ISSN 2616-5910 Vol. 4, Issue 1: 54-61, DOI: 10.25236/AJALS.2023.040108

# Research progress in artificial gene circuit

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***Abstract:*** *A gene circuit is a genetic device consisting of gene regulatory elements and regulated genes. The basic theory of gene circuits includes the theory of synthetic biology and the regulation of gene expression. Gene circuits consist of logic gate gene circuits, bistable switches, and gene oscillators. The biggest challenge facing artificial gene circuits today is predictability. The behavior of artificial gene circuits within chassis cells may deviate from the predetermined behavior due to chassis-circuit coupling, the complexity of the circuits themselves, and other reasons. Rational design of gene circuits can be achieved by using mathematical modeling tools and computer support. Artificial gene circuits play an important role in chemical production, medical treatment, and life science research. This paper summarizes the knowledge about artificial gene circuits and describes the existing results and future developments in this field.*

***Keywords:*** *Synthetic Biology, Artificial Gene Circuits*

## 1. Introduction

There are a large number of natural genetic circuits in nature, which are an indispensable part of the living system [1].

The development of synthetic biology has led to a focus on using engineering concepts to achieve standardization, modularization, and systematization of biological research, and more and more standardized biological components have been explored. Artificial genetic circuits are an important element of synthetic biology, and many researchers have borrowed the design concept of electronic engineering to construct artificial genetic circuits using standardized components in organisms. The bistable switch designed by Collin [2] at the transcriptional level and the gene oscillator designed by Elowitz [3] at the transcriptional level are not only the "milestones of synthetic biology", but also demonstrate the possibility of rationally designing artificial genetic circuits. In synthetic biology genetic circuits, basic biological components have been used to design and construct synthetic devices such as gene switches [2], oscillators [3], and logic gates [4] to realize the reprogramming of living systems and make them meet the needs of human production and life.

Unlike electronic circuits, genetic circuits designed in vitro are difficult to function as intended by the designer after transfer to the organism due to the complex environment inside the cell and the complexity of the biological system itself. To solve these problems, scientists have proposed optimization schemes such as orthogonalization [5] and isolating elements [6] using synthetic biology modeling and computational research to make genetic circuits behave predictably and stably in cells.

The design and construction of artificial genetic circuits has not only greatly contributed to the understanding of the basic laws of life regulation, but also further enriches the means of modifying and designing natural biological systems from scratch, and provides brand-new solutions for practical needs in the fields of medicine and health [7,8], material design [9], and industrial fermentation [10], etc.

This paper will provide an overview of the theories and models regarding artificial genetic circuits within the given space. At the same time, this paper extensively references mathematical methods, such as differential equations, to quantitatively analyze the model of the artificial gene circuit. To conclude, the applications of artificial gene circuits in life science theoretical research, medical treatment, and other fields are summarized, and the future of gene circuits is discussed.

ISSN 2616-5910 Vol. 4, Issue 1: 54-61, DOI: 10.25236/AJALS.2023.040108

## 2. The Basic Theory of Gene Circuits

### *2.1 Gene Expression Regulation*

The mechanisms and principles of gene expression regulation in prokaryotes and eukaryotes are the basis for the construction of synthetic gene circuits. Gene expression regulation refers to the manner and mechanism by which the entire process of regulating the flow of genetic information from DNA to proteins in an organism is regulated.

#### *2.1.1 The main features of gene expression regulation in prokaryotes:*

(1) Regulation of operon. A operon is usually composed of several related structural genes encoding proteins and control genes controlling regulatory proteins. In 1961, F. Jacob and J. Monod proposed the lactose manipulator model based on the utilization of lactose by Escherichia coli. This model explicitly introduced the concept of gene expression regulation for the first time [11]. Based on the theory of Lac operon, the later proposed the model of tryptophan operon, arabinose operon and so on.

(2) Transcription and translation are continuous in space and time. Prokaryotes do not have a structure similar to the nuclear membrane, and their transcription and translation are continuous in the same space.

#### *2.1.2 Main characteristics of gene expression regulation in eukaryotes:*

(1) Characteristics of interrupted genes. Most eukaryotic genes consist of protein coding sequences and non-coding sequence, the coding sequences are called exons and the non-coding sequences become introns. Selective splicing of introns is an important step in the transcription and processing of eukaryotic genes to produce mature mRNA molecules.

(2) Temporal and Spatial Specificity of Gene Expression. Eukaryotic cells turn on different gene expressions in different locations at different times.

(3) Transcription and translation are spatially and temporally separated. The existence of the nucleus makes the eukaryotic cell divisible into two parts: nucleus and cytoplasm. The complex structure makes eukaryotic gene expression more complex and hierarchical than that of prokaryotes.

Based on the theory of gene expression regulation, one can optimize gene circuits from different expression levels. For example, the concept of riboswitch was proposed in 2002 [12]. Based on the theoretical study of riboswitch, Ogawa [13] and others artificially constructed an aptamer-based riboswitch to regulate transcription by exploiting conformational changes in the RBS (ribosome-binding site) sequence of mRNA.

### *2.2 Synthetic biology*

Synthetic biology is a young interdisciplinary field that combines biology with engineering technologies that can benefit the agricultural, manufacturing, fuel, environmental and medical sectors. Synthetic biology focuses on the design and construction of novel biological components or systems as well as the re-engineering and application of pre-existing biological systems in nature. The engineering character of synthetic biology is what distinguishes it from traditional life science disciplines. This characteristic is mainly reflected in its "bottom-up" positive engineering "strategy", which is manifested in the use of standardized biological components to construct universal biological modules that are adapted to the chassis cells, including the understanding of the pathways of life processes, the composition of networks and their regulation, and the "orthogonalization of life", which is the key to the development of new biological components or systems. The construction of genomes (including the synthesis of prototype cells and other "cell engineering") is the most important engineering platform [14].

An important goal of synthetic biology is to realize the plug-and-play of genetic circuits, so as to achieve complex network regulation with basic genetic elements. This concept is best embodied in standardized biological modules——bioblocks. Bioblocks include gene modules, subcellular modules, gene networks for biosynthesis, metabolic pathways and signaling pathways, and transporter mechanisms.

The construction of bioblocks is to realize the standardized combination and construction of biomodules with corresponding functions in living cells, so as to construct biological systems. As long as the standardized biological modules with standard enzymatic sites, they can be called bioblocks. Currently, bioblocks have formed a corresponding module database - iGEM Registry (http://Parts.igem.org/Catalog).

ISSN 2616-5910 Vol. 4, Issue 1: 54-61, DOI: 10.25236/AJALS.2023.040108

### *2.3 Composition of gene circuits*

Gene circuits consist of logic gate gene circuits, switch gene circuits, and gene oscillators. The theory related to logic gate gene circuits is developed based on the knowledge related to the discipline of electronic circuits, and researchers use biological components to build logic circuits that can be rationally analyzed inside the chassis cells. Switching gene circuits can realize gene state transitions under exogenous stimuli, of which bistable switching is one of the most typical models [2]. Gene oscillators are important regulatory elements that have been synthesized based on basic biological components to achieve artificial programming of gene circuits and even living systems [3].

#### *2.3.1 Logic Gate Gene Circuit*

Just as Boolean logic gates are widely used in electronic circuits to build digital devices, logic operations are encoded in gene regulatory networks. Logic-gated gene circuits in synthetic biology have their origins in the logical operations of digital circuits, and their control theory and the design rules of logic circuits are used to study the logical relationships and regulatory methods of gene circuits. In the logic gate gene line, complex biology is abstracted into the relationship between 0 and 1, which helps us better understand the function of gene regulation.

Using standardized biological components, logical operations such as AND gate, OR gate, NON gate, NOR gate, NAND gate and XOR gate can be implemented. Based on logic gate operations, researchers can design different gene circuits for their experimental purposes. For example, Liu et al [4] constructed an E. coli AND gate circuit to study the interaction of exogenous genetic circuit with chassis cells. Moon et al [15] demonstrated the feasibility of constructing large-scale integrated circuits in single cells based on AND gate circuits.

#### *2.3.2 Bistable Switch*

Bistable switches can be artificially regulated to realize the switching of genetic circuit between two different stable states. Gardner et al. designed a bistable switch at the transcriptional level based on the role of specific proteins in regulating the expression of specific genes [2]. As shown in Figure 1, arrows indicate promoters, rectangles indicate coding genes, and straight lines indicate repressive relationships, promoter 1 is used to express repressor 2, and promoter 2 expresses repressor 1. The switching state can be changed by adding inducers. Fluorescent proteins are used as reporter genes to represent the switching state of the circuit. The bistable switch can be switched between two steady states when the strengths of the two promoters are balanced by setting up a differential equation for simulation.

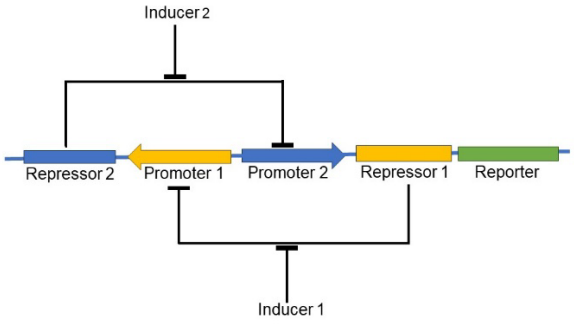


*Figure 1: Simplified Model of a Bistable Switch.*

#### *2.3.3 Gene Oscillator*

Gene oscillation is a gene regulatory mechanism that determines the timing of gene expression by the amplitude and period of the oscillation. This temporal control of gene expression allows a relatively large number of genes in a large gene network to be regulated by only a small number of regulatory factors, thereby enabling the regulation of complex cellular behavior. A feature of the classical oscillator is that each promoter is followed by a repressor. Elowitz [3] et al. constructed gene oscillators at the transcriptional level. They connected three gene modules whose expression products repress each other in series to form a ring structure, and the function of the oscillator can be realized by using the repression and de-repression of the gene modules to repress each other. As shown in Figure 2, the arrows indicate promoters, the straight lines indicate repression relationships, and the rectangles represent coding genes. The three promoters slowly interlock to form a complete oscillator, the repressor proteins oscillate from week to week, and the expression of the output fluorescent proteins is constantly changing. The behavior of this model can also be simulated with differential equations, and the simulation results show that the

ISSN 2616-5910 Vol. 4, Issue 1: 54-61, DOI: 10.25236/AJALS.2023.040108

oscillator can continue to oscillate stably under stable conditions.

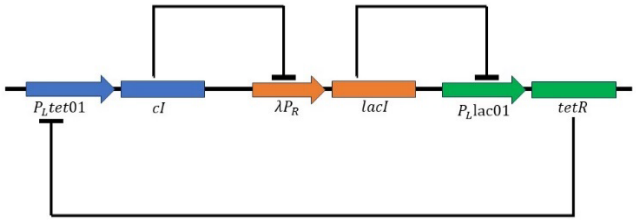


*Figure 2: Simplified Model of a Genetic Oscillator*

## 3. The Challenges and coping strategy of Artificial Gene Pathways

### *3.1 Predictability of gene circuits*

Conventional approaches to genetic circuit construction typically involve either constructing circuit prototypes and then modifying the underlying components through iterative trials to finally achieve the desired performance, or using directed evolutionary approaches to mutate and screen out target genetic circuit. The disadvantage of these approaches is that they lack predictability and require significant time and effort to obtain functional circuits. The predictability of genetic circuits is limited by the function of the circuit and its intended use. Factors affecting the predictability of circuits include the complexity of the environment in which the gene circuits are located and the complexity of the circuits themselves [16]. The former of these is mainly reflected in the coupling of the chassis to the loop.

#### *3.1.1 Chassis-loop coupling*

In recent years, a large amount of experimental evidence has shown that gene circuits cannot be strictly isolated from host cells, and the two will inevitably be coupled into a whole. Artificial gene circuits can have unpredictable and interfering effects on cell physiology, and these interfering effects also make the theoretically "modular" biological components and gene circuits lose their predictability and are no longer "modular". This leads to the fact that artificially designed components in one organism are not directly usable in another organism, as the behavior of the components may deviate from the expected once they are in the informative environment. This results in synthetic gene circuits that cannot be "plug-and-play" like electronic components where simple components are used to build complex circuits step-by-step, but rather require a lot of time and effort to optimize the components in individual chassis organisms on a point-to-point basis.

The gene expression of synthetic circuits is regulated by the resource allocation mechanism of chassis cells, and the expression of synthetic gene circuits consumes the resources of chassis cells. The expression of synthetic gene circuits occupies the resources related to the growth and metabolism of chassis cells and affects the normal growth of cells [17], which leads to changes in the physiological state of chassis cells and affects the function of the circuits. Competition for enzyme and protein machinery by exogenous gene circuits is a major factor contributing to this stress. For example, when yellow fluorescent protein and cyan fluorescent protein with the LAA protein degradation tag are overexpressed in the same E. coli, the intracellular ClpXP degradation machinery may be overloaded, and the two different fluorescent proteins must enter the degradation channel of ClpXP sequentially at this moment just as the queuing behavior of human beings does, resulting in the interference of the degradation process of the two unrelated proteins [18]. This mechanism is known as the queuing effect. Similarly, when the number of ribosomes in a cell is insufficient, there is competition for ribosomes for mRNA translation, resulting in a queuing effect of unrelated mRNA translations.

The expression of exogenous genes perturbs the chassis cell as a system, leading to increased physiological heterogeneity of the chassis cell. This heterogeneity leads to a relatively low expression of a portion of the cell to obtain a relatively fast growth rate, this type of cell can gain an advantage in the successive generations of culture, and ultimately lead to the failure of the synthetic gene circuits, the evolutionary stability of the whole system is greatly reduced [19].

#### *3.1.2 Complexity of circuit function and validation.*

Competition for protein machinery and enzymes also occurs between different gene circuits. When genes in exogenous circuits are all competing for these limited resources, unintended interactions

ISSN 2616-5910 Vol. 4, Issue 1: 54-61, DOI: 10.25236/AJALS.2023.040108

between genes can occur. These interactions can dramatically alter the expected behavior of the gene circuits, leading to experimental results that are completely at odds with model predictions. In addition, complex gene circuits require more time for testing. For example, gene oscillators and bistable switches differ from simple logic circuits in that the former require a sufficiently long period of time to measure the output and the latter require multiple inputs. This can further increase the difficulty of applying genetic circuits.

### *3.2 Coping strategies*

#### *3.2.1 Orthogonalization of synthetic gene circuits*

Element orthogonalization of gene circuits is an effective way to avoid coupling of exogenous gene circuits to chassis cells. Ideally, gene elements should be as orthogonal as possible to their chassis cells to facilitate reuse and reliability in different cellular environments, i.e., minimal interference with host gene expression and low metabolic load on host growth. A number of orthogonal genetic devices and circuits have been constructed to achieve various functions and have demonstrated the great potential of using orthogonal elements to generate robust host cell behavior [5, 15, 20]. For example, an orthogonal AND gate circuit designed by Liu et al. [5] has been shown to work reliably in almost all seven commonly used E. coli strains, whereas the same circuit using an alternative endogenous promoter as an input (i.e., $P_{lac}$ replaced by $P_{lux}$) failed to work in six of the seven host strains [5].

#### *3.2.2 Optimization of Gene Circuit Feature Quantity*

Rationally designing the composition of gene circuits can effectively address predictive issues. For example, previous studies have shown that reducing the number of repetitive sequences in gene circuits and the expression of genes increases the genetic stability of the whole system. There may be a threshold level of gene circuit expression below which the predictability of gene circuits can be improved [19]. Quantifying the expression capacity can provide designers with a reference for constructing stable gene circuits [21]. In addition, Hossain et al. developed a novel non-repetitive parts calculator that efficiently designs thousands of highly non-repetitive gene parts based on specified constraints. A generalized algorithmic solution is provided for bioengineering without the need to introduce repetitive DNA, thus improving the robustness of biological evolution [22].

#### *3.2.3 Using Mathematical Modeling to Analyze and Predict the Behavior of Gene Circuits*

Gene circuits can be regarded as a system, and we can predict the behavior of gene circuits before implanting them into chassis cells by extracting the relevant variables to build a mathematical model, which can guide the construction of gene circuits in a quantitative way.

For example, Becske et al [23] constructed a differential equation model to analyze the linear stability of their constructed gene circuit based on a simple control system, and constructed the following differential equation model:

$$\boldsymbol{S_{unreg} = f'_{unreg}(R^*) = -k_{deg}}$$

$$\boldsymbol{S_{auto} = f'_{unreg}(R^*) = -\frac{nk_pPk_Iak_r}{\left(1 + k_pP + k_rR^*\right)^2} - k_{deg}}$$

$$\boldsymbol{S_r = \frac{S_{auto}}{S_{unreg}}}$$

The equations represent the absolute value of the stability of the unregulated system and the autoregulated system and the ratio of the stability of the two systems, respectively, where R is the concentration of the inhibitor ($\boldsymbol{R^*}$ at steady state), $\boldsymbol{a}$ is the constant of the ratio of the mRNA and protein concentrations, $\boldsymbol{P}$ is the concentration of RNA polymerase, $\boldsymbol{k_p}$ and $\boldsymbol{k_r}$ are the binding constants of polymerase and inhibitor, respectively, and $\boldsymbol{n}$ is the copy number of the gene, $\boldsymbol{k_I}$ is the rate of promoter degradation from the closure complex to the initiation complex, $\boldsymbol{k_{deg}}$ is the degradation rate of the inhibitor.

At the same time, based on this model, random perturbations can be applied to the steady state of the cell population to simulate the unique perturbed state of the cells and further simulate the real cellular environment [23].

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#### *3.2.4 Computer aided optimization*

More advanced control functions require more complex artificial genetic circuits, which can make design more difficult. One of the best ways to do this is to introduce computer-aided design. The addition of computer technology and system design simulations can greatly speed up the genetic circuit design process. For example:

GenoCAD is a web-based design software for designing protein expression vectors, artificial gene networks, and other gene constructs. The variables in this software are composed of basic biological components with biological functions. GenoCAD guides the user in the design process to design the desired DNA sequence by designing rules and strategies for DNA sequence syntax. GenoCAD treats each biological component as a variable to be used in the design of desired synthetic biology units by compiling the appropriate syntax. GenoCAD includes a flexible system for managing both public and user-defined genetic components. The variety of models in it allows users to design according to their individual needs [24].

Christopher A. Voigt's team at MIT combined engineering and biology to build the first generation of the Cello platform by introducing computer-aided design (CAD) software, widely used in engineering design, to genetic circuit design. Cello is an automated gene circuit design software that automatically compiles the target functional gene circuits into DNA sequences based on sensor inputs, circuit function, and corresponding chassis cell information. With this system, the research team realized the dynamic process prediction and steady-state switching of large-scale gene circuits, and realized the automated design of gene circuits in eukaryotes for the first time [25].

## 4. Application of Gene Circuits

The design and construction of artificial genetic circuits not only enriches people's understanding of the working mechanism of living systems, but also further promotes the design and modification of natural biological systems according to production needs. Genetic circuits have brought new solutions to the fields of production, medical treatment, and life sciences.

### *4.1 Application of Artificial Gene Circuits in Production.*

Dynamic regulation is an effective strategy to regulate the metabolism of engineered cells to maximize the synthesis of target products. Through gene circuits, it can be realized that engineered cells can sense the accumulation of a key intermediate metabolite in the cell and automatically regulate the expression of the pathway as well as the metabolic activity according to environmental changes. The Key Laboratory of Carbohydrate Chemistry and Biotechnology, Ministry of Education, Jiangnan University [10] established an autonomous dual control system (ADS) by combining the pathway with CRISPRi-based NOT gate and a new biosensor for key metabolites. Using the GlcN6P biosensor and the ADC system to initiate a feedback loop, the metabolic flux in Bacillus subtilis was balanced and optimized by detecting the level of the intermediate product glucosamine-6-phosphate (GlcN6P) and accordingly self-regulating the expression level of target genes for the production of the nutrient-rich n-acetylglucosamine (GlcNAc). This self-regulatory approach requires no external control, and the study also suggests that programmable genetic circuits can be extended to design other microbial cells and metabolic pathways. Peng [9] and others have incorporated E. coli with built-in artificial genetic circuits into the production of living materials to express pigmented and fluorescent proteins for fiber staining and contamination monitoring.

### *4.2 Application of Artificial Genetic Circuits in Health Care.*

In the field of disease prevention, gene circuits can be used to control disease transmission. For example, Fu et al [7] constructed a conditionally dominant lethal gene circuit in Aedes aegypti. In this gene circuit, the flight muscle-specific promoter P_FM controls the expression of the tetracycline transactivator (tTA) containing introns. Due to the sex-specific splicing pattern of Aedes aegypti individuals for the intron of the gene in question, this ultimately leads to normal development of male mosquitoes and flightlessness of female mosquitoes, thus inhibiting reproduction in wild populations and blocking disease transmission. Immune cells using gene circuits can realize diverse signal sensing and logic processing in complex tumor microenvironments to achieve precise treatment of diseases [8].

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### *4.3 Application of Artificial Genetic Circuits in Life Science Research.*

Artificial gene circuits are used to reprogram natural gene circuits to construct artificial life processes that are different from the known laws of evolution, which can be used to explore some fundamental scientific problems that are difficult to study in traditional biology. This approach is known as "knowledge engineering. For example, adaptive learning is a complex sequential logic function that is widespread from microorganisms to primates, and Zhang et al constructed a genetic circuit in Escherichia coli that enables a function similar to conditioned reflexes. They used logic gates and toggle switches to construct an artificial conditioned reflex gene circuit in E. coli. Andrews et al constructed an artificial gene circuit that can indicate the cell cycle of E. coli based on NOT gates and nonlinear kinetic analysis, providing a convenient tool for studying cell division and other life activities.

## 5. Summary and Prospect

This paper introduces the basics of gene circuits, explains important components such as logic gates and bistable switches, and points out the current challenges related to chassis circuit coupling and circuit complexity faced by synthetic gene circuits, and summarizes a series of countermeasures, such as orthogonalization and quantitative dataset rational guidance for artificial circuit design. Finally, we summarize the practical applications of gene circuits in industrial production, medical treatment, life science and other fields. In today's world where synthetic biology is continuously emphasized, artificial gene circuits will become an integral part of the future biological field. Microbial synthesis factories, green bio-manufacturing, novel medical therapies, etc. all require mature artificial gene circuit systems. In order for the artificial gene circuits to be applied in large-scale industrial production, it is necessary to make the gene circuits function stably in the complex biological environment. In this regard, we should make efforts in the following directions: (1) Continue to explore standardized gene components and establish a larger and more widely applicable standard gene component library based on experimental data and quantitative analysis, so as to realize the "plug and play" of gene circuits and reduce the influence of the genetic background of chassis cells on gene circuits. (2) Realize the rational design of gene circuits based on engineering principles and mathematical analysis, and establish a stable gene circuit system to meet production needs. (3) Develop more mature theoretical models to guide the artificial synthesis of gene circuits with the help of computer technology, and at the same time develop more convenient and efficient applications to meet the needs of artificial gene circuit design. In conclusion, we need to develop a more mature artificial gene circuit system to realize the efficient transformation from theory to actual production.

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