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Regulation of *Escherichia coli* Biofilm Formation (Review)

J. A. Markova[a, c, *], E. V. Anganova[b, c], A. L. Turskaya[a], V. A. Bybin[a], and E. D. Savilov[b, c, **]

[a] *Siberian Institute of Plant Physiology and Biochemistry, Siberian Branch, Russian Academy of Sciences, Irkutsk, 664003 Russia*

[b] *Scientific Center of Family Health and Human Reproduction, Irkutsk, 664003 Russia*

[c] *Irkutsk State Medical Academy of Postgraduate Education, Irkutsk, 664003 Russia*

**e-mail: juliam06@mail.ru*

***e-mail: savilov47@gmail.com*

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Abstract—The review considers the regulatory mechanisms controlling the formation of biofilms by bacteria *Escherichia coli*. Under harsh conditions, microbial populations transfer to the structured mode of existence by building biofilms. The regulation of biofilm formation is a complex multistage process. Environmental signals are perceived by two-component signaling systems, which fulfill their transduction to the genome. This switches microbial cells from the planktonic motile lifestyle to the sessile.

Keywords: biofilm, cAMP, c-di-GMP, sigma-factor, sRNA, two-component system, quorum sensing

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INTRODUCTION

Bacterial cells exist in an unstable medium. They are exposed to various stressors, such as nutrient deficiency, changes in pH and osmolarity, and DNA damage by reactive oxygen species. Depending on the environment, bacteria have evolved at least three behavioral strategies, namely, free swimming of single cells, swarming, and a sessile state associated with biofilms. In a liquid medium with sufficient amounts of nutrients, bacteria live in the well-known unicellular planktonic state. In this case, the flagellum-driven motility and ability to chemotax help bacterial cells to choose the most favorable ecological niches. Under certain conditions, they may create highly differentiated swarm cells that exploit the dynamics of collective motion to occupy new niches [1]. At the stationary phase, bacteria adhering to a surface form a biofilm (Fig. 1). This state is the dominant living form of microbes typical of almost all studied bacterial species. The cells integrated in a biofilm are more resistant to antibiotics, disinfectants, and various detergents used in a food industry [2]. A considerable feature of the bacteria inside a biofilm is their immobility. The flagella promote the initial contact of cells with the surface due to the overcoming of a repulsive force and participation in bacterial distribution over the substrate surface [3]. This is not an obligatory requirement, since flagellum-free bacteria are also able to form biofilms [4].

Biofilm morphology depends on the surrounding hydrodynamic conditions. In response to mechanical irritation caused by a flow of liquid, the matrix acquires properties of an elastic body. Under a weak flow, biofilms that are less bound to the substrate appear. These changes may be accounted for by a biosurfactant secretion controlled by a quorum sensing [5].

Cells of *Escherichia coli* adhere to the substrate in response to certain factors of the environment. Adhesion initiates several signaling cascades, entailing the syntheses of matrix components. The adhesion of bacterial cells depends on the substrate material. For example, silicon appears to be more effective than steel or polypropylene [6].

ADHESINS

The primary contact of bacteria with the surface depends on the physicochemical and electrostatic cell–substrate interactions [7]. This is determined to large extent by the amounts and nature of nutrients in the medium [8]. Organic molecules accumulate near a surface that is favorable to bacteria, especially upon a deficit of these compounds in a bulk medium [9].

The adhesion changes the transcription rates of certain genes [10] due to the activation of the two-component system Cpx (see below). However, the nature of the activator signal is still obscure. This feature, together with the multiplicity of signals (including those received from the host-macroorganism), is discussed in the review [11].

Key players in the adhesion and intercellular aggregation are bacterial adhesins—fimbriae and amyloid

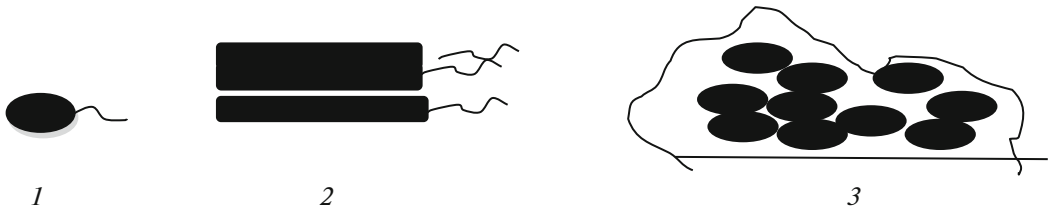

Fig. 1. Outline of different living forms of bacterial cells: (*1*) planktonic form; (*2*) swarm form, and (*3*) biofilm.

fibrils (curli). These structures belong to factors of bacterial virulence, because they are involved not only in the adhesion to abiotic substrates but also in recognition of the host cells and adherence to the superficial structures favoring the colonization process [12].

Bacterial fimbriae, or pili, are widely diverse. They are classified by schemes that change from time to time. Most of the schemes are not in use now, but some terms are adopted in the official nomenclature. One classification is based on a division of pili into mannoresistant and mannosensitive [13]. The second category includes type 1 pili, which promote adhesion to host tissues, in particular, bladder epithelium [10]. As shown in experiments with adhesin FimH inhibition, these pili participate in biofilm formation [14].

F-pili play roles in the initial adhesion and biofilm maturation: they nonspecifically adhere to abiotic surfaces and subsequently maintain intercellular contacts stabilizing the biofilm structure. Contact with the surface is reported to positively influence a plasmid transfer dynamics [15]. Presumably, the conjugative and nonconjugative plasmids carry determinants of initiation of biofilm formation and development; they, in turn, act on the intensity of plasmid-mediated horizontal gene transfer within them [16].

Adhesin AidA of enterohemorrhagic *E.coli* and TibA adhesin/invasin associated with enterotoxigenic *E. coli* are two glycosylated surface proteins involved in bacterial adhesion to various eukaryotic cells. These proteins also take parts in biofilm formation on abiotic surface [17].

Antigen 43 (Ag 43) is involved in the initial nonspecific adhesion to an abiotic surface and intercellular adhesison. It is necessary to create biofilms on minimal media. Ag43 is also a participant in yielding interspecific biofilms from *E. coli* and *Pseudomonas aeruginosa* [18].

Fimbriae Yad, which are constitutively expressed in *E. coli* K-12, participate in adhesion to human cells and abiotic surfaces. It was recently found that these adhesins are able to bind to xylose, the chief component of the plant cell wall, which gives rise to seed colonization and survival of bacteria in rhizosphere. This adhesin is expressed at temperatures below 37°C; thus, Yad enables *E. coli* survival outside the warm-blooded organism [19].

One more adhesin, AAF/I from *E. coli* O104:H4, participates in the specific adhesion to fibronectin, one of the key proteins of the intercellular matrix of vertebrates. Deletion of the gene encoding this adhesin reduces the aggregation capacity of the microbial cells [12].

Type IV pili of *E. coli*, which are encoded by *bfp* operon [20], and type P pili encoded by *pap* operon [21], as well as nonfimbrial adhesin TosA, are also involved in biofilm development [22].

Another important kind of adhesin, termed curlin, had been initially identified in *E. coli* and was later found in different representatives of the family Enterobacteriaceae—*Shigella, Citrobacter*, and *Enterobacter* [2, 23]. Curlin filaments consist of repeating subunits of the CsgA protein [13]. At present, curlin-like structures were found in *Bacteroidetes*, *Proteobacteria*, *Firmicutes*, and *Thermodesulfobacteria* [24]. They are composed of protein threads that form curled structures on the microbial cell surface.

Curlins possess properties in common with amyloid fibers of eukaryotes. Recent studies make it possible to hypothesize that the presence of amyloid peptides predispose the organism to such diseases as systemic amyloidosis of higher animals and humans, as well as Alzheimer's and Parkinson's diseases. However, unlike these proteins, amyloids of microbial cell surfaces are qualified as "functional amyloids" with a strictly controlled expression [13, 25].

Curlin of *E. coli* binds to many human proteins, including fibronectin, lamininin, type I collagen, molecules of the chief complex of the Class I histocompatibility, plasminogen, etc. [25, 26]. Their roles are also important in plant tissue colonization [12].

Curlins favor biofilm formation on biological and abiotic surfaces [13]. They are stained with Congo red and thioflavin likewise other amyloid structures [13, 27].

Curlin-coding genes are located in two operons. The first, *csgBA*, carries genes of structural components of curli, and the second, *csgDEFG*, encodes the transcriptional regulator CsgD and the system CsgE-G exporting these components from the cell [23, 25]. They are usually expressed at temperatures below 30°C, low concentrations of nutrients, high osmotic pressure, and the stationary phase of the growth, which helps the survival of bacterial pathogens outside

the host organism [2, 13, 25]. At the same time, certain strains of uropathogenic *E. coli* express curlin at 37°C [13].

Therefore, *E. coli* possesses a broad set of surface adhesins, some of which are still unstudied. These adhesins vary in their specificity and are expressed in response to changes in the environment [8]. They are components of the matrix and are involved in both the adhesion process and the formation of biofilm structure [28].

MATRIX EXOPOLYSACCHARIDES

One of the most important features that distinguish biofilms from the planktonic living form is the presence of an extracellular matrix that surrounds the bacterial cells and determines the structure of a mature biofilm [8]. The matrix is the hydrated mucilaginous layer protecting bacteria from drying. It also hinders recognition of the pathogen by the host and, hence, protects the former from the immune system of the latter. It may also be a significant protective barrier for such toxic molecules as antibiotics, disinfectants, and reactive oxygen species. The matrix is composed of exopolysaccharides, proteins, nucleic acids, lipids/phospholipids, adsorbed nutrients, and low-molecular-weight metabolites [29]. The substantial constituents of the matrix are adhesins, which are discussed in the previous section, and DNA [5].

The analysis of extracellular exopolysaccharides (EPS) is relatively difficult; hence, their exact content is not satisfactorily characterized. Particular EPS are known to determine the physicochemical properties of the matrix [30].

In the *E. coli* biofilm, β-1,6-*N*-acetyl-D-glucosamine polymer (PGA), colonic acid, and cellulose predominate [18, 29, 31]. Poly-β-1,6-*N*-acetylglucosamine (β-1,6-GlcNAc) is the polysaccharide involved in intercellular adhesion and attachment to surfaces [20]. The proteins operating in the PGA synthesis of *E. coli* are encoded by the operon *pgaABCD* (*ycdSRQP*). This operon is capable of horizontal transfer and is present in various species of eubacteria [32].

Cellulose, which belongs to major components of the plant cell wall, is also present in the matrix of *Enterobacteriaceae, Pseudomonas, Gluconacetobacter hansenii, Rhodobacter sphaeroides*, etc. [33]. The genes involved in the cellulose synthesis are organized into two differently transcribed operons, *bcsABZC* and *bcsEFG* [34].

Colanic acid consists of deoxihexose units with a majority of fucose and glucuronic acid. The physical barrier created by colanic acid, together with its negative charge, makes *E. coli* biofilms resistant to osmotic, oxidative, and temperature stresses [35]. The operon *wca,* which consists of 19 genes and is also known as *cps*, encodes the enzymes that necessary for colonic acid synthesis.

The polysaccharides of the capsule and lipopolysaccharides (LPSs) plays also serious roles in the biofilm matrix. In a supernatant of bacterial culture, group II capsule polysaccharides display antiadhesive activity and thus prevent biofilm formation [36]. LPS favor *E. coli* adhesion to an abiotic surface [37].

Most *E. coli* strains form biofilms both inside the host, including the cell interior of its bladder and kidneys [38, 39], and in the environment. Since the corresponding conditions greatly differ (in temperature, pH, availability of nutrients, oxygen, etc.), these microorganisms possess a complicated system that regulates the process depending on the particular location of the prokaryote. As a consequence, the matrix content differs depending on the environment, that enables high adaptivity and survival of the bacteria [18]. At 37°C, bacteria living in the host organism or on abiotic surfaces employ type I fimbriae or adhesin Ag43 for attachment and PGA and carlin as a dominant components of the matrix [31]. In bacteria that inhabit abiotic substrates at a low temperature (below 30°C), the content biofilm is different. They use flagella for the initial attachment and carlin, cellulose, and colonic acid as the matrix of the mature biofilm [40]. It is revealed that the synthesis of colonic acid is induced by certain β-lactamic antibiotics [41].

Taken together, these facts allow the conclusion that the regulation of the biofilm-forming process is rather complex and includes multiple elements. Ambient stimuli are perceived by two-component signal systems and induce subsequent signal transduction to the genome, enabling the rapid adaptation of microbial cells. The sigma-factor of the stationary phase activates the transcription of the genes recruited for the biofilm formation. Small RNAs carry out regulation at the post-transcription level. Cyclo-di-GMP (c-di-GMP) takes part in the allosteric regulation of the enzymes synthesizing components of the biofilm matrix. Quorum sensing systems also play indispensable roles [42].

The switch from the planktonic to biofilm lifestyle of *E. coli* is regulated by two cascades: FlhDC + σ^{70}/σ^{F} and σ^{S}/MlrA/CsgD, which operate, respectively, at the postexponential and stationary growing phases. Flagella synthesis and the formation of components of the biofilm matrix are mutually exclusive processes [43]. This switch is controlled by such signals as limitation in nutrients, low temperature, and changes in characteristics of the cell wall [40, 44].

TWO-COMPONENT SIGNALING SYSTEMS

Most bacterial adaptive reactions involve two-component regulatory systems. The system consists of two (or, rarely, three) associated proteins working as a sensor and a regulator of the response. Histidine pro-

teinkinase, as a sensor, detects extracellular signals. This changes its activity through phosphorilation of the conservative histidine residue in its molecule, followed by the transfer of this residue to the associated molecule of the response regulator (to the residue of asparaginic acid) [45]. The response regulator often functions as a transcription factor that enables the rapid expression of genes related to the adequate cell reaction to the stress.

These sensor systems are present in the majority of bacteria, especially those inhabiting the environment. They are absent in *Mycoplasma genitalium*, which is an obligate intracellular pathogen. Bacteria *E. coli* have approximately 30 such systems [46].

At least, three two-component signaling systems—CpxA/CpxR, EnvZ–OmpR, and RcsC/RcsD/RcsB—take part in the regulation of biofilm formation. Their regulons overlap and regulate such different cell processes as the synthesis of flagella and matrix components, along with pathogenicity factors.

CpxA/CpxR SYSTEM

The system reacts to changes in the ambient environment surrounding the periplasm, outer membrane, and cell wall. It is activated under low levels of nutrients, high osmolarity, and high temperature. Thus, CpxA/CpxR may be interpreted as a stress response system programmed for reactions towards the cell wall damage. The expression of the CpxA/CpxR correlates with activation of the genes involved in antibiotic resistance, for example, gene *mdtA,* which is responsible for the removal of β-lactamic antibiotics from the cell and genes *dsbA* and *degP,* which encode periplasmic foldases and chaperons [23].

The participation of the CpxA/CpxR system is different at different steps of the biofilm formation. In *E. coli* K-12, cell contact with a surface is mediated by the lipoprotein of the outer membrane NlpE, which activates the CpxA/CpxR system due to the processes referred to as surface sensing [47]. This activation modifies the chemical content of cell surfaces by regulation of the expressions of the surface proteins OmpC and by repression of the flagella and chemotaxis genes [48]. Activation of the *Cpx* genes decreases curlin synthesis. It was hypothesized that the system may diminish in the biofilm the expression of the energetically expensive adhesive molecules, which occurs just after the initial contact of the bacterium with the surface [49]. Protein CpxR is a dominant regulator in the hierarchy of signal transduction systems, since it controls such regulatory systems as components of the Rcs and *rpoH* system (an alternative sigma-factor involved in the gene transcription in response to heat shock) [49].

EnvZ/OmpR SYSTEM

The system modifies the expressions of the porins ompC and ompF of the outer membrane in response to changes in osmotic properties of the ambient [50]. It also induces curlin production [23]. The effect is mediated by activation of the regulator CsgD phosphorilated by OmpR [8].

RcsCDB REGULATOR

This system (*Regulator of capsule synthesis*) regulates the synthesis of colanic acid [23]. It is a representative of nonstandard two-component systems [51] and consists of three proteins indicated as RcsC, RcsD, and RcsB. The first protein is a hybrid sensor kinase located on the inner membrane; the signals to which it reacts are not exactly characterized. However, by some evidence, the protein RcsC percepts complex signals comprising loss of water and change in osmolarity [52]. Protein RcsD is also membrane-associated; it possesses the histidine-containing phosphotransmitter domain Hpt. The protein belongs to the classical cytoplasmic response regulators; after its phosphorilation by sensor kinase RscC, the regulator of transcription RcsA connects with RcsB and activates transcriptions of the corresponding genes [23].

Transcription analysis revealed more than 150 genes related to the regulon Rcs [52]. At least 50% of the regulated Rcs genes encode proteins with functions associated with the cell surface. This is evidence of the important role of Rcs in rearrangements of the *E. coli* cell surface [46]. The RcsCDB system inhibits expression of *flhDC* and thus blocks cell motility [53]. This also represses the formation of such surface structures as fibers of curlin and Ag43 [52].

PROTEINS ArcB/ArcA

This two-component system coordinates the synthesis and proteolysis of the *E. coli* stress factor σ^S (RpoS). Activation of the system unites the information on the availability of oxygen and energy for the cell [54].

PROTEINS BarA/UvrY

Homologs of the two-component system BarA/UvrY of *E. coli* are identified in different species of Gram-negative bacteria *Salmonella enterica* (BarA/SirA), *Erwinia carotovora* (ExpS/ExpA or GacS/GacA), and *Pseudomonas* (GacS/GacA). As ascertained rather recently, the two-component system BarA/UvrY participates in the regulation of *csrA* expression with the use of small noncoding RNAs CsrB and CsrC [55]. It is also known that mutations in genes *barA* or *uvrY* weaken the biofilm formation [55].

ALARMONES

These intracellular signal molecules are produced by cells under unfavorable conditions and play significant roles in regulation of the biofilm formation processes. Alarmones cAMP and (p)ppGpp are the main effectors of the *E. coli* response to starvation. An accumulation of (p)ppGpp in the cell inhibits RNA biosynthesis, reduces the growth rate, and activates the genes associated with the stress response [56]. In *E. coli*, the synthesis of (p)ppGpp involves protein RelA (under amino acid starvation) or SpoT (under other stress impacts). This alarmone induces the expression of the alternative sigma-factor σ^S of RNA-polymerase. The influence of (p)ppGpp is more pronounced with the help of the protein DksA, which can interact with RNA polymerase. It is shown that (p)ppGpp enhances the expression of the recombinase gene *fimB* and, this way, stimulates biosynthesis of the type 1 fimbriae. The effect does not actually depend on RpoS, H-NS, and NanR—the regulators of the *fimB* expression described earlier [57]. An increase in the production of type 1 fimbriae may promote colonization by bacterial societies of more favorable areas.

The occurrence of another alarmone, cAMP, in the cell depends on the presence of glucose in the medium. In the absence of glucose or other rapidly metabolized sugars, the protein $EIIA^{Glc}$ of the phosphoenol pyruvate–phosphotransferase system is phosphorilated and activates adenylate cyclase. This entails an increased level of cAMP, which forms a complex with the receptor protein CRP. The latter is also called CAP (catabolite gene activator protein) and acts as a transcription factor. This complex binds to DNA and stimulates the expressions of the several genes involved in metabolism of energy and catabolism of secondary carbon sources. Transcritome analysis of *E. coli* showed that 65% genes, including those related to activation of transcription of *csgD* (transcription factor, see below), are regulated by CRP/cAMP [58]. In addition, CPR participates in such processes as osmoregulation [59], stringent response [60], virulence [59], nitrogen assimilation, and resistance to antibiotics [61]. CPR inhibits formation of the type 1 pili in uropathogenic strains of *E. coli* [58].

Protein CsrA (*carbon storage regulatory*) modulates bioflm formation by repressing the glycogen metabolism and by regulation of the master-regulator *flhDC* of the flagellum motility [62]. The expression of *csrA* is sharply decreased several hours after the onset of bacterial biofilm growth on a surface and resumed after its maturation. This also resumes flagellum mobility, which favors biofilm destruction. At the posttranscription level, CsrA participates in the regulation of PGA production (*pgaABCD*) [32]. One such regulatory mechanism is the inhibition of the diguanilate cyclase genes *ycdT* and *ydeH* [63]. CsrA is also involved in the activation of UvrY expression, which is a component of the two-component signal system BarA/UvrY [64, 65].

Cycling-di-GMP (c-di-GMP) is the essential switch of microbial cells from the planktonic to biofilm mode of existence [66]. This secondary messenger is synthesized by diguanilate cyclases containing the catalytic domain GGDEF; its degradation occurs by means of the EAL domains of diphosphoesterases [34, 47]. Domains GGDEF and (or) EAL are present in 29 proteins of *E. coli* K-12 [34, 47, 67]. Cycling-di-GMP activates the specific proteins at the posttranscription level. The domains called PilZ and GIL, which are its receptors, were identified with the use of bioinformatic methods [68, 69]. The new nomenclature was recently proposed for diguanilate cyclases and phosphoesterases. It is used in this review; conventional names of enzymes are given in parentheses in addition [47].

In *E. coli*, flagellum motility is controlled by the c-di-GMP-dependent protein YcgR, which is named flagellum brake. It interacts with the basal body of the flagellum retarding its rotation [47]. Diguanilate cyclases DgcE (YegE) and DgcM (YdaM) and phosphodiesterases PdeH (YhjH) and PdeR (YcjR), together with the MerR-like transcription factor MlrA, regulate the transcription of *csgD*—the key regulator of the biofilm formation [70]. DgcC (YaiC) and receptor of c-di-GMP—BcsE—activate cellulose synthesis without effects on curlin gene expression [69].

DgcZ (YdeH) regulates the synthesis of poly-β-1,6-*N*-acetylglucosamine in *E. coli* [71] and is activated by the transcription regulator CpxR [72]. In the carbon-rich medium, the global regulator CsrA blocks the mRNA translation of this protein at the posttranscription level [73]. Molecule DgcZ contains a zinc-binding domain with a connection to the metal that diminishes the activity [33, 47].

Two diguanilate cyclases DosC (YddV) and DosP (YddU) possess an oxygen sensor similar to heme by its structure. Hyperexpression of the gene *dosC* elevates the c-di-GMP level in the cell and consequently stimulates biofilm formation [67], which, in contrast, is inhibited upon hyperexpression of the gene *dosP* [74].

The different cycling dinucleotide c-di-AMP was recently discovered in many bacteria and archaea. Likewise c-di-GMP, it controls such cellular processes as gene expression, DNA reparation, synthesis, and metabolism of the cell wall. Hybrid molecules of adenosine and guanosine—3',3'-cGAMP and 2',3'-cGAMP—were also found [75].

Over recent decades, it has been established that cyclic nucleotides are actually a new widespread class of secondary messengers that actively participate in various vital processes of the prokaryotic cell. The number of proteins containing domains that are involved in the metabolism or recognition of these messengers is evergrowing. All the same, the biological functions of most of them have not been elucidated

so far [75]. The investigation of this complicated signaling network may be of use in search of a novel means of control of infectious diseases.

Bacteria sense changes in the environment, not only by two-component signaling systems but also by so-called ribo-switches represented by a part of the 5'-nontranslated region of mRNA, which directly interacts with signaling molecules. This interaction may switch protein synthesis on or off. Ribo-switches control the expression of noncoding RNAs. Ligands for ribo-switches are certain ions (Mg^{2+}, Ni^{2+} / Co^{2+}, and Mn^{2+}), vitamins, cofactors, amino acids, and nucleotides including c-di-GMP. These ribo-switches are situated in front of the genes encoding diguanilat cyclases, phosphodiesterases, and different proteins capable of interaction with c-di-GMP [76].

SMALL RNAs

Small RNAs (sRNAs) exist as small noncoding RNA molecules (from 50 to 250 nucleotides) that are produced by bacteria and participate in the regulation of cell responses to stress impacts. Small RNAs may attach to informational RNA (and modulate gene expression) or interact with protein (affecting its function). They may also act at the level of transctiption termination [77]. The interaction of small RNAs with a target object often involves RNA-chaperon Hfq [78]. Small RNAs may be classified into four groups according to their roles in processes of the biofilm formation. Group I stimulates biofilm development and inhibits flagellum formation, Group II stimulates flagellin expression and disturbs synthesis of the biofilm matrix, Group III inhibits both functions, and Group IV controls formation of flagella and biofilms.

Group 1 includes ArcZ (*arc*-associated sRNA Z), which inhibits *flhDC* and type 1 fimbriae. In this way, it counteracts the motility and (or) initial attachment of bacteria. The two-component system ArcA/ArcB is activated in the *E. coli* cells under oxidative stress. This leads to a reduction in the transcription of sigma subunit RpoS and small RNA ArcZ. At the same time, ArcZ destabilizes mRNA-kinase ArcB and thus regulates its level [79]. The small RNA DsrA (downstream region of *rcsA*) may also belong to Group I, since it stimulates *rpoS* translation and hinders the expression of H-NS (mediated activator of FlhDC expression). The elevated expression of DsrA at low temperature (25°C) contributes to the increase in σ^S level under these conditions. As was recently found, it inhibits mRNA translation of the genes *mreB* and *rbsD* involved in ribose metabolism [80].

GadY (*gad* gene-related sRNA) indirectly inhibits FlhDC. At the same time, it positively controls GadX expression and, consequently, the expression of the response genes at low pH of medium [81]. The process may proceed in mature biofilm; some areas of these biofilms, in which the acid-yielding fermentation exceeds respiration, contain low oxygen.

Group II includes the small RNA McaS (*multicellular adhesive sRNA*), which belongs to the CRT regulon controlling carbon metabolism and takes part in the regulation of motility and biofilm formation [82]. McaS was found to bind to the chaperon Hfg resulting in inhibition of mRNA *csgD* and activation of mRNA *fhlDC*. This small RNA also activates mRNA *pga* in interaction with the chaperon CsrA [83].

MicA (regulator of *ompA* mRNA) inhibits certain porins of *E. coli* [40].

Group III is represented by OmrA/B (OmpR-regulated sRNAs A and B), which are also involved in the regulation of porins and affect biofilm formation through inhibition of the translation from mRNA *csgD* [84]. OmrA/B is under the control of the two-component system EnvZ/OmpR, which, as stated above, reacts to changes in medium osmolarity [85].

Small RNA OxyS (oxidative stress-related) is induced by the response factor OxyR, which senses the presence of hydrogen peroxide. It decreases the expression of flagellum genes and indirectly blocks σ^S expression [86].

Group IV comprises RprA (RpoS regulator A), which, when hyperexpressed, inhibits mRNA *csgD* but also has indirect multifaceted effects on cascades of flagellum synthesis. As an activator of σ^S, this small RNA may stimulate induction of the σ^S-dependent genes under still unknown conditions. RprA may inhibit the expression of diguanilate cyclase *ydaM* and, hence, prevent *csgD* transcription. RprA transcrtiption is activated by the two-component system of Rcs signal transduction, which also down-regulates *flhDC* transcription [87].

Therefore, small RNAs contribute to the reliability of expression switching from the flagellum genes to genes of components of the biofilm matrix associated with exponential growth and stationary phase, respectively. A common function of Group III small RNAs is that they switch off the expressions of big structures in the cell, irrespective of the structures' involvements in motility or biofilm formation. This model may work under a sudden stress [40].

BACTERIAL RNA POLYMERASE

The enzyme is an aggregate protein that consists of a minimal or core enzyme composed of five subunits $\beta\beta'\alpha 2\omega$ and the sixth subunit σ responsible for promoter recognition. All genes encoding σ-factors have an abbreviation rpo (RNA polymerase); thus, four-letter abbreviations (for example, RpoE) are also used for sigma-factors.

As opposed to so-called housekeeping σ-factors, which are responsible for the transcription of the genes essential for the main cell functions, the alternative

σ-factors provide the transcriptions of the few genes related to specific functions. Six alternative σ-factors, σ^E, σ^F, σ^H, σ^I, σ^N, and σ^S, are identified in *E. coli* [88]. These factors are often functionally associated with such specific stresses as nitrogen deprivation (σ^N) or heat shock (σ^E and σ^H). In the processes of biofilm emergence, the key role belongs to the alternative sigma-factor of the stationary phase RpoS, which is a master-regulator of the so-called general stress response—the global physiological adaptation of the bacterial cell to various unfavorable conditions [89]. The gene *rpoS* of the σ^S-factor is not obligatory for bacterial growth, but the mutant strains lacking in its activity are extremely sensitive to various stressors [90].

The level of σ^S-factor increases during transition to the stationary phase of microbial growth with an inadequate supply of nutrients. In this case, the growth rate, which reflects the energy status of the cell (determined by intracellular ATP concentration), may be the chief physiological signal for *rpoS* gene expression [54, 90].

The regulon *RpoS* of *E. coli* constitutes nearly 500 genes that are approximately 10% of the total bacterial genome [91]. Nevertheless, about only 140 genes are defined as core *rpoS* regulon, i.e., the genes induced by increased σ^S irrespective of a character of the stress impact [92]. This indicates that auxiliary regulators are mobilized for genes corresponding to the regulon *rpoS* [66]. The gene *rpoS* itself and protein σ^S are also governed by multiple factors at the level of gene expression (translation, activity, and degradation of a protein).

The induction of σ^S protein synthesis is affected by various stress conditions, which send signals to the cell via complex pathways. In this regard, the energy and redox status of a cell deficit in nutrients (especially glucose, etc.) may be mentioned. A link exists between the regulon *rpoS* and the system toxin–antitoxin (TA), which plays a significant role during long starvation. These are the systems MqsR/MqsA and YafQ/DinJ [93]. The partial death of a bacterial population due to TA results in cell lysis, which may release nutrients that are helpful to live cells.

At the posttranscription level, the expression of *rpoS* is regulated by some small RNAs (see above). RNAse III, which is encoded by the gene *rnc,* takes part in the modulation of the *rpoS* translation. The cold shock protein (RNA helicase) is involved in maintenance of the secondary structure of *rpoS* mRNA at a low temperature. This structure is stabilized by proteins CspC and CspE, which play the roles of RNA chaperones. Protein HU stimulates the translation of *rpoS* [90]. In competition with other σ-factors, the association of σ^S with core RNA polymerase requires the presence of proteins and alarmons, such as Crl [94], ppGpp, DksA [95], and Rsd [90]. The protease ClpXP performs proteolysis of σ^S [90], which must join with the ATP-dependent protein RssB. Antiadaptive proteins IraP, IraM, and IraD prevent the degradation of σ^S by its isolation from RssB in response to a deficit of phosphates (IraP) or magnesium (IraM) or to DNA damage (IraD) [56] (Fig. 2). RpoS is situated at the top of the signaling cascade that regulates the synthesis of biofilm matrix components. Its expression changes the physiology and morphology of the microbial cell. Activation of the transcription factors MlrA and CsgD induces the synthesis of curlin, colonic acid, and cellulose (Fig. 3).

Transcription factor CsgD occupies the central position in the signaling cascade governing biofilm formation. It is activated under conditions of low concentration of glucose, high osmolarity, and a temperature below 30°C. CsgD is controlled by many factors at both transcription and posttranscription levels, as well as by the system c-di-GMP (see above). CsgD controls expressions of 13 genes, including those responsible for curlin formation, and gene *iraP*, which is involved in RpoS stabilization and represses the genes of flagellum synthesis *fliE* and *fliF*. CsgD activates the synthesis of cellulose BcsA via interaction of the domain PilZ of this enzyme with c-di-GMP. A novel CsgD- and AdrA-independent pathway of activation of cellulose production with the participation of diguanilate cyclase DgcQ (YedQ) [18] was recently identified.

Transcription factor BolA is present in the majority of Gram-negative bacteria. It is involved in regulation of the balance of proteins and OmpF/OmpC porins (which diminishes the permeability of cell membrane), regulates gene expression related to pyruvate metabolism and Krebs cycle, controls the paths of the formation of some amino acids, and activates curlin synthesis [6].

QUORUM SENSING SYSTEM

Quorum sensing plays a substantial role in the regulation of bacterial biofilm formation [96].

The regulatory system based on acyl homoserine lactones (AHL) consists of *luxI* gene of AHL synthase and *luxR* of receptor. Homologs of these genes are present in the genomes of some Gram-negative microorganisms. *E. coli* has the *sdiA* gene, which is a homolog of *luxR*, but has no *luxI* homolog. Consequently, this organism cannot synthesize AHL but is able to react to autoinducers of other species. The content of the protein SdiA regulon comprises the genes *uvrY* and *csrA,* which participate in the regulation of biofilm formation, motility, and virulence [28].

The quorum sensing system based on type 2 autoinducers (AI-2) is considered universal, since this autoinducer is synthesized by most bacterial species. Synthase AI-2 (protein LuxS) is also involved in the methyl cycle of microbial cells. AI-2 regulates biofilm formation by means of the TA system MqsAR, which regulates flagellum motility with the use of the two-component signaling system QseBC [97]. The extra-

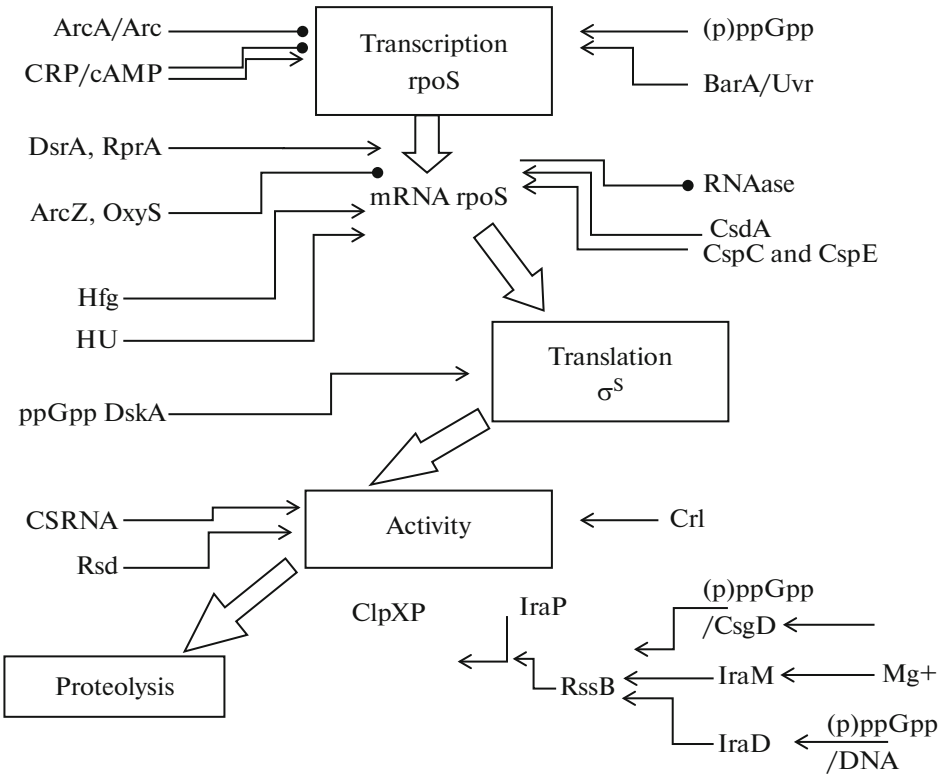

Fig. 2. Regulation of the synthesis of alternative sigma-factor.

cellular AI-2 achieves its maximal concentration at the middle or end of the exponential phase and decreases during transfer to the stationary phase of bacterial growth.

An indole-dependent quorum sensing system was recently reported. The system allows bacterial adaptation to starvation conditions at the stationary growth phase, when amino acid catabolism is a source of energy. Indole favors antibiotic resistance via participation in the formation of persister cells and is able to induce genes encoding proteins exporters.

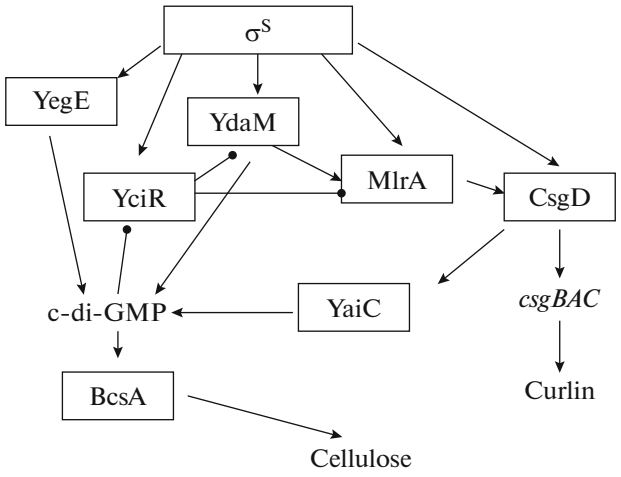

Fig. 3. Regulation of the synthesis of matrix components of the biofilm.

Indole is synthesized from tryptophan by tryptophanase. The expression of this protein increases with an increased cAMP concentration and pH; it is suppressed if glucose, pyruvate, and acetate are present in the medium. It was recently shown that the indole concentration in a supernatant of a cultural medium enhances at the late exponential–early stationary phase of the bacterial growth. Not all bacteria are capable of indole synthesis. Nonproducing ones (*Pseudomonas*) can transport exogenous indole. In general, its role in the metabolism may differ in indole-producing and nonproducing microorganisms. The data on the indole contribution to the regulation of biofilm building are controversial. This substance was shown to hinder the emergence of *E. coli* biofilm, but the details of its action as an autoinducer are still uncertain [98]. It was assumed that indole binds to the receptor SdiA, which is also the receptor of AHL. However, more recent evidence casts doubt on this [98].

Interestingly, microbial cells interact with each other within the biofilm by means of electric signals propagated by potassium ionic channels [99, 100].

To sum up, it should be stated that biofilms are prevalent everywhere in nature. They pave oil pipe-

lines, aquaria, permanent catheters, internal implants, contact lens, and prostheses. Both Gram-positive (*Enterococcus* spp., *Staphylococcus* spp., and *Streptococcus viridians*) and Gram-negative (*E. coli, Klebsiella pneumoniae, Proteus mirabilis,* and *Pseudomonas aeruginosa*) microorganisms produce biofilms on medical equipment. Sterilization is often ineffectual because of the high resistance to it by the bacteria integrated in biofilms; this requires the use of disposable instruments. Problems of infections within hospitals are closely related to the biofilms appearing on walls, floors, and bed surfaces. Biofilms can also cover organs and tissues of humans and animals or roots and other parts of plants. In humans, more than 500 bacterial species form biofilms; all representatives of normal microflora exist in this form. Many biotopes of the organism differ in living conditions for microbes and their colonization. Macroorganisms employ some machinery that directly (through immune protection) or indirectly (through nonspecific protection) regulates biotope colonization. This ability is termed colonization resistance. In turn, it strongly depends on the biofilms of mucosal tunic, including normal human microflora [101].

Biofilms of pathogenic bacteria help them to affect most organs (upper air passages, lungs, heart, kidneys, skin, and the digestion system). In addition to direct damage to macroorganisms, biofilms are able to create serious difficulties in economy, medicine, etc. Biofilm formation in pipelines is one of the principal problems in current industrial enterprises. These include not only water contamination but also disturbances of the technical characteristics of the tubes, for instance, from a reduction of the effectiveness of heat exchange and the deterioration and breakdown of equipment [102]. In the food industry, biofilms on food products enhances the risk of contamination with pathogenous microorganisms, leading to food infections [103]. In nature, biofilms may spoil ecological conditions. For instance, the cyanobacterial film of a water surface hinders the oxygen supply of aquatic organisms in water bodies [104].

In some cases, a human may get a benefit from bacterial biofilms. For example, in manufacturing, including long biotransformation of toxic substances, they increase bacterial tolerance to xenobiotics. Microbial societies in biofilms effectively degrade harmful compounds in systems for the biological cleaning of water, air, and other media [105]. Bacteria that are used for biocontrol of infective plant diseases better withstand attacks of phytopthogenic microorganisms and better survive in the rhizosphere [106]. The formation of bacterial biofilms is useful for the case of medical preparations having probiotic activity of living bacteria [107].

All of the aforesaid is evidence of the need for thorough and versatile research on microbial biofilms, the means of their regulation, and genetic control of their formation, along with the development of methods to control microbes pathogenous to human beings [104]. The problem of how to govern these processes has not yet been elucidated in many aspects.

The processes of biofilm formation are highly complicated. They are initiated by many factors of the environment and are regulated by multiple signaling systems. The proteins participating in the synthesis of matrix components are under strict control at any level. The present review considers the functions of elements of the regulatory network of the most studied microorganism—*E. coli*. However, the discussed data suggest that a significant part of the elements are universal and operate in different species of Gram-positive and Gram-negative bacteria.

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