) systems [17, 18]. In gram-negative bacteria, the signal compounds, or autoinducers (AI), are represented by N-acyl-homoserine lactones (AHLs or AI-1), which, in different species, have different side acyl chains; furanosyl borate diester (AI-2); hydroxyalkylquinolines (HAQ, or PQS, derived from *Pseudomonas quinolone signal*); and methyl palmitate. In gram-positive bacteria, the signal compounds are usually linear or cyclic oligopeptides or gamma-butyrolactones [18, 19]. The signal compounds may be strictly specific to a given microbial species. However, some of them have broad specificity and are sensed by many species of bacteria. Thus, furanosyl borate diester (AI-2) and the recently revealed autoinducer-3 (AI-3) may serve as signals for many species of both gram-negative and gram-positive bacteria [19]. It should be noted that signal compounds are not necessarily compounds specially intended for this function. Such compounds as amino acids and purine derivatives may play the signal role. For example, in myxobacteria (Myxococcales), the role of signals that trigger coordinated behavior of a cell population is played by a mixture of six definite amino acids [20]. In addition, various metabolites that are effectors of global regulators may accumulate in the medium and induce systemic rearrangement of the metabolism of the whole cell population.

The cellular processes regulated by the quorum sensing mechanism are diverse and include bioluminescence in some of the representatives of the genus *Vibrio*, virulence and the formation of biofilms in *Pseudomonas aeruginosa* and other pathogenic bacteria, the formation of fruit bodies in myxobacteria, development of genetic competence in *Bacillus subtilis* and *Streptococcus pneumoniae*, and the production of antibiotics in different species of bacteria [18, 19]. In recent years, it has been discovered that QS may also regulate the transition of cells to the stationary growth phase [21–23]. In the stationary phase, the expression of many of the genes ceases, whereas the expression of other genes is activated and provides for cell survival under the conditions of the lack of nutrients and exposure to unfavorable environmental factors. In *Escherichia coli*, for example, the tolerance to toxic compounds and antibiotics, as well as the resistance to stresses (high osmolarity or temperature, a change in pH, decreased aeration, etc.) increases in the stationary phase [21, 23]. Both on solid and liquid media, a culture usually enters the stationary phase after reaching a considerable cell number. The cells sense the increase in their population density as a signal to prepare for the transition to the stationary phase. This transition is controlled by the key transcriptional factors, whose formation is connected with starvation for amino acids or sources of carbon, nitrogen, or phosphorus or with the effects that slow down the growth rate through the activation of the synthesis of guanosine-3',5'-bisdiphosphate (ppGpp), a global cell metabolism regulator [24–26]. Such factors include the alternative RNA polymerase subunit, which recognizes promoters of certain genes (σ^S subunit) [21–23], and the Lrp protein [27]. It was shown for *P. aeruginosa* that ppGpp directly activates the two QS systems of this bacterium [26].

Recently, papers have been published linking QS with the transport of compounds from the cell [28–31]. Although the translocation of homoserine lactones through the cytoplasmic membrane may occur merely as a result of nonfacilitated diffusion, some of the efflux systems that confer upon cells multiple drug resistance may also export these signal compounds [28, 29]. The release of *N*-(3-oxododecanoyl)-homoserine lactone from *P. aeruginosa* cells through the activation of the MexAB–OprM efflux system significantly decreased its synthesis and accumulation in the cells and diminished the QS-dependent formation of extracellular virulence factors [28]. MexEF–OprN is another efflux system. It is capable of exporting *N*-butyryl-homoserine lactone and HAQ. With its activation, the formation of virulence factors also decreases, and, in addition, the resistance of bacteria to β-lactam antibiotics decreases as well [30]. This is connected with the fact that *N*-butyryl-homoserine lactone induces the QS-dependent expression of the MexAB–OprM system, which drives these antibiotics out of the cells and ensures resistance to them [31].

The cells of *E. coli* are not capable of synthesizing AHLs. However, they possess the SdiA protein, which is homologous to the transcriptional regulators of the bacteria interacting with AHLs. It was shown that this regulator is able to activate the AcrAB–TolC efflux system homologous to the MexAB–OprM system of *P. aeruginosa* [32]. In turn, not only does AcrAB

Table 1. Effect of increased expression or inactivation of the *rhtA*, *rhtB*, and *leuE* genes on the resistance of *E. coli* strains to threonine, homoserine, and homoserine lactones

E. coli strain	Minimal inhibiting concentration (mg/ml)				
	L-threonine	*L*-serine	*L*-homoserine	*L*-homoserine lactone hydrochloride	α-amino-γ-butyrolactone hydrobromide
N99 (control)	30	5	0.75	0.5	1
N99 *rhtA23* (*rhtA* overexpression)	100	40	>100	10	10
N99 (pNPZ16) (*rhtA* overexpression)	70	20	50	10	10
N99 *rhtA*::*kan* (*rhtA* inactivation)	20	5	0.75	0.5	1
N99 (pNZ42) (*rhtB* overexpression)	50	15	40	5	ND[a]
N99 *rhtB*::*cat* (*rhtB* inactivation)	30	5	0.25	0.25	ND[a]
leuE overexpression	50	ND[a]	3	+[b]	ND[a]
leuE inactivation	30	ND[a]	0.75[b]	–[b]	ND[a]

[a] ND stands for "not determined".

[b] According to [36], overexpression of *leuE* increases the resistance of *E. coli* to L-threonine, L-homoserine, and L-homoserine lactone, and in activation of *leuE* decreases its resistance to L-threonine and L-homoserine lactone, but not to L-homoserine.

ensure multiple drug resistance and, in particular, increase the resistance of bacteria to quinolone derivatives, but it also seems to be involved in the efflux of signal molecules from cells [32]. Thus, the efflux processes play an active role in intercellular interactions.

As has already been mentioned, genes have recently been revealed that encode proteins involved in the efflux of amino acids and purines from *E. coli* cells. The biological meaning of the existence of systems responsible for the efflux of these valuable metabolites from cells is unclear. One of the possible functions of these systems is the involvement in the processes related to intercellular interactions and cell adaptation to the stationary phase conditions and stresses.

THE RhtA AND RhtB PROTEINS INVOLVED IN THE EFFLUX OF THREONINE AND HOMOSERINE MAY ALSO EXPORT HOMOSERINE LACTONES

An increase in the level of expression of a number of genes encoding membrane proteins involved in efflux processes enhances the resistance of bacteria to certain amino acids and their analogues [5, 8], as well as to purine derivatives and their analogues [12–14]. It has been established that representatives of the DMT and RhtB/LysE protein families may be involved in the efflux of several different metabolites. Thus, for example, the RhtA, RhtB, and RhtC proteins export from the cells threonine and its metabolic precursor homoserine, as well as some other amino acids and their analogues [5, 7, 8]; YdeD (EamA) exports cysteine, *O*-acetylserine, glutamine, and asparagine [6]; YfiK (EamB) exports proline [7], as well as cysteine and *O*-acetylserine [33]; YeaS (LeuE) exports leucine, methionine, histidine, and some other amino acids [11]; YggA (ArgO) exports lysine and arginine, like its orthologue LysE from *Corynebacterium glutamicum* [34]. YddG is involved in the efflux of aromatic amino acids [9]; YedA, of several different amino acids [10]. Thus, these proteins have a wide, partially overlapping, specificity and resemble the MDR transporters in this respect. However, as distinct from the latter, an increased expression of the amino acid transporters does not noticeably influence resistance to most antibiotics.

Along with this, it turned out that the overexpression of *rhtA*, *rhtB*, and *leuE* increases the resistance of *E. coli* cells not only to amino acids but also to L-homoserine lactone and α-amino-γ-butyrolactone (Table 1). These compounds are not amino acid precursors, but their derivatives are signal substances inducing QS in many gram-negative bacteria. The existence of QS in *E. coli* is beyond question [37–39]. Although, as mentioned above, these bacteria are not capable of AHL synthesis on their own, such QS regulation (at least that involving AI-2) does operate in them [39]. Along with this, it has recently been shown that synthetic AHLs are also able to modulate the level of expression of many genes in *E. coli* by interacting with the transcriptional regulator SdiA [40]. Obviously, the RhtA and RhtB proteins, which export homoserine lactones from the cell, are able to take part in the processes related to intercellular interactions. Unfortunately, nothing can be said about the involvement of these proteins in the export of AI-2, although their wide specificity does not exclude such a possibility.

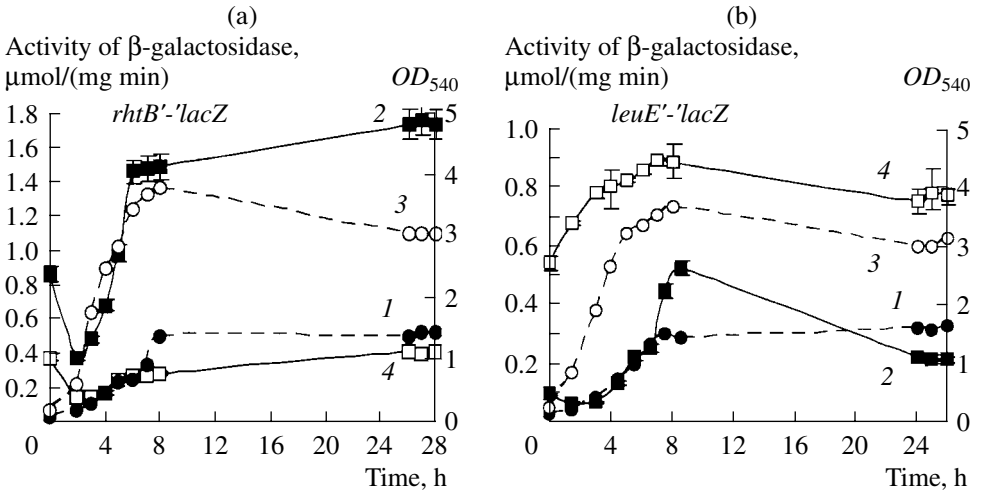

Fig. 1. Dependence of the expression of the genes (a) *rhtB'–lacZ'* and (b) *leuE'–lacZ'* on the culture growth phase and composition of the cultivation medium: (*1*) optical density (OD_{540}) in minimal (M9) medium; (*2*) activity of β-galactosidase in M9 medium; (*3*) OD_{540} in complete (LB) medium; (*4*) activity of β-galactosidase in LB medium.

THE EXPRESSION OF THE DMT AND RhtB/LysE GENES DEPENDS ON THE GROWTH PHASE AND IS INDUCED UNDER STRESSES

Only a small number of the genes encoding the RhtB proteins are expressed constitutively. The only gene with a constitutive expression revealed, *cmaU* [41], is located in the cluster of genes involved in the biosynthesis of ethylcyclopropyl amino acid (2-ethyl-1-aminocyclopropane-1-carboxyl), a biosynthetic precursor of coronatine, a *Pseudomonas syringae* virulence factor that is a mimetic of growth phytohormones such as methyl jasminic acid. The substrate of the predicted CmaU protein is unknown. The activity of all of the other known genes encoding the DMT and RhtB/LysE proteins is regulated. Among these, two groups significantly differing in the mode of regulation can be distinguished.

Group I includes the genes whose activity is regulated by the exported substrate or by chemically similar compounds. This regulation is mediated by the LysR-type transcriptional regulators, e.g., the LysG protein in the case of the *lysE* gene of *C. glutamicum* [34] and the ArgP protein in the case of the *yggA* (*argO*) gene of *Escherichia coli* [35]. Binding of these proteins to operator DNA depends on allosteric interaction with the inducer molecule.

Group 2 genes do not depend on the presence of substrates for their expression. At least, we did not succeed in revealing the induction of these genes by amino acids transported by the proteins encoded by them. Thus, the *rhtA* expression is not induced by either threonine or homoserine, exported by protein RhtA. However, the expression of group 2 genes is induced by signal molecules or factors triggering cell response to different stresses. For example, the *rhtA* (*ybiF*) gene is markedly induced by exogenous AI-2 [39]. The *rhtA* expression is increased in the logarithmic growth phase [8], i.e., simultaneously with the synthesis of AI-2 in the cells [38]. The PA0239 and PA0485 genes, distant *rhtA* homologues, and PA2306, an orthologue of *P. aeruginosa yfiK*, are induced by homoserine lactones [42, 43].

In many cases, the factors triggering the expression of these genes at the molecular level remain unrevealed, but their activation under different stresses is observed. Experiments on translational fusion of the regulatory regions of the RhtB genes with the structural part of the *lacZ* marker gene showed their activation upon cell transition to the stationary growth phase (Fig. 1a). A similar pattern is observed in the case of the *mexAB* genes from *P. aeruginosa*, whose expression is regulated by QS [30]. Moreover, the expression of many genes from the RhtB family increases during growth in the minimal M9 medium where the cells are deprived of nutrients (Fig. 1a), under osmotic shock (Figs. 2a–2c), at low pH (Fig. 3), and under oxygen deficiency (data not shown). At first sight, it seems especially paradoxical that the amino acid efflux system is activated during starvation. Under stresses, not only the RhtB genes are activated but also those of the DMT family (Fig. 2d).

As mentioned above, the transition of cells to the stationary phase is regulated by global regulators which modify the expression of a large number of genes in a coordinated manner. The RNA polymerase σ^S subunit is one of these global regulators. However, we did not find any substantial difference in the expression of the RhtB genes in the wild-type and *rpoS*-mutant strains (data not shown). Another global regulator is the Lrp protein, which controls gene expression upon transition from rich to depleted medium and upon the reverse transition [27, 44]. According to our data, the expression of gene *rhtB* (Fig. 4a), as well as genes *rhtC*, *yfiK*,

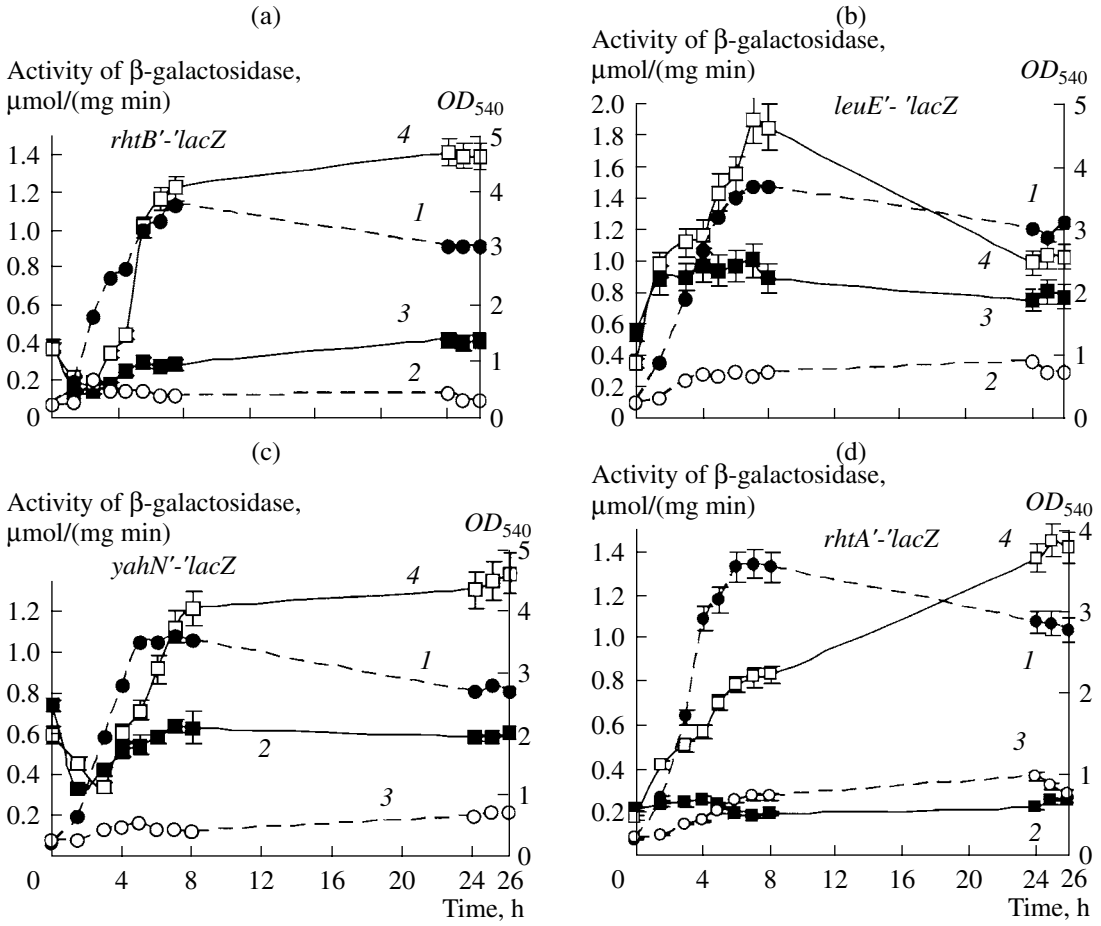

Fig. 2. Effect of osmotic stress on the expression level of the genes (a) *rhtB*, (b) *leuE*, (c) *yahN*, and (d) *rhtA*: (*1*) OD_{540} in LB medium; (*2*) activity of β-galactosidase in LB medium; (*3*) OD_{540} in LB medium supplemented with 0.6 M NaCl; (*4*) activity of β-galactosidase in LB medium supplemented with 0.6 M NaCl.

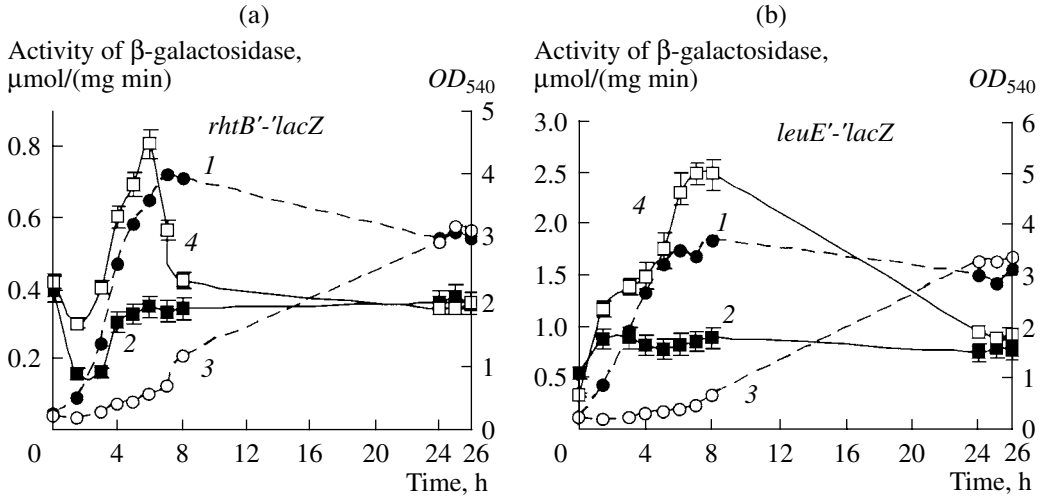

Fig. 3. Effect of low pH on the expression level of the genes (a) *rhtB* and (b) *leuE* during cultivation in complete LB medium: (*1*) OD_{540} in LB medium; (*2*) activity of β-galactosidase in LB medium; (*3*) OD_{540} in LB medium with pH 4.2; (*4*) activity of β-galactosidase in LB medium with pH 4.2.

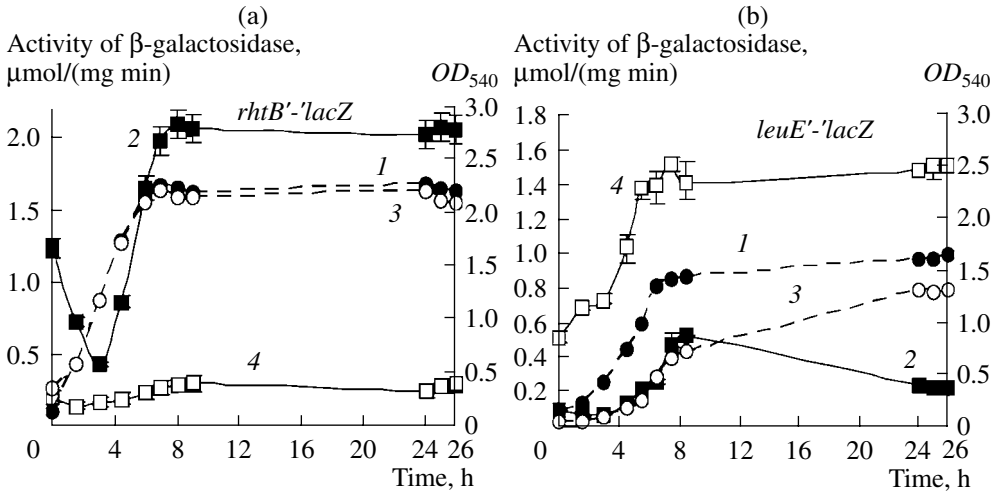

Fig. 4. Effect of the *lrp* gene inactivation on the expression level of the genes (a) *rhtB* and (b) *leuE* during cultivation in the minimal M9 medium: (*1*) OD_{540} of the *lrp*+ strain; (*2*) activity of β-galactosidase of the *lrp*+ strain; (*3*) OD_{540} of the Δ*lrp* strain; (*4*) activity of β-galactosidase of the Δ*lrp* strain.

and *yahN*, is activated by Lrp: in an *E. coli* strain with an *lrp* deletion, the expression of these genes either did not occur at all or was maintained at a very low level.

The *leuE* gene occupies a special place in the proposed classification. On the one hand, it could be assigned to the first group because its expression is activated by leucine, the exported substrate [11]. On the other hand, *leuE* expression is suppressed by the Lrp global regulator (Fig. 4b). Thus, the character of *leuE* regulation can be considered intermediate. This can be explained by the fact that leucine, the most common amino acid occurring in proteins, is also used by bacteria as a signal molecule. Leucine, by binding directly to Lrp, triggers the activation or repression of the Lrp regulon genes. In relation to *leuE*, the Lrp protein may be considered as a negative regulator whose functions could, in principle, be fulfilled by a specialized LysR-type repressor. The *E. chrysanthemi pecM* gene (the DMT family) may be assigned to the same intermediate group: it is controlled by the MarR/EmrR-type translational regulator PecS, which also influences the expression of many other genes [15] and, in turn, may be regulated by a large number of different substrates.

A question arises as to which regulation type, type 1 or type 2, was the initial one for the RhtB/LysE genes. Type 2 is mainly inherent in the RhtB subfamily, while type 1 is typical of the LysE subfamily. However, the RhtB family should be considered as the initial one: it is more diverse and is represented in a wider taxonomic spectrum. Archaea have genes that may be attributed to this subfamily and lack genes of the LysE subfamily. This indirectly testifies in favor of the primary nature of the regulation that is characteristic of RhtB; later in the course of evolution, depending on specific physiological needs, it may have been replaced by regulation dependent on one or another substrate, as in the case of LysE. The expression of *leuE* is regulated by the substrate [11], does not depend on the growth phase (Fig. 1b) and starvation, but retains its relict elements: it responds to high osmotic strength (Fig. 2b) and acidification of the medium (Fig. 3b).

The facts concerning the substrate specificity and the modes of regulation of the DMT and RhtB genes agree with the hypothesis about their role in cell response to stresses and their possible involvement in the transduction of chemical signals between cells [5]. The possibility of specialization of certain proteins of the family in the performance of more restricted functions does not contradict this hypothesis. For example, ArgO is endowed with the role of the factor of bacterial resistance to the phytotoxin canavanine, an antimetabolic arginine mimetic [35]. In the case of *Corynebacterium*, incapable of catabolizing lysine and arginine, the efflux of excessive amounts of these amino acids by LysE is necessary for maintaining the intracellular balance of amino acids when growth occurs on peptide-rich substrates [34]. The diverse applications of the export systems are determined by their initial polyfunctional nature. Responding to unfavorable conditions by transporting various organic compounds out of the cell, they provide cells with multiple drug resistance. In addition, by exporting metabolites, including short-lived intermediate products of biosynthesis, they enrich the medium with biogenic substances, which may act as chemical signals evidencing the density and physiological state of the cell population (provided that the signal is recognized). One of the directions of specialization of the efflux systems is the recognition and selective efflux of the most common toxins (a probable example is ArgO). Another direction of specialization is the enhancement of the communicative function of such systems. What may be expected in this case is a gradual selection from the metabolite pool of certain compounds that assume the role of signals, the develop-

ment of receptors to them, and specialization of the transport proteins for these substrates. Obviously, homoserine, an intermediate product in the biosynthesis of three amino acids—threonine, methionine, and isoleucine—may play the role of both an intracellular metabolic regulator and extracellular regulator. It should be noted that the presence in the medium of millimolar concentrations of homoserine impairs amino acid metabolism. Chemically related substances (acylhomoserine lactones, biogenic amines, regulatory peptides) are not intermediate products of amino acid synthesis. These specialized substances of intercellular communication may synthesized by enzyme systems independent of basal metabolism. But even such signal transmitters as the mediators of the nervous system of multicellular organisms retain functions not connected with synaptic transmission. For example, glutamic acid and glycine are involved in the synthesis of proteins (although γ-aminobutyric acid is a specialized neurotransmitter); acetylcholine, adrenaline, dopamine, noradrenaline, and serotonin are involved in regulating blastomere division and cell motility in embryogenesis and, apparently, in the process of fertilization [45, 46], as well as in the regulation of division in unicellular eukaryotes. There is no doubt that the most complex intercellular communications in neuronal networks originate from the transport of unspecialized metabolic intermediates by low-specialized systems of export of organic compounds, already developed in bacteria [47].

THE FUNCTIONS OF DMT PROTEINS IN EUKARYOTIC CELLS

RhtB homologues in eukaryotes have been revealed in the chloroplast and mitochondrial DNA of plants as constituents of more extensive open reading frames. Their functions are unknown, and they are not helpful in clarifying the functions of their bacterial homologues. On the contrary, DMT proteins are widespread in eukaryotes. Among them, the transporters of carbohydrates in the form of nucleoside phosphate sugars [48] or hexosephosphates and triosephosphates [49] are the best studied. They use nucleoside monophosphates and phosphate, respectively, as a counterion. Nucleoside phosphate sugars are transported from the cytosol into the Golgi apparatus (GA). Morphologically, the GA may be considered as a part of the external environment, so the analogy with bacterial RhtA and YdeD homologues, which excrete metabolites out of the cells, is evident. In the GA, the sugars activated by nucleoside phosphate are used in the glycosylation of macromolecules, which are later exposed on the cell surface to be involved in the intercellular interactions mediated by the Wnt/Wingless, Hedgehog, and Notch signal pathways [50–52]. Mutations in the genes responsible for the transport to the GA lead to the impairment of intercellular interactions and, consequently, to the development of embryonic malformations (shown for humans and the nematode worm *Caenorhabditis elegans*), impaired adhesion to the host cells of the cells of *Leishmania*, the causative agent of visceral leishmaniasis kala-azar [53], etc.

The other DMT functional class affords an exchange of metabolites between the plastid—an intracellular endosymbiont of bacterial origin—and the host component of the eukaryotic cell [48]. Formally speaking, this exchange is not a signal because it provides for massive transfer of metabolites (photosynthesis products) between the compartments; however, it is controlled by the plant cell general regulatory systems, which depend on the intensity of illumination and other general factors. Additionally, it has been shown [54] that the inactivation of the glucose-6-phosphate transporter gene, responsible for carbon import by starch-containing plastids in the nonphotosynthesizing parts of the plant, results in impairments of pollen and embryo sac development; i.e., it exerts a regulatory effect on the development, and this effect is not directly connected with the glucose-6-phosphate transport into plastids.

PURINE DERIVATIVES AS REGULATORY MOLECULES AND THEIR EXPORT FROM THE CELL

As we have seen, some eukaryotic DMT proteins transport nucleotide-activated carbohydrates through the membrane, using nucleotides as counterions. These processes are important for glycosylation and, in the final analysis, for correct intercellular interactions. We performed a search for the genes involved in the efflux of purine derivatives from *E. coli* cells. As a result, a number of genes encoding membrane proteins were cloned; upon amplification, these genes ensured resistance of *E. coli* to the purine base analogues [12–14].

Among the genes that we identified by using this approach were genes encoding the DMT superfamily proteins, including the already familiar proteins RhtA, YdeD, and YedA, involved in the efflux of amino acids (Table 2). Subsequent studies showed that these proteins are involved in the efflux of purine bases and nucleosides ([12–14] and unpublished data). Thus, the specificity of these proteins appeared to be very broad: they are able to transport not only a number of diverse amino acids but also purine derivatives.

In addition, the resistance to purine analogues was afforded by overexpression of the YicM, YdhC, and YdeAB proteins, belonging to the MFS superfamily (Table 2); in the latter case, this phenotype was observed only in the presence of both proteins, YdeA and YdeB. The *yicM* gene was studied by us more thoroughly. It encodes the YicM (NepI) protein, which transports purine ribonucleosides from *E. coli* cells [55]. It turned out that the activity of *yicM* does not depend on the substrate transported; this gene is activated in the stationary growth phase, like many genes

Table 2. Effect of increased expression of the *rhtA*, *ydeD*, *yijE*, *yedA*, *yicM*, *ydhC*, *ydeAB,* and *ydhE* genes on the resistance of *E. coli* strains to purine base analogues

Purine base analogue, mg/l	Growth of *E. coli* TG1 and its derivatives with overexpression of a gene encoding a protein of a superfamily (family)								
	No	DMT				MFS			MATE
		rhtA	*ydeD*	*yijE*	*yedA*	*yicM*	*ydhC*	*ydeAB*	*ydhE*
No	+	+	+	+	+	+	+	+	+
Purine, 80	–	–	–	+	±	+	–	–	+
6-Methylpurine, 20	–	–	–	±	±	±	–	–	+
8-Azaadenine, 150	–	+	+	+	+	+	–	+	+
2,6-Diaminopurine, 200	–	–	–	–	+	+	–	–	+
6-Mercaptopurine, 150	–	±	–	+	–	+	+	–	+
6-Thioguanine, 300	–	±	–	+	–	+	+	–	+

Note: "+", good growth; "±", poor growth; "–", no growth.

of the RhtB family. This was shown in experiments on *yicM'–'lacZ* translational fusion.

The resistance to many purine base analogues also increases upon amplification of the *ydhE* (*norE*) gene (Table 2), which encodes a MATE family membrane protein involved in the efflux of quinolone derivatives from cells [56]. Recently, data were obtained suggesting that this protein exports signal compounds involved in intercellular communication [57]. During overexpression of the *ydhE* (*norE*) gene, a premature induction of the RNA polymeraseσ^S subunit synthesis was observed; the biomass yield of the culture decreased; and the cell-free conditioned medium(CM) from cells overexpressing *ydhE* (*norE*) repressed cell growth stronger than CM from the wild-type cells. Obviously, this was connected with increased accumulation in the medium of a signal compound mediating QS. The authors suggest that the transport of the signal substances involved in intercellular communication is a natural function of the YdhE (NorE) protein, as well as of the MDR-type AcrAB/TolC transport system [57].

Purine derivatives are well known as signal molecules in prokaryotes and eukaryotes. Cyclic adenosine-5'-phosphate (cAMP) plays an important role in the phenomenon of catabolite repression in bacteria [58] and in intercellular signaling in eukaryotes [59]. ATP and adenosine, along with uridine-5'-phosphate and uridine, serve as signals for intercellular interactions in humans [60] and, evidently, other multicellular organisms. Recently, another purine-based signal compound—bis-(3',5')-cyclic dimeric guanosine monophosphate—was revealed. This compound regulates the preparation of cells for cell-to-cell interaction during cell aggregation and biofilm formation and may play the role of a signal in intercellular interactions [61, 62].

In order to perform the functions of signal compounds that induce coordinated responses of a cell population, all these purine derivatives must be transported from the cell. The transporters revealed by us may play a role in this translocation.

Evidently, the interaction of bacterial cells in a population includes not only QS but a wider range of phenomena and not only autoinducers but a wider spectrum of signals. The metabolites accumulated in the medium during culture growth may also play the role of such signals. Thus, induction of the σ^S subunit of RNA polymerase under the action of acetate was shown [21]; this metabolite often accumulates in the medium during the growth of bacteria. It should be noted that this induction may also occur at neutral or close-to-neutral pH values of the medium [63]. Ribosome degradation and, as a consequence, accumulation in the medium of purine and pyrimidine derivatives is known to occur when cells lack nutrients and protein synthesis therefore slows down [64]. These compounds might also serve as a signal for coordinated responses of a cell population.

Thus, there exists direct evidence of the involvement of DMT transporter proteins in the peripheral part of the most important systems of intercellular communication in animals and plants and a significant body of indirect evidence of the participation of their bacterial homologues, as well as of a number of RhtB proteins, in global regulation, where they occupy not a central but, apparently, a fairly important place.

ACKNOWLEDGMENTS

V.V.A. acknowledges the support of the Russian Foundation for Basic Research and the Kaluga oblast administration (project nos. 04-04-97245-r2004 naukograd and 06-04-49288).

REFERENCES

1. Jack, D.L., Yang, N.M., and Saier, M.H., The Drug/Metabolite Transporter Superfamily, *Eur. J. Biochem.*, 2001, vol. 268, no. 13, pp. 3620–3639.
2. Aleshin, V.V., Zakataeva, N.P., and Livshits, V.A., A New Family of Amino Acid Efflux Proteins, *Trends Biochem. Sci.*, 1999, vol. 24, pp. 133–135.
3. Vrljić, M., Garg, J., Bellmann, A., Wachi, S., Freudl, R., Malecki, M.J., Sahm, H., Kozina, V.J., Eggeling, L., and Saier, M.H.., The LysE Superfamily: Topology of the Lysine Exporter LysE of *Corynebacterium glutamicum*, a Paradigm for a Novel Superfamily of Transmembrane Solute Translocators, *J. Mol. Microbiol. Biotechnol*, 1999, vol. 1, no. 2, pp. 327–336.
4. Vrljić, M., Sahm, H.., and Eggeling, L., A New Type of Transporter with a New Type of Cellular Function: L-Lysine export from *Corynebacterium glutamicum, Mol. Microbiol.*, 1996, vol. 22, pp. 815–826.
5. Zakataeva, N.P., Aleshin, V.V., Tokmakova, I.L., Troshin, P.V., and Livshits, V.A., The Novel Transmembrane *Escherichia coli* Proteins Involved in the Amino Acid Efflux, *FEBS Lett.*, 1999, vol. 452, no. 3, pp. 228–232.
6. Daßler, T., Maier, T., Winterhalter, C., and Böck, A., Identification of a Major Facilitator Protein from *Escherichia coli* Involved in Efflux of Metabolites of the Cysteine Pathway, *Mol. Microbiol.*, 2000, vol. 36, pp. 1101–1112.
7. Livshits, V.A., Zakataeva, N.P., Nakanishi, K., Aleshin, V.V., Troshin, P.V., and Tokmakova, I.L., An *Escherichia coli* DNA Fragment Determining Increased Production of *L*-Amino Acids (Variants); a Method for Obtaining *L*-Amino Acids, RF Patent no. 2175351, October 2001.
8. Livshits, V.A., Zakataeva, N.P., Aleshin, V.V., and Vitushkina, M.V., Identification and Characterization of the New Gene *rhtA* Involved in Threonine and Homoserine Efflux in *Escherichia coli, Res. Microbiol.*, 2003, vol. 154, pp. 123–135.
9. Livshits, V.A., Vitushkina, M.V., Mashko, S.V., Doroshenko, V.G., Biryukova, I.V., Katashkina, Zh.I., Skorokhodova, A.Yu., and Belareva, A.V., A Method for Obtaining *L*-Amino Acids; an *Escherichia coli* Strain Producing *L*-Amino Acids (Variants), RF Patent no. 2222596, January 2004.
10. Livshits, V.A., Vitushkina, M.V., Gusyatiner, M.M., Ziyatdinov, M.Kh., Akhverdyan, V.Z., Savrasova, E.A., Doroshenko, V.G., and Mashko, S.V., A Method for Obtaining L-Amino Acids; an *Escherichia coli* Strain Producing L-Amino Acids (Variants), RF Patent no. 2229513, May 2004.
11. Kutukova, E.A., Livshits, V.A., Altman, I.P., Ptitsyn, L.R., Zyiatdinov, M.H., Tokmakova, I.L., and Zakataeva, N.P., The *yeaS* (*leuE*) Gene of *Escherichia coli* Encodes an Exporter of Leucine, and the Lrp Protein Regulates Its Expression, *FEBS Lett.*, 2005, vol. 579, pp. 4629–4634.
12. Livshits, V.A., Zakataeva, N.P., Gronskiy, S.V., Vitushkina, M.V., and Novikova, A.E., A Method for Obtaining Purine Nucleosides and Nucleotides; a Strain Producing Purine Nucleosides (Variants), RF Patent no. 2239656, November 2004.
13. Zakataeva, N.P., Livshits, V.A., and Gronskiy, S.V., A Method for Obtaining Purine Inosine and 5'-Inosinic Acid; an *Escherichia coli* Strain Producing Inosine, RF Patent no. 2244003, January 2005.
14. Gronskiy, S.V., Zakataeva, N.P., and Livshits, V.A., A Method for Obtaining Purine Inosine and 5'-Inosinic Acid; an *Escherichia coli* Strain Producing Inosine, RF Patent no. 2244004, January 2005.
15. Praillet, T., Reverchon, S., Robert-Baudouy, J., and Nasser, W., The PecM Protein Is Necessary for the DNA-Binding Capacity of the PecS Repressor, One of the Regulators of Virulence-Factor Synthesis in *Erwinia chrysanthemi, FEMS Microbiol. Lett.*, 1997, vol. 154, no. 2, pp. 265–270.
16. Reverchon, S., Rouanet, C., Expert, D., and Nasser, W., Characterization of Indigoidine Biosynthetic Genes in *Erwinia chrysanthemi* and Role of This Blue Pigment in Pathogenicity, *J. Bacteriol.*, 2002, vol. 184, no. 3, pp. 654–665.
17. Zavilgel'sky, G.B. and Manukhov, I.V., Quorum Sensing, or How Bacteria "Talk" to Each Other, *Mol. Biol.* 2001, vol. 35, no. 2, pp. 268-277 [*Mol. Biol.* (Engl. Transl.), vol. 35, no. 2, pp. 224-232].
18. Visick, K.L. and Fugua, C., Decoding Microbial Chatter: Cell–Cell Communication in Bacteria, *J. Bacteriol.*, 2005, vol. 187, no. 16, pp. 5507–5519.
19. Kaper, J.B. and Sperandio, V., Bacterial Cell-to-Cell Signaling in the Gastrointestinal Tract, *Infect. Immun.*, 2005, vol. 73, no. 6, pp. 3197–3209.
20. Kaplan, H.B. and Plamann, L., A *Myxococcus xanthus* Cell Density–Sensing System Required for Multicellular Development, *FEMS Microbiol. Lett.*, 1996, vol. 139, nos. 2–3, pp. 89–95.
21. Hengge-Aronis, R., Signal Transduction and Regulatory Mechanisms Involved in Control of the σ^S (RpoS) Subunit of RNA Polymerase, *Microbiol. Mol. Biol. Rev.*, 2002, vol. 66, no. 3, pp. 373–395.
22. Venturi, V., Control of *rpoS* Transcription in *Escherichia coli* and *Pseudomonas*: Why So Different?, *Mol. Microbiol.*, 2003, vol. 49, no. 1, pp. 1–9.
23. Khmel, I.A., Regulation of Expression of Bacterial Genes in the Absence of Active Cell Growth, *Genetika*, 2005, vol. 41, no. 9, pp. 1183–1202 [*Russ. J. Genet.* (Engl. Transl.), vol. 41, no. 9, pp. 968-984].
24. Magnusson, L.U., Farewell, A., and Nystrom, T., ppGpp: A Global Regulator in *Escherichia coli, Trends Microbiol.*, 2005, vol. 13, no. 5, pp. 236–242.
25. Braeken, K., Moris, M., Daniels, R., Vanderleyden, J., and Michiels, J., New Horizons for (p)ppGpp in Bacterial and Plant Physiology, *Trends Microbiol.*, 2006, vol. 14, no. 1, pp. 45–54.
26. van Delden, C., Comte, R., and Bally, A.M., Stringent Response Activates Quorum Sensing and Modulates Cell Density-Dependent Gene Expression in *Pseudomonas aeruginosa, J. Bacteriol.*, 2001, vol. 183, no. 18, pp. 5376–5384.
27. Calvo, J.M. and Matthews, R.G., The Leucine-Responsive Regulatory Protein, a Global Regulator of Metabolism in *Escherichia coli, Microbiol. Rev.*, 1994, vol. 58, no. 3, pp. 466–490.
28. Evans, K., Passador, L., Srikumar, R., Tsang, E., Nezelzon, J., and Poole, K., Influence of the MexAB–OprM

Multidrug Efflux System on Quorum Sensing in *Pseudomonas aeruginosa, J. Bacteriol.*, 1998, vol. 180, pp. 5443–5447.

29. Pearson, J.P., van Delden, C., and Iglewski, B.H., Active Efflux and Diffusion Are Involved in Transport of *Pseudomonas aeruginosa* Cell-to-Cell Signals, *J. Bacteriol.*, 1999, vol. 181, no. 4, pp. 1203–1210.
30. Maseda, H., Sawada, I., Saito, K., Uchiyama, H., Nakae, T., and Nomura, N., Enhancement of the MexAB–OprM Efflux Pump Expression by a Quorum-Sensing Autoinducer and Its Cancellation by a Regulator, MexT, of the *mexEF–oprN* Efflux Pump Operon in *Pseudomonas aeruginosa, Antimicrob. Agents Chemother.*, 2004, vol. 48, no. 4, pp. 1320–1328.
31. Sawada, I., Maseda, H., Nakae, T., Uchiyama, H., Nomura, N., A Quorum-Sensing Autoinducer Enhances the *mexAB–oprM* Efflux-Pump Expression without the MexR-Mediated Regulation in *Pseudomonas aeruginosa, Microbiol. Immunol.*, 2004, vol. 48, no. 5, pp. 435–439.
32. Rahmati, S., Yang, S., Davidson, A.L., and Zechiedrich, E.L., Control of the AcrAB Multidrug Efflux Pump by Quorum-Sensing Regulator SdiA, *Mol. Microbiol.*, 2002, vol. 43, no. 3, pp. 677–685.
33. Franke, I., Resch, A., Daler, T., Maier, T., and Bock, A., YfiK from *Escherichia coli* Promotes Export of *O*-Acetylserine and Cysteine, *J. Bacteriol.*, 2003, vol. 185, no. 4, pp. 1161–1166.
34. Bellmann, A., Vrljic, M., Patek, M., Sahm, H., Kramer, R., and Eggeling, L., Expression Control and Specificity of the Basic Amino Acid Exporter LysE of *Corynebacterium glutamicum, Arch. Microbiol.*, 2001, vol. 147, no. 7, pp. 1765–1774.
35. Nandieni, R. and Gowrishankar, J., Evidence for an Arginine Exporter Encoded by *yggA* (a*rgO*) That Is Regulated by the LysR-Type Transcriptional Regulator ArgP in *Escherichia coli, J. Bacteriol.*, 2004, vol. 186, no. 11, pp. 3539–3546.
36. Eggeling, L. and Sahm, H., New Ubiquitous Translocators: Amino Acid Export by *Corynebacterium glutamicum* and *Escherichia coli, Arch. Microbiol.,* 2003, vol. 180, no. 3, pp. 155–160.
37. Surette, M.G., Miller, M.B., and Bassler, B.L., Quorum Sensing in *Escherichia coli, Salmonella typhimurium*, and *Vibrio harveyi*: A New Family of Genes Responsible for Autoinducer Production, *Proc. Natl. Acad. Sci. USA*, 1999, vol. 96, no. 4, pp. 1639–1644.
38. Ahmer, B.M.M., Cell-to-Cell Signaling in *Escherichia coli* and *Salmonella enterica, Mol. Microbiol.*, 2004, vol. 52, no. 4, pp. 933–945.
39. DeLisa, M.P., Wu, C.-F., Wang, L., Valdes, J.J., and Bentley, W.E., DNA Microarray–Based Identification of Genes Controlled by Autoinducer 2–Stimulated Quorum Sensing in *Escherichia coli, J. Bacteriol.*, 2001, vol. 183, no. 18, pp. 5239–5247.
40. van Houdt, R., Aertsen, A., Moons, P., Vanoirbeek, K., and Michiels, C.W., *N*-Acyl-*L*-Homoserine Lactone Signal Interception by *Escherichia coli, FEMS Microbiol. Lett.,* 2006, vol. 256.
41. Ullrich, M. and Bender, C.L., The Biosynthetic Gene Cluster for Coronamic Acid, an Ethylcyclopropyl Amino Acid, Contains Genes Homologous to Amino Acid–Activating Enzymes and Thioesterases, *J. Bacteriol.*, 1994, vol. 176, no. 24, pp. 7574–7586.
42. Schuster, M., Lostroh, C.P., Ogi, T., and Greenberg, E.P., Identification, Timing, and Signal Specificity of *Pseudomonas aeruginosa* Quorum-Controlled Genes: A Transcriptome Analysis, *J. Bacteriol.*, 2003, vol. 185, no. 7, pp. 2066–2079.
43. Wagner, V.E., Bushnell, D., Passador, L., Brooks, A.I., and Iglewski, B.H., Microarray Analysis of *Pseudomonas aeruginosa* Quorum-Sensing Regulons: Effects of Growth Phase and Environment, *J. Bacteriol.*, 2003, vol. 185, no. 7, pp. 2080–2095.
44. Tani, T.H., Khodursky, A., Blumenthal, R.M., Brown, P.O., and Matthews, R.G., Adaptation to Famine: A Family of Stationary-Phase Genes Revealed by Microarray Analysis, *Proc. Natl. Acad. Sci. USA*, 2002, vol. 99, no. 21, pp. 13471–13476.
45. Buznikov, G.A. and Turpaev, T.M., Intracellular Functions of Neurotransmitters: Phylogenetic and Ontogenetic Aspects, *Zh. Evol. Biokhim. Fiziol.*, 1987, vol. 23, no. 4, pp. 423–432.
46. Buznikov, G.A., Shmukler, Y.B., and Lauder, J.M., Changes in the Physiological Roles of Neurotransmitters during Individual Development, *Neurosci. Behav. Physiol.*, 1999, vol. 29, no. 1, pp. 11–21.
47. Oleskin, A.V. and Kirovskaya, T.A., Research on Population Organization and Communication in Microorganisms, *Mikrobiologiya*, 2006, vol. 75, no. 4 [*Microbiology* (Engl. Transl.), vol. 75, no. 4].
48. Berninsone, P.M. and Hirschberg, C.B., Nucleotide Sugar Transporters of the Golgi Apparatus, *Curr. Opin. Struct. Biol.*, 2000, vol. 10, no. 5, pp. 542–547.
49. Knappe, S., Flugge, U.-I., and Fischer, K., Analysis of the Plastidic Phosphate Translocator Gene Family in *Arabidopsis* and Identification of New Phosphate Translocator-Homologous Transporters, Classified by Their Putative Substrate-Binding Site, *Plant Physiol.*, 2003, vol. 131, no. 3, pp. 1178–1190.
50. Hirschberg, C.B., Golgi Nucleotide Sugar Transport and Leukocyte Adhesion Deficiency II, *J. Clin. Invest.,* 2001, vol. 108, no. 1, pp. 3–6.
51. Hwang, H.Y. and Horvitz, H.R., The *SQV-1* UDP-Glucuronic Acid Decarboxylase and the *SQV-7* Nucleotide-Sugar Transporter May Act in the Golgi Apparatus To Affect *Caenorhabditis elegans* Vulval Morphogenesis and Embryonic Development, *Proc. Natl. Acad. Sci. USA*, 2002, vol. 99, no. 22, pp. 14218–14223.
52. Ishikawa, H.O., Higashi, S., Ayukawa, T., Sasamura, T., Kitagawa, M., Harigaya, K., Aoki, K., Ishida, N., Sanai, Y., and Matsuno, K., Notch Deficiency Implicated in the Pathogenesis of Congenital Disorder of Glycosylation IIc, *Proc. Natl. Acad. Sci. USA*, 2005, vol. 102, no. 51, pp. 18532–18537.
53. Sacks, D.L., Modi, G., Rowton, E., Späth, G., Epstein, L., Turco, S.J., and Beverley, S.M., The Role of Phosphoglycans in *Leishmania*–Sand Fly Interactions, *Proc. Natl. Acad. Sci. USA*, 2000, vol. 97, no. 1, pp. 406–411.
54. Niewiadomski, P., Knappe, S., Geimer, S., Fischer, K., Schulz, B., Unte, U.S., Rosso, M.G., Ache, P., Flugge, U.-I., and Schneider, A., The *Arabidopsis* Plastidic Glucose-6-Phosphate/Phosphate Translocator GPT1 Is Essential for

Pollen Maturation and Embryo Sac Development, *Plant Cell*, 2005, vol. 17, no. 3, pp. 760–775.

55. Gronskiy, S.V., Zakataeva, N.P., Vitushkina, M.V., Ptitsyn, L.R., Altman, I.B., Novikova, A.E., and Livshits, V.A., The *yicM* (*nepI*) Gene of *Escherichia coli* Encodes a Major Facilitator Superfamily Protein Involved in Efflux of Purine Ribonucleosides, *FEMS Microbiol. Lett.*, 2005, vol. 250, no. 1, pp. 39–47.
56. Yang, S., Clayton, S.R., and Zechiedrich, E.L., Relative Contributions of the AcrAB, MdfA and NorE Efflux Pumps to Quinolone Resistance in *Escherichia coli, J. Antimicrob. Chemother.*, 2003, vol. 51, no. 3, pp. 545–556.
57. Yang, S., Lopez, C.R., and Zechiedrich, E.L., Quorum Sensing and Multidrug Transporters in *Escherichia coli, Proc. Natl. Acad Sci. USA*, 2006, vol. 103, no. 7, pp. 2386–2391.
58. Ullmann, A. and Danchin, A., Role of Cyclic AMP in Bacteria, *Adv. Cyclic Nucleotide Res.*, 1983, vol. 30, pp. 1–53.
59. Mann, S.K. and Firtel, R.A., cAMP-Dependent Protein Kinase Differentially Regulates Prestalk and Prespore Differentiation during *Dictyostelium* Development, *Development*, 1993, vol. 119, no. 1, pp. 135–146.
60. Williams, M. and Jarvis, M.F., Purinergic and Pyrimidinergic Receptors as Potential Drug Targets, *Biochem. Pharmacol.*, 2000, vol. 59, no. 10, pp. 1173–1185.
61. Jenal, U., Cyclic Di-Guanosine-Monophosphate Comes of Age: A Novel Secondary Messenger Involved in Modulating Cell Surface Structures in Bacteria?, *Curr. Opin. Microbiol.*, 2004, vol. 7, no. 2, pp. 185–191.
62. Römling, U., Gomelsky, M., and Galperin, M.Y., c-di-GMP: The Dawning of a Novel Bacterial Signaling System, *Mol. Microbiol.*, 2005, vol. 57, no. 3, pp. 629–639.
63. Arnold, C.N., McElhanon, J., Lee, A., Leonhart, R., and Siegele, D.A., Global Analysis of *Escherichia coli* Gene Expression during the Acetate-Induced Acid Tolerance Response, *J. Bacteriol.*, 2001, vol. 183, no. 7, pp. 2178–2186.
64. Rinas, U., Hellmuth, K., Kang, R., Seeger, A., and Schlieker, H., Entry of *Escherichia coli* into Stationary Phase Is Indicated by Endogenous and Exogenous Accumulation of Nucleobases, *Appl. Environ. Microbiol.*, 1995, vol. 61, no. 12, pp. 4147–4151.