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REVIEWS

Synthetic Biology: Current State and Applications

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Abstract—This review is devoted to synthetic biology. In this field, molecular biology methods and techniques are applied to develop a predetermined cell behavior based on theoretically constructed regulatory networks using engineering approaches. A cell can be considered the device that performs several types of basic functions. These are sensory, information processing, and response formation functions. Endogenous and exogenous physical or chemical factors that trigger information processing cascades are input signals. These include signaling and metabolic pathways. As a rule, changes in these pathways lead to the modification of the transcriptional profile. The response includes alterations in the metabolic profile followed by physiological changes. Taking this into account, a cell and a computer can be considered analogs. Therefore, the principles of engineering can be applied to natural biological systems in general and genetic circuits in particular. In addition to the main areas of synthetic biology, as well as their principles and methods, specific examples of the application of the synthetic biology approach in medicine and other fields were discussed.

Keywords: synthetic biology, artificial gene circuits, logic gates, orthogonality, expression regulation
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HISTORY OF SYNTHETIC BIOLOGY

The control of cell functions, development, and behavior, has been of interest since the early stages of the development of biology as a separate branch of science. In the 20th century, attempts of directed changes in the phenotype were limited to the application of an external factor. As a result, cells changed their phenotype either temporarily or uncontrollably. The situation changed with the emergence of synthetic biology (SB). Like many other areas, SB developed nonlinearly, representing a global trend in the development of biotechnology or passing through the stage of stagnation. Moreover, the definition of SB did not appear immediately. Today, it is generally accepted that SB is a field in which methods and techniques of molecular biology are applied to develop a programmed cell behavior based on theoretically constructed regulatory networks using engineering approaches (this is not the only definition; however, we believe that it most capaciously determines this field). Engineering approaches include the construction of complex genetic networks from simple functional elements (blocks) that interact with each other depending on external conditions. Engineering, which is represented by the design and construction of such elementary blocks at the initial stages, as well as the construction of complex genetic circuits with feedback from them at later stages, distinguishes SB from other methods for the construction of organisms possessing certain features. For instance, in contrast to the classical application of molecular biosensors to measure physical, mechanical, or chemical stress in bioreactors, the synthetic biology approach involves the construction of regulatory networks based on these biosensors, which integrate responses from several simultaneous signals. Moreover, the minimum detection threshold of the target molecule can be varied without the interaction of the network components with the normal intra- and intercellular environment and other networks.

F. Jacob and J. Monod were the first to report on SB in 1961. The first gene circuit consisting of several functional blocks capable of responding to certain environmental factors was shown in bacterial cells for the first time [1].

The period from 1961 to 2000 was characterized by the accumulation of knowledge in the field of structural and functional molecular biology; new technologies and methods appeared. The development of recombinant DNA technologies and subsequent rapid development of genetic engineering methods, which were fundamental in the technologies for the practical implementation of engineering principles in synthetic biology, can be considered a special stage in the development of SB [2].

The first synthetic regulatory network was reported in 2000. The study presented the results on the artificial gene circuit (AGC) in which several genes repressed each other [3]. This was the first bistable AGC dependent on the amplified external factor.

At the same time, M. Elowitz and S. Leibler announced the results of their study on the design of the artificial oscillatory network based on repression, which was named the repressilator by the authors [4].

Studies on the intercellular AGC, the blocks of which were located in different cells forming a common functionality due to the exchange of expression products of these blocks, have become important milestones in the development of SB [5]. Subsequently, the intercellular networks in bacteria were precisely regulated using quorum sensing, which allowed the development of even more complex AGCs [6]. The first studies on the control of the behavior of mammalian cells appeared only in the mid-2000s. At the same time, yeasts were used for this purpose already in the early 2000s, although the number of networks designed for them was significantly lower than that for bacteria.

ENGINEERING OF BIOLOGICAL NETWORKS

Circuitry in Synthetic Biology

A cell can be considered a device that fulfills several types of basic functions: sensory function (perception of incoming signals), processing of incoming information, and response formation. The input signals are endogenous and exogenous physical or chemical factors that launch information processing cascades. These include signaling and metabolic pathways. As a rule, changes in them lead to the modification of the transcriptional profile. The response includes alterations in the metabolic profile followed by physiological changes. Taking this into account, a direct analogy between a cell and a computer, which allows the application of the engineering principles to natural biological systems in general and gene circuits in particular, is possible.

In 1995, H. McAdams and L. Shapiro were the first to use an analogy between electrical and gene circuits, introducing the term "gate" and the concept of circuitry into genetics [7]. This allowed systematization and schematization of intracellular processes. As a result, a more technical understanding of occurring processes was achieved, the procedures for the AGC design were simplified, and it was possible to form various complex genetic functions. For instance, the approach using the electric circuitry classification allows systematization of the types of interaction schemes applied in synthetic biology.

In digital networks, signal levels that are below or above the background value are considered 0 or 1, respectively. A logic gate, which is an abstract device that reacts in a certain way depending on the sum of conditions, is the main term used for these networks. When considering a cell, the sum of conditions are structural (qualitative) or quantitative characteristics of certain molecules A and B (inputs A and B) inside the cell or in the external environment, which are required for qualitative or quantitative changes in the molecule Y (output Y). There are 3 main logic gates (NOT, OR, and AND) and several combinations of them. The presence of at least two conditions to receive a response (except for the NOT gate) is essential. From the point of view of electric circuitry, an example of lactose operon discovered by F. Jacob and J. Mono is the ANDN gate (a combination of AND and NOT). For the expression of beta-galactosidase, the conditions for the simultaneous presence of lactose and the absence of glucose should be met (Fig. 1) [1].

The XOR logic gate is often represented by more complex interactions. The output signal of this gate is observed only when one of the substrates is present. Figure 2 shows an example of the artificial network in which a green fluorescent protein (GFP) is expressed in the presence of either isopropyl β-D-1-thiogalactopyranoside (IPTG) (input A) or anhydrotetracycline (aTc) (input B) [8, 9].

The NOR logic gate describes a situation where an output signal is observed only if there are no certain stimuli at the input. A network in which the NOR gate was constructed by adding the inducible promoter to the gene encoding the effector gene repressor could be such an example. Upon activation of any of the promoters of this gene or both promoters, the expression of the repressor is immediately induced; the latter binds to the promoter of the effector gene, inhibiting its expression [10].

The remaining logic gates are also applied in SB, and their various combinations expand the scope of AGCs, mediating the development of engineering in biology [11].

Multilayer and Analog AGCs

Examples of simple AGCs were given above. However, it is necessary to design more complex systems to implement a more precise regulation. As a rule, in such networks, the outputs of some gates are inputs for others. These AGCs are usually called multilayer AGCs. As in the case with simple AGCs, multilayer systems are also aimed at a single result: the activation or repression of the gene expression or regulation of a particular enzyme function.

The complication of multilayer AGCs occurs through an increase in the number of inputs (more than two for the first layer) and outputs (more than one for the first or last AGC layer). For a long time, the design of such AGCs has remained difficult due to a large number of cross interactions of the components during their simultaneous application. In 2017, a principle for the construction of monolayer AGCs in

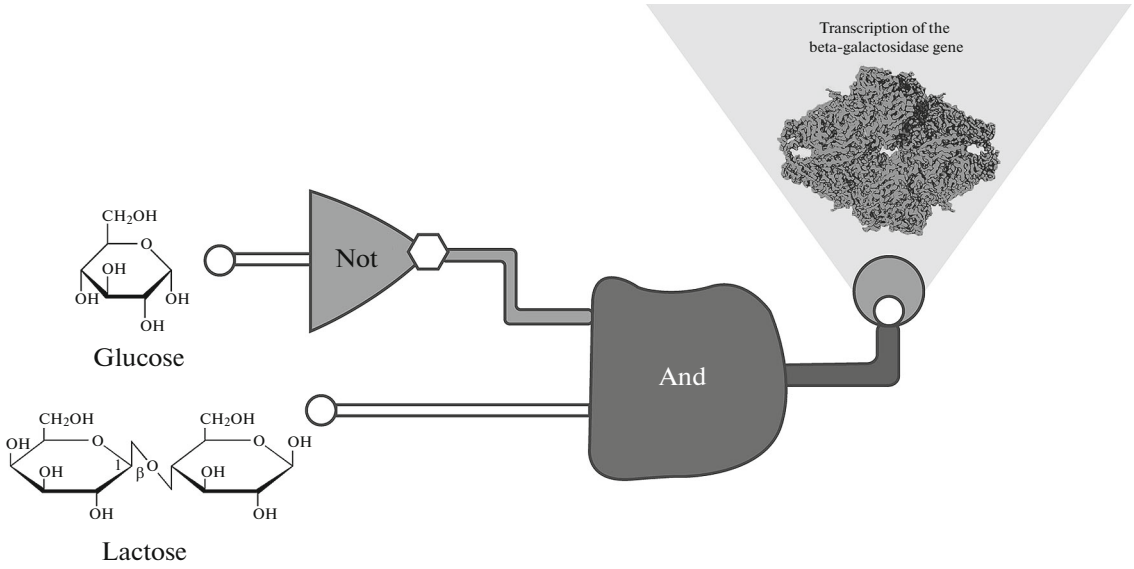

Fig. 1. Complex logic gate ANDN using an example of lactose operon. Beta-galactosidase transcription occurs only in the presence of lactose and the simultaneous absence of glucose.

mammalian cells, which was based on the mechanisms of site-specific recombination, was proposed. In this case, tyrosine recombinases were used to excise sites surrounding regulatory sequences, while serine sites were used to invert these fragments, which resulted in the activation of the promoter or terminator. This approach allowed the construction of all 16 logic gates [11].

It should be noted that most AGCs are based on the discreteness principle, like electrical networks in which two states may occur: the presence of a signal (1) or its absence (0). However, biological systems cannot be completely discrete and are mostly analog. When constructing discrete networks, insignificant background values and signal amplitude are usually neglected if they do not affect the overall functionality of the network. As a result, the design of analog AGCs is one of the main vectors in SB development. According to some authors, analog AGCs will make it possible to create "cellular computers" [12]. In 2013, AGCs that implemented mathematical functions, the digital representation of which would require the construction of an extremely complex multilayer network with a large number of gates, were proposed. Analog implementation included only two transcription factors, the functions of which were modulated by a wide range of inducer concentrations. The modulation was based on positive feedback loops. In this case, the activation of both feedback loops was interpreted by the cell as a relationship between them [13].

Thus, the challenges of modern biology require the design of both analog AGCs, the potential of which can be successfully applied, e.g., in the field of biosensors (which is described in the section "Applications" in detail), and complex multilayer networks that allow the implementation of more complex functions inside the cells.

Half Adders

Examples of such multilayer networks are, in particular, half adders and half subtractors. Two connected half adders form an adder. In digital computing, adders are key components of shift registers, binary counters, and serial-to-parallel converters. A half adder is a multilayer logic circuit consisting of basic gates but containing two outputs. The first gate reflects the sum of the inputs (SUM); the second gate also reflects SUM and, at the same time, transfers the result of the sum to the next half adder (CARRY) as an input. The design of such systems in prokaryotes allows the improvement of cell control and decision making in the production of drugs and biofuels, biosensors, probiotics, etc. At the same time, half adders in eukaryotes can contribute to therapeutic applications. For instance, it was possible to implement an orthogonal half adder in *E. coli* using arabinose and rhamnose as inputs (the presence of each of them was equal to 1, and the absence was equal to 0), a red fluorescent protein as the SUM output (its appearance in the system corresponded to a sum equal to 1), and a green fluorescent protein as the CARRY output (its appearance in the system was equal to 2) [14]. Orthogonality in the context of SB usually means the absence of the influence of artificial regulatory elements on the native components of the cell. Unexpectedly for the authors themselves, their study on the design of these circuits allowed determination of the optimal topology of the network elements for its more efficient functioning [14].

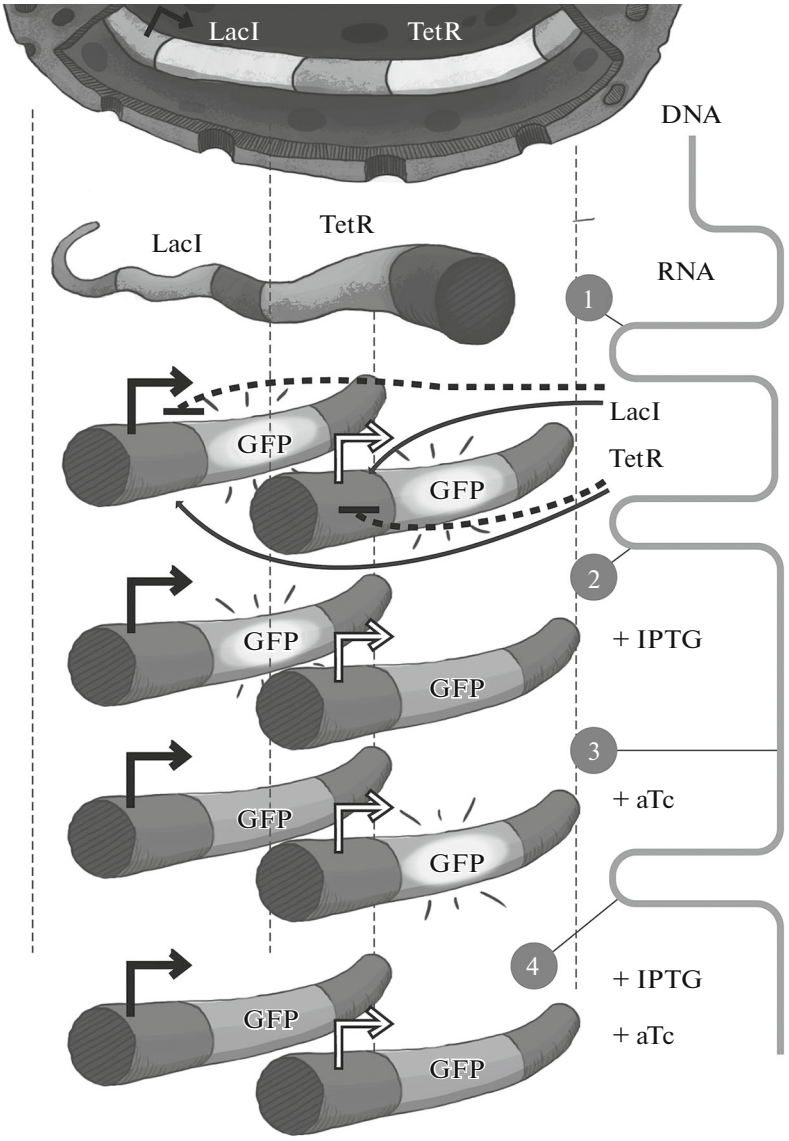

Fig. 2. Scheme of cross induction/repression. From top to bottom: the LacI and TetR repressor genes on the DNA template; mRNA repressors. Sharp arrows indicate induction; T-shaped dashed arrows show repression. Expression of two proteins LacI and TetR occurs constitutively from one promoter. The system contains two genes encoding GFP under the control of different promoters. LacI and TetR are inducers of one of the promoters and repressors of the other. Thus, when both proteins are active, they inhibit corresponding promoters. When IPTG is added, it binds to LacI, which removes repression from the TetR-activated promoter. When aTc is added, it binds to TetR, which leads to the release of repression from the promoter activated by LacI. If both IPTG and aTc are present in the medium, the repression is removed from both promoters. However, the expression of GFP does not occur in this case, since the induction is absent.

The possibility of implementing fundamental arithmetic operations with half adders and half subtractors using a mammalian cell culture was shown in 2012 [15]. Antibiotics and fluorescent proteins were used as inputs and outputs of the system, respectively, while the so-called RNA controllers (i.e., proteins or nucleic acids that affect translation by binding to specific mRNA motifs) served as the core of the multilayer network. For instance, the MS2 phage coat protein (the MS2 box binding the RNA motif) and the archaeal ribosomal protein inhibit the translation of mRNAs containing the corresponding boxes. Using these characteristics, as well as antibiotic-dependent transcription transactivators, it is possible to construct complex networks possessing predetermined properties. An important result of a series of works devoted to half adders was the implementation of the full adder using the example of a 3D mammalian cell culture [16]. Due to the almost unlimited total computing power of cell cultures, as well as a deterministic response to various endogenous and exogenous stimuli, full adder-based AGCs can provide new possibili-

ties in tissue engineering and can significantly simplify the screening of potential drugs using 3D cultures in the future.

As mentioned above, the engineering of biological networks is driven by the idea of a direct analogy between a cell and a computer. According to such engineering logic, a molecular toolkit used to design AGCs and functioning in a wide range of possibilities should be developed. Since engineering involves the formation of networks from interacting elementary blocks (functional parts), the approaches to obtain these blocks should be determined.

Elementary Blocks and Regulation Levels

Currently, two approaches to obtain new functional parts of gene circuits are used:

(1) An approach that includes searching for natural functional elements in databases of gene sequences (part mining).

(2) An approach that is based on the design of targeted regulators, such as chimeric transcription factors linked to the nuclease protein of the system of short palindromic repeats, which are regularly clustered (CRISPR/Cas) or contain transcriptional activator-like TALEN effectors. Such regulators can also be represented by toehold riboswitches and others.

At the same time, the first approach is complicated by the requirement of orthogonality when selecting the regulatory parts of the AGC. The specificity of the second approach is determined by the initial requirements of orthogonality to the constructed parts. The result of applying any of these approaches is a molecule or a set of molecules that perform their function at one of the possible levels of regulation: gene, transcriptional, post-transcriptional, translational, or post-translational levels.

Gene Regulation

Regulation at the genetic level occurs due to the modification of the DNA sequence of the regulatory element or its environment. Recombinases are among the most commonly used tools to design artificial gene circuits with predetermined properties. The convenience of using recombinases is associated with their orthogonality, on the one hand, and their high diversity, on the other hand [17]. The autonomy of recombinases and the variety of their functions (deletion, integration, and inversion of DNA fragments) are other advantages. It is also noteworthy that technologies for producing artificial recombinases specific to certain sequences currently exist. They give more opportunities for constructing AGCs. Recombinases can be used to design both simple circuits in which an inverted region of the promoter is often used for reversible inhibition of transcription and complex networks via cascade activation or multilayer algorithms for activating AGC elements. At the same time, the use of recombinases makes it possible to avoid constructing multilayer networks based on logic gates. For instance, a regulatory network associated with the formation of genetic memory has been recently tested. The design of this network is also an example of the engineering of biological circuits. In the network described, chemical stimuli were used to induce the expression of recombinases (a separate stimulus for each recombinase). Depending on the expression of the particular recombinase, the latter performed either inversion or excision of the specific site, which resulted in the changed register and memory formation for the latter state [18].

Directed genome editing systems belong to another subtype of regulation at the genetic level. The proven TALEN systems based on zinc finger proteins (ZFN) and CRISPR/Cas have become widespread in the design of gene circuits [19]. Proteins act as modules that recognize target sequences in ZFN and TALEN. For most cases of gene circuit design, this is not convenient since any changes in the design of the network require a complete "reassembly" of guiding proteins. At the same time, CRISPR-based systems are much more convenient, since the guiding element in them is RNA. Today, the design and production of the latter is a routine procedure [20, 21].

Transcriptional Regulation

Regulation at the transcriptional level is considered the most important aspect, regarding both the spatial and time-dependent organization of the functionality of regulatory gene circuits. In contrast to the gene level, the transcriptional level allows both qualitative and quantitative regulation of the expression of individual circuit blocks. Most AGCs are based on the interaction between the blocks through the spatiotemporal regulation of transcription.

Protein transcription factors represented by transcriptional activators or repressors are commonly used in the design of artificial circuits. At the same time, not every transcription factor can become an element of the synthetic regulatory network. Regarding SB, only orthogonal transcription factors are of importance, which affects their diversity during AGC design. In the case of prokaryotes, *LacI*, *TetR*, and *cI* are most commonly used, while in eukaryotes, these are *VP16*, *Gal4*, *KRAB*, and *tTA*. Therefore, numerous research groups have focused on obtaining artificial orthogonal pairs of the promoter and transcription factor. Some of these studies are very successful. In particular, 12 variants of transcription factor *cI* of bacteriophage λ have been obtained. These variants exhibit both repression and activation activity towards the controlled genes with bidirectional promoters. Moreover, 270 different sequences of artificial promoters have also been proposed for this transcription factor. This allows the design of a complex network

using only different *cI* variants and combinations of promoters as elementary blocks [22].

Post-Transcriptional Regulation

Protein regulators represent a large class of regulatory macromolecules that modulate gene expression depending on external conditions, but they are not the only class. Another class combines regulatory RNAs the diversity of which allows the design of both "digital" and "analog" AGCs. For instance, the integration of a protein-binding aptamer into the 5'-untranslated region of eukaryotic mRNA allows regulation of its translation [23]. Autocatalytic ribozymes, which are represented by relatively short RNA motifs that can catalyze RNA cleavage upon the conformational change, are applied in SB. In particular, the artificial integration of the ribozyme into various regions of eukaryotic RNA was shown to lead to the cleavage of the latter and a decrease in the expression of the corresponding gene [24]. Ribozyme activity can be controlled by integrating an aptamer into its structure, inducing the activity of the obtained aptazyme by specific ligands. At the same time, the integration of the aptazyme into the mRNA structure makes it possible to control the expression of a certain gene by these ligands [25]. Most AGCs that function as logic devices are constructed on the basis of protein regulators interacting with low molecular weight compounds (as a rule) that determine their activity. However, RNAs can also play the role of all elements of signal transmission systems: sensors, processors, and actuators. A study supporting this fact has been reported. In this study, the main logic gates were based on RNA: the AND gate was constructed by integrating two aptazymes into the 3'-untranslated region of mRNA; each of them interacted with its ligand. A similar effect was achieved by the formation of the aptazyme controlled by two aptamers specific to different ligands [26].

Translational Regulation

The creation of artificial functional elements of the cell also focuses on translational machinery. Studies carried out in this area include both the design of orthogonal ribosomes interacting with certain mRNAs [27] and the construction of the translational apparatus oriented towards an artificial genetic code [28]. In particular, it was shown that natural ribosomes could incorporate more than 150 noncanonical amino acids into synthesized proteins [29–31] and catalyze the polymerization of nonpeptide polymers (for instance, polyesters) [32]. The possibility of using the strategy of multiplex automated genome engineering (MAGE) to produce highly selective and orthogonal aaPC-tRNA pairs facilitates the incorporation of noncanonical amino acids into the polypeptide chain in vivo [33].

Post-Translational Regulation

Engineering principles are also used to reconfigure native biological networks. For instance, artificial switching of MAPK activation pathways has been shown [34] (Fig. 3). Such a switch is possible if a common element, which binds to scaffold proteins, is present in signaling pathways. Later, the same research group proposed the concept of artificial scaffold proteins. The specificity of binding of different cascade elements by these proteins can be predetermined by linking the amino acid sequences that show affinity to the scaffold domain and the target proteins. At the same time, due to the predetermination of the strength of binding of the amino acid sequence to the scaffold, it was possible to achieve precise regulation of this signaling pathway, for instance, by adding units or, conversely, through their deletion [35].

Inteins are other important protein tools of SB. Cis- or trans-splicing of inteins is used for the delivery of large constructs into mammalian cells [36], the detection of protein–protein interactions in vivo [37], ligand-dependent activation of proteins [38], redox-dependent activation [39], etc. With zinc finger transcription factors (ZF-TF), intein trans-splicing was used to construct NAND and AND logic circuits [40]. Using a similar scheme for TALE, the simplest AND network with three INPUTs was designed. To achieve this, trans-splicing of three segments of the dnaB mini-intein was used [41]. A bit later, inteins were used to construct a band-pass filter, which is widely applied in SB for the product expression only at the medium concentrations of INPUTs. Such a filter often requires the presence of the AND gate, which was a designed system [42].

Applications

The greatest expectations for the use of synthetic biology technologies are associated with medicine. Created and developed concepts of SB enable optimistic forecasts of the formation of new classes of diagnostic and therapeutic solutions. In some cases, these solutions have already been implemented in medical practice. Both molecular components of gene circuits and modified cells are applied. At the moment, registration procedures for new types of test systems based on toehold RNA switches are underway. The characteristic feature of these systems is the possibility of accurate detection of a certain RNA sequence. In particular, similar test systems have been proposed for the rapid detection of Zika and Ebola viruses [43, 44]. The same technology with minor modifications was proposed to assess the profile of the microbiota and identify associated biomarkers of diseases [45].

Today, various aspects of the application of the principles and tools of SB, ranging from a deeper understanding of cellular functions to diagnostic and therapeutic systems, are considered. In contrast to the

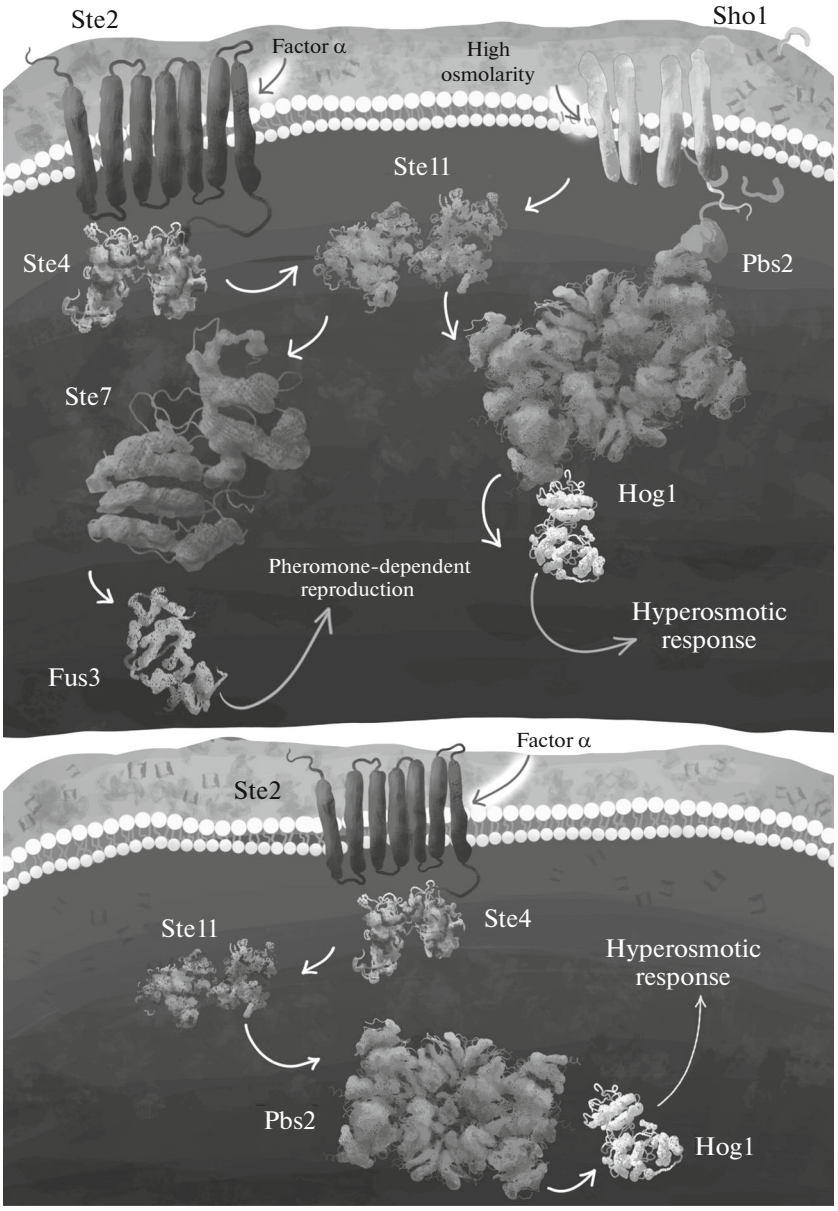

Fig. 3. MAPK pathway switching. Two different signaling cascades in yeasts are mediated by the Ste11 protein: (1) signaling an increase in osmolarity and (2) pheromone-dependent reproduction. Using this fact, a hybrid pathway was designed, the beginning of the signaling cascade in which proceeded to the Ste11 unit through the pheromone-dependent pathway, and then the signal was transmitted via the pathway of response to high osmolarity, i.e., cells responded to the pheromone as in the case of the hyperosmotic response. Upper left part: the pathway when cells respond to pheromone; upper right part: cells respond to hyperosmotic conditions. The combined pathway (due to the common Ste11 unit) is shown below.

early approaches of "blind" design in which alterations in cells often led to unpredictable results, the principle of directed design allowed the prediction of the behavior of the cell using certain tools to change the genome and phenotype of cells. This made it possible to increase the efficiency of the design, mainly due to the reduction of side effects arising from a large number of errors in blind design. For instance, the orthogonality of products of AGCs significantly reduced their impact on the intracellular environment. On the other hand, tools, such as the Boolean language, have increased the efficiency of the theoretical design phase, making AGCs more complex in structure and multifunctional.

The principle of orthogonality was also taken into account when creating molecular tools such as RNA switches, effector genes, recombinases, artificial promoters, etc.

One of the main achievements of SB is the absence of discreteness when making any modifications to the

genome. At present, we can predict the quantitative characteristics of the introduced changes with high accuracy, which is extremely important for medical and biotechnological applications of the products obtained using genome modification approaches. Currently, AGCs have become more and more "analogous." The level of expression of individual parts of AGCs can be regulated by external factors and through feedback loops.

Microbiota is also an important object in the development of new therapeutic approaches, which is explained by the relative simplicity of constructing bacteria that possess predetermined characteristics and by reduced risks compared to the intervention in human cells. Several approaches for the therapy of inflammatory bowel disease based on the secretion of anti-inflammatory cytokines by members of intestinal microbiota (*Lactococcus lactis*), including IL-10 [46], or antibodies targeted at proinflammatory cytokines [47], have been proposed. Approaches based on the use of the ability of bacteria to produce desired compounds, including therapeutic ones, are also considered in antitumor strategies. A solution based on *E. coli* modification to transfer a plasmid vector into human cells was proposed. This plasmid vector encodes short hairpin RNAs (shRNAs) that inhibit the production of beta-1 catenin, which is overexpressed in some cancer types [48]. The therapeutic potential of bacterial cells is not only their ability to produce specific compounds but also the possibility of targeted delivery of these compounds to the foci of the disease using bacterial cell wall modifications aimed at recognizing specific antigens on the surface of eukaryotic cells. As a result, the ability of modified *E. coli* cells to colonize tumor proliferation foci has been shown [49].

SB technologies for the modification of bacterial representatives of human microbiota give opportunities for the treatment of not only local intestinal diseases. Researchers also focus on infectious and metabolic diseases. A therapy using AGCs applied to microbiotic consortia is being developed. For instance, the strategy of reprogramming intestinal cells into insulin-producing cells by constructing the GLP-1 secreting *Lactobacillus gasseri* strain has been demonstrated using animal models [50]. The microbiota-based approach provided an effective strategy for preventive anti-HIV therapy. Modification of the vaginal microbiota member, *Lactobacillus jensenii*, aimed at constructing a cyanovirin-producing strain that was active against HIV, reduced HIV abundance in experimental macaques by 63% [51].

CAR-T-cell therapy is among the most important examples of the introduction of SB technologies into clinical practice in modern world healthcare. It is based on the artificially constructed antigen receptor, the induction of which leads to antitumor activation of T cells [52]. At present, numerous studies aimed at increasing the efficiency of CAR-T and developing alternative (including ligand-induced) variants of this method are carried out. One of the options is the use of synthetic Notch receptors (SynNotch), the activation of which by surface antigens allows designing more precise systems activated by free ligands or surface antigens and ultimately inducing the transgene expression [53]. SB concepts as therapeutic strategies are most actively developed in the area of immune cell modification through a combination of various soluble and transmembrane receptors. It is mainly aimed at achieving the orthogonality of artificial cascades of high specificity. The development of theranostic approaches for the diagnosis and therapy of metabolic disorders is also performed. For instance, a gene circuit that produces insulin in response to a decrease in pH has been designed [54]. At the same time, a strategy for the regulation of blood glucose levels has been proposed. It is based on gene circuits containing optogenetic sensors and has been successfully tested in animal models [55]. One more AGC that functions according to the principle of a feedback loop to maintain the balance of fatty acids in blood has also been developed [56].

The imbalance of metabolites, hormones, and cytokines is considered to be the main cause of metabolic endocrine and inflammatory diseases [57]. The majority of these compounds are extremely important for the normal functioning of the body, and, therefore, a possible therapeutic strategy is to restore their balance through AGCs that function according to the feedback loop principle. The application of this strategy allowed for designing of a system to control blood glucose levels by constructing an AGC in which insulin expression was activated by a glucose sensor: a glucose-sensitive calcium channel [58].

Due to the convergence of SB with other areas of science and technology, approaches to the diagnosis of acute conditions are developed. For instance, a combined sensor composed of bacteria carrying a heme-sensitive AGC and an electronic part that sends a radio signal in the case of activation of the gene circuit by the heme has been developed [59]. The designed sensor detects bleeding in the gastrointestinal tract and is a breakthrough in the diagnosis of this condition. Despite the success, applications of SB solutions in medicine are at the very beginning, which is demonstrated by the concepts that are currently implemented [60].

However, the use of SB is not limited to medical applications. International commercial companies also use advances in SB, for instance, to develop bio-isoprene produced from recycled raw materials and used to make car tires without refined products. To achieve this, the modified bacteria that efficiently process sugars obtained from sugar cane, corn, millet, and other biomass to produce isoprene are used. The most important step in this process is the tuning of metabolic pathways to obtain 3,3-dimethylallyl pyrophos-

phate (DMAPP) from which the final product is obtained using the isoprene synthase enzyme.

The principles and methods of SB allow reconstruction of the processes of cell differentiation controlling the cell fate. The approach [61] demonstrates the possibility of modifying transcription factors using transactivating domains linked to the ends of proteins (for instance, OCT4, SOX2, NANOG, and KLF4). Their fundamental role in the reprogramming of fibroblasts into induced pluripotent stem cells (iPSCs) is widely known.

Another example is the study on the production of glucose-sensitive insulin-secreting beta cells from iPSCs by constructing a synthetic regulatory network [62].

Sensors of toxic substances in water, soil, and food were among the earliest applications of SB. To detect heavy metals and antibiotics, simple schemes, which include a reporter protein and a sensitive element that transmits a signal to the reporter, are used. For instance, the concept of the bacterial biosensor with oscillating fluorescence was proposed; the intensity and frequency of the fluorescence indicated the ambient arsenic concentration [63].

At the same time, it is possible to program microorganisms to remove harmful organic substances and heavy metals from the environment. The introduction of a metal-binding peptide into rhizobacteria degrading trichloroethylene (TCE) results in the simultaneous binding of metals and destruction of TCE [64].

SB is also widely applied in the biofuel sector. Various microalgal species are successfully programmed using the achievements of metabolic engineering to produce biofuel [65].

The Massachusetts Institute of Technology, in collaboration with several other universities, has been involved in the development of Cyberplasm: a microrobot, the functioning of which is based on the principles of SB. It is a complex system of mammalian muscle cells. This system obtains energy from bacteria. Optogenetically modified muscle cells are required to move the microrobot in water, mimicking an undulating movement. The coordination of contractions is provided by the electronic biomimetic central pattern generator (CPG). In nature, the CPG is a neural ensemble, the members of which generate the joint output activity of the ensemble ordered in time and according to targets, which serves as a command for the muscles [66]. An electronic analog sends signals to muscle cells through an organic light-emitting diode (OLED) obtained by 3D printing using the Kapton polyimide film. OLED is responsible for coupling the excitation/contraction processes. The electronic device functions due to a "microbial fuel cell" integrated into the "body" of the robot. The entire system is in the hydrogel, which separates it from the external environment [67]. According to the idea of the project executors, the microrobot should imitate the behavior of the sea lamprey.

CONCLUSION AND PERSPECTIVES

Thus, the current stage in the development of synthetic biology is characterized by the formation of engineering principles for the design of regulatory networks, as well as the development of tools that include all known types of biological macromolecules and supramolecular structures. The main task in this field is the search or design of orthogonal systems, the functioning of which inside cells will not affect the native metabolic and biogenetic pathways. We believe that the trend of SB engineering will continue to develop and will ultimately result in the formation of a common base of elementary blocks. Potentially, combinations of these blocks will implement any functionality, and the task of researchers will be reduced to the correct selection of elements of the developed network.

The abundance of possibilities of synthetic circuits, even minimally illustrated in this review, shows their wide application in various fields, although SB emerged relatively recently as a science and is at the initial stage of its development. Therefore, in the medium term, the further success of SB in medicine, ecology, biotechnology, and other industries is expected.

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COMPLIANCE WITH ETHICAL STANDARDS

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ADDITIONAL INFORMATION

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