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Review Article

Synthetic biology: Recent progress, biosafety and biosecurity concerns, and possible solutions

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ABSTRACT

Synthetic biology is a new interdisciplinary research area that uses engineering principles as guidelines for biological investigation. With research goals to modify existing biological systems or to create new ones, the recent applications of synthetic biology have expanded approaches and tools for conventional biological research. In this article, we first briefly review the development and progress of synthetic biology over the past decade. Although the contributions of synthetic biology to basic life science research, human health, environmental protection, and even economic growth have been widely observed, potential biosafety, biosecurity, and ethical risks related to synthetic biology have also emerged in recent years as technology becomes less expensive, more mature, and more accessible. We provide a brief assessment of the risks associated with the possible misuse or abuse of this technology in various areas and discuss concerns from three points of view: biosafety, biosecurity risks, and ethics. Finally, to address challenges arising from the rapid progress of synthetic biology, technical, ethical, and regulatory measures were developed or discussed in recent years, including laboratory level precautionary measures for biosafety and biosecurity related to synthetic biology (such as genetic safeguards and firewalls), ethical codes of conduct for biological scientists, and regulations or oversight rules from personal, national, and international perspectives. A brief summary of these efforts is provided.

1. History of synthetic biology

Rational design and cell engineering have been desirable to biological researchers for decades. As the first step, recombinant-DNA technologies developed in the 1970s allowed scientists not only to engineer cells and create new biological functions for the first time but also to accelerate elucidation of the physiological and biochemical characteristics of cells. In the past 10 years, key technologies crucial to cell engineering, such as genome sequencing or synthesis, became less expensive and more accessible to researchers around the world. Based on these technical breakthroughs, synthetic biology, a new discipline that uses engineering principles as guidelines for biological research, has emerged to either modify existing biological systems or to create new ones. Since the advent of synthetic biology, significant progress has been made in two research areas of this field.

1.1. Creation of standard genetic parts or circuits to engineer natural organisms for new functions

In 2000, the first genetic circuits, a genetic toggle switch[1] and an oscillating network,[2] were established. Both circuits consisted of well-known DNA regulatory elements and were constructed based on simple mathematical models. These reports represent the birth of synthetic biology.[3] From 2004 to 2007, engineering principles were applied further. Several research groups made further attempts, introducing electrical or mechanical engineering principles into the study of biological systems. Many genetic stan-

Abbreviations: iGEM, international genetically engineered machine competition; CRISPR-Cas, clustered regularly interspaced short palindromic repeats with associated proteins; Sc. 2.0 project, synthetic yeast genome project; RBS, ribosome-binding site; 5′-UTR, 5′ untranslated region; BWC, Biological and Toxin Weapons Convention; HGP-Write, Human Genome Project-Write.

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dard databases, such as the Registry of Standard Biological Parts[4] and OpenWetWare[5] were established. The international conference on synthetic biology and international genetically engineered machine competition (iGEM) were first held in 2004, and synthetic biology has received widespread attention thereafter. Since 2008, various genetic circuits with increased complexity, robustness, and precision have been developed. Computational mathematical models were also applied to quantitate the interaction of cells or stimulate metabolic flux analysis to balance the physiological burden in a living organism. For example, a synchronized quorum of genetic networks with global intercellular coupling, which can generate synchronized oscillations of a growing population of cells, was reported, and computer modeling was set up to quantitatively describe the mechanisms driving bulk synchronization and wave propagation.[6] Successful construction of layered logic gates in *Escherichia coli* was completed by engineering a multiple input logic network, and a mathematical model was utilized to guide the connection of each circuit.[7] In 2013, a revolutionary genome-editing method, CRISPR-Cas (clustered regularly interspaced short palindromic repeats with associated proteins), was invented. dCas9, which lost the cleavage activity of Cas9 but retains the DNA-binding specificity, greatly facilitates the design of synthetic circuits.[8]

1.2. The synthetic genome

The first synthetic genome, the poliovirus genome, was successfully constructed in 2002. This work indicated the feasibility of *de novo* chemical synthesis of genomes from existing genomic sequences without reliance on natural templates. In 2008, a minimal prokaryotic genome, the chromosome of *Mycoplasma genitalium* JCVI-1.0, was successfully chemically synthesized and assembled.[9] In 2008, one-step assembly of 25 chemical DNA fragments into an intact *M. genitalium* genome was achieved in yeast by the same research group.[10] In 2010, an artificial *M. mycoides* cell with the expected phenotype and self-replication capability, "Synthia," was created.[11] These historical breakthroughs represent a research shift in biology research from the exploration of life to the creation of an organism with a desired phenotype. In 2009, the first synthetic yeast genome project (Sc. 2.0 project) was launched to redesign and chemically synthesize the entire *Saccharomyces cerevisiae* genome. In 2011, the right arm of chromosome 9 and the left arm of chromosome 6 were successfully chemically synthesized.[12] In 2017, five redesigned yeast chromosomes were completed; this accomplishment represents over one-third of the Sc. 2.0 project.[13] During this project, scientists gradually established basic principles and methodologies for the design and synthesis of yeast artificial chromosomes.[12] BioStudio, a software for design and reprogramming of a complete genome on computers, has also been published.[14] Recently, 16 natural chromosomes of *S. cerevisiae* were successfully fused into a single chromosome, similar to that found in prokaryotic cells; the artificial *S. cerevisiae* still has normal cellular functions.[15] These works suggest that boundaries between natural and artificial life have been broken, and that natural living systems can be simplified by synthetic biology approaches, providing new directions for exploring natural life. In June 2016, scientists announced that approximately $100 million will be raised to launch the Human Genome Project-Write (HGP-Write) with the goal of synthesizing a complete human genome within 10 years.[16] Aside from the *de novo* synthesis of genomes, new artificial versions of building blocks (nucleotides and amino acids) have been developed, which can yield orthogonal life forms using artificial genetic alphabets.[17] For example, researchers have successfully constructed a synthetic bacterium containing six kinds of nucleotides, including an artificial base pair.[18]

Although there are multiple definitions of synthetic biology, one common view is that synthetic biology is a multidisciplinary research area that combines biology with chemistry, mathematics, computer science, and engineering and focuses on engineering of biological systems by modifying, designing, and *de novo* constructing biological components with new functions. With rapid progress in the relevant technologies, it is expected that synthetic biology can overcome the limitations of natural evolution and create synthetic organisms with desired properties. This approach's development and application will not only benefit basic studies on the fundamental laws of life activities but also provide new tools and approaches to address many key problems facing human beings, e.g., energy shortages and environmental pollution.

2. Recent progress of synthetic biology

2.1. Tools

Some progress has been made in the development of synthetic biology tools to design and optimize biological systems. Transcriptional tools, such as synthetic promoters or RNA-based transcriptional regulation, are widely utilized for the precise control of gene expression. Via expansion of flanking sequences upstream and downstream of core promoters and through an increase in the promoter copy number, expected transcription efficiency can be achieved.[19] Many studies have shown that the translation initiation region of mRNA, including a ribosome-binding site (RBS) and the 5′ untranslated region (5′-UTR), plays important roles in the determination of translation efficiency of a particular mRNA.[20] During the design of a synthetic RBS or 5′-UTR, tools for post-transcriptional regulation to balance the expression levels of individual genes have been developed, such as RBS or UTR calculators.[21,22] Post-translation tools are also constructed to maximally control metabolic fluxes. A "codon harmonization" algorithm has been devised to improve functional protein expression.[23] In addition, scaffold strategies have been adopted to facilitate intermediate metabolite conversion for efficiency or redirection of metabolic fluxes.[24]

2.2. Biopharmaceuticals production

Microbes have been used as "cell factories" to produce valuable chemicals for decades, and the advancement of synthetic biology provides new tools and strategies to improve the efficiency and capabilities of these cell factories. One successful example is the production of artemisinic acid in yeast. In 2006, scientists engineered yeast by increasing farnesyl pyrophosphate production and introducing the amorphadiene synthase and cytochrome P450 genes from *Artemisia annua* to efficiently convert simple sugars to artemisinic acid;[25] the technology was later commercialized by the biotechnology company Amyris in 2013. Vaccine development by means of attenuated pathogens has benefited from synthetic biology too. For example, the replication and infectivity of poliovirus can be reduced through genome scale changes in codon sets in the genome; this approach makes the poliovirus vaccines safer.[26] In addition, synthetic vaccines may be safer than natural vaccines if artificial bases are utilized because such bacteria and viruses cannot replicate in the human body: it lacks the corresponding artificial base materials.

2.3. Biotherapy

In therapeutic research, a synthetic circuit that produces invasin in *Yersinia pseudotuberculosis* has been devised to cause nonin-

vasive bacteria to invade tumor cells.[27] The invading bacteria have been successfully programmed to trigger drug expression to repress tumor growth in mice.[28] That study offered new therapeutic ideas for cell-based therapies. For phage therapy, scientists have successfully engineered a bacteriophage to express enzymes capable of lysing biofilms.[29,30]

2.4. A sustainable chemical industry

Synthetic biology provides an exciting opportunity to fabricate biomaterials or biofuels by means of engineered microbes with high efficiency, typically using available and inexpensive materials to produce a broader array of valuable chemical products. Engineered bacteria that live on cornstarch have been constructed to generate high-tech fabrics. Furthermore, microalgae that accumulate oil utilizing only sunlight, carbon dioxide, and water are a promising alternative to fossil fuels. The efficiency of photosynthetic complexes (chloroplasts) is key to improving the oil content of microalgae. A synthetic chloroplast genome and the chloroplast genome of *Chlamydomonas reinhardtii* have been successfully introduced into yeast cells, allowing for rapid manipulation or evolution of chloroplasts for higher photosynthetic efficiency.[31] In addition, photosystem proteins have been successfully expressed in microalgae to optimize photosynthesis.[32] Besides, efforts have been made to engineer *E. coli* as a biocatalyst for bioisobutanol or biodiesel production.[33,34]

2.5. Biosensing and bioremediation

The robustness and long-term stability of biosensors can be addressed by designing synthetic molecules or constructing engineered biological systems.[35] Additionally, a cell-free synthetic gene circuit and other materials can be freeze-dried into sterile paper. The transcription and translation properties of this cell-free synthetic gene circuit can later be activated by adding water to the paper. Rapid, low-cost detection of Ebola and Zika viruses has been achieved by implementing biosensor gene networks on paper.[36,37] The *in vivo* expression yields of multicopper oxidase have been limited by the copper concentration in the microbe. Li et al. demonstrated that high titers of soluble multicopper oxidases can be produced by the simple addition of copper ions into cell-free protein synthesis for application in wastewater decolorization and pulp delignification.[38]

2.6. Funding of synthetic biology

Because synthetic biology has an enormous potential in various applications, its funding from governmental sources and private venture capital has significantly increased. Since 2005, between 500 million and 1 billion dollars have been invested in synthetic biology research in the U.S.A.[39] From 2004 to 2013, approximately 450 million euro were invested into the synthetic-biology field by the European Union. Over 300 million pounds have been invested in synthetic biology research in the United Kingdom.[39] In China, over 200 million dollars were invested in synthetic biology research from 2011 to 2015, and an increased investment of one and half billion dollars has been proposed for 2018–2022.

In addition, as a strategic focus area, several federal agencies in the U.S.A. have published synthetic-biology strategic roadmaps to identify key challenges and make recommendations. For example, the report "Synthetic Biology" was submitted to the U.S. Congress by the U.S. Department of Energy in 2013. Three technical challenges in the synthetic-biology field were formulated: *i*) Genome scale engineering tools, DNA synthesis and assembly, and analytical tools; *ii*) Biological design principles, genetically tractable organisms/chassis, a minimal cell and in *vitro* systems, tools for plant systems, and biocontainment mechanisms; and *iii*) Computational tools, information standards, and databases.[40] In 2009, the European Molecular Biology Organization released the report "Making the Most of Synthetic Biology Strategies for Synthetic Biology Development in Europe" to introduce a strategic roadmap for European synthetic biology research.[41] The European roadmap covers a wide range of topics such as supervision, funding, and knowledge transfer. Furthermore, it noted the need to integrate various European R&D plans and formulate a comprehensive development strategy to strengthen European competitiveness in synthetic biology. In 2009, the Royal Academy of Engineering in the United Kingdom released the report "Synthetic Biology: Scope, Applications and Significance." The report summarized the basic technologies and development status of synthetic biology and predicted the application impact on technology, economy, and society for the next 5, 10, and 25 years.[42] In 2010, the Chinese government released a national strategic roadmap on synthetic biology. The roadmap specifies a timeframe for research on synthetic parts, commercial application of engineering parts, and clinical application of devices and systems for the next 5, 10, and 20 years.

From 2009 to 2015, over 350 synthetic-biology companies across America and the European Union raised over 3.3 billion in venture capital dollars.[43] In 2016 alone, over 192 American synthetic-biology firms received approximately 828 million dollars in private investments.[43] In 2017, 50 American synthetic-biology companies secured over 1.7 billion dollars to develop innovative synthetic-biology technologies.[44] The global synthetic-biology market was valued at 2.1 billion dollars in 2012 and reached nearly 2.7 billion dollars in 2013; this market is expected to grow to 11.4 billion dollars by 2021.[45] With the enhanced support of governmental and private investments, synthetic biology has revolutionized the traditional biotech industry. A new term, "bioeconomy," has been coined, suggesting that biotechnology can make a significant contribution to economies at both national and international levels.[39]

3. Potential risks of synthetic biology

As discussed above, synthetic biology opens up new possibilities for modifying or creating living organisms. Nonetheless, synthetic biology encounters the "dual-use dilemma" of technologies, which means that technology can be either used for good or misused for nefarious purposes. Although the possibility of abuse of synthetic biology cannot be completely eliminated, risks can be minimized by full awareness of the hazards and via suitable application of relevant ethical and regulatory measures. Below, we discuss the risks from three perspectives: biosafety, biosecurity, and ethics.

3.1. Biosafety concerns

According to the traditional definition, biosafety issues include "containment principles, facility design, practices and procedures to prevent occupational infections in the biomedical environment or release of the organisms to the environment," as stated by the American Biological Safety Association.[46] Similarly, during the 2006 conference of the Biological and Toxin Weapons Convention (BWC), the German representative, speaking on behalf of the European Union, provided the following definition of biosafety: "a biosafety risk classification system is based on the inherent capability of microorganisms to cause disease, of greater or lesser severity, in humans, animals and plants".[47] With the rapid development of synthetic biology, biosafety risks related to dual-use biotechnology have attracted attention as well.

Currently, it is argued that insufficient work has been conducted to identify or assess related biosafety risks in the synthetic-biology field.[48] A comparative approach is a common method for evaluation of risks; however, risk assessment based on a comparison is difficult due to the complexity of synthetic biology. Traditional genetic modification approaches usually involve manipulation of known genes in a donor organism; therefore, it is easy to find an appropriate comparator. In contrast, designs and procedures in synthetic biology are usually more complex and typically involve construction of a new pathway consisting of multiple genes or involve a gene with an unknown function. In addition, an important branch of synthetic biology, xenobiology, deals with construction of life by means of noncanonical base pairs or amino acids. These components do not exist in nature; therefore, no comparator can be found naturally for these cases.

Nevertheless, after reviewing more than 200 documents, Hewett et al. identified 44 discrete risks in synthetic biology, which can be categorized into four risk types related to human health and environmental pollution. The problems are allergies, antibiotic resistance, carcinogens, and pathogenicity or toxicity among human-health–related risks; and changes to or depletion of the environment; competition with native species, horizontal gene transfer, and pathogenicity or toxicity as environmental risks.[49]

One important biosafety concern in synthetic biology is the intentional or unintentional release of synthetic organisms into the environment during research and application, although it has been argued that there is no risk owing to the homeostasis of biological ecosystems and the vulnerability of synthetic organisms to displacement by native organisms.[50] In recent years, the European Union has funded several research efforts on the environmental impact of a deliberate release of genetically engineered microbes for plant growth enhancement or bioremediation.[50] The authors of these studies have concluded that the environmental impact was approximately the same between genetically engineered microbes and native ones. The studies have revealed that synthetic microbes can attain a transient advantage in a population, but it is difficult for them to survive in the long run because they can be rapidly destroyed by competitors or predators, and strong constraints for their proliferation are also imposed by ecological conditions involved; this notion is consistent with the fact that most attempts to genetically engineer microbes for environmental applications have had little success to date.[51,52]

Another important issue related to moving synthetic biology from the realms of laboratory-confined research to real-world applications is horizontal gene transfer, a common phenomenon in nature. It is estimated that there is up to 1 μg of nucleic acids per gram of soil and 80 μg per liter of marine water, owing to natural lysis of microbes.[53,54] Extracellular DNA can exist stably for months before being assimilated by prokaryotic or eukaryotic cells in nature. Although the natural transformation frequency of microbes is only approximately 1×10^{-7} per bacterial cell,[55] synthetic DNA circuits consisting of mobilized genes or sequences during conjugation or transduction may achieve a significantly high rate of horizontal gene transfer, with high risks to the genetic structure of environments.

The third biosafety issue is the formation of antibiotic-resistant superbugs. Plasmids used for DNA or pathway assembly typically contain antibiotic resistance genes as selective markers. Under conditions without selection pressure, superbugs can escape from host cells and enter the environment. Because these entities are capable of self-replication, they can enter and survive in other bacteria, and therefore generate antibiotic-resistant "superbugs" in nature.

3.2. Biosecurity concerns

Biosecurity is defined as "security against the inadvertent, inappropriate, or intentional malicious or malevolent use of potentially dangerous biological agents or biotechnology, including the development, production, stockpiling, or use of biological weapons, as well as outbreaks of newly emergent and epidemic disease".[56] During the 2006 BWC conference, the German representative on behalf of the European Union noted that a biosecurity risk classification system (should be) initiated to address the potential of a microorganism or toxin being used as a weapon.[47] Currently, biosecurity risks are mostly linked to possible bioterrorism activity.[46]

With the progress of synthetic biology, the possibility of bioterrorism via the dual-use synthetic-biology technology is also increasing. First, synthetic biology provides technical support for reviving and constructing dangerous bacteria or viruses. It is now easy to obtain the genetic sequences of highly pathogenic bacteria and viruses because such information can be downloaded freely from websites, such as GenBank, EMBL, and DDBJ; meanwhile, various viral, prokaryotic, and eukaryotic genomes can be synthesized at low prices using commercial services. In addition, methods for improving the pathogenicity and transmission of dangerous viruses or bacteria have been made available in many academic journals. The technical barriers for the artificial design and chemical synthesis of dangerous bacteria or a viral genome have almost vanished. Second, synthetic-biology technologies are now more accessible than ever. The commercialization of the synthetic-biology technology has given birth to many technical service companies that can provide support from experimental design to products through network orders. In addition, international biological academic competitions such as iGEM and the rapid expansion of amateur biological groups have made it easy to find and utilize relevant professional knowledge and skills. Third, traditional regulation related to pathogen or laboratory management is not sufficient to meet the challenges raised by synthetic biology. For example, given that they can be synthesized according to genome information, the need to obtain active bacteria or viruses that are typically under strict laboratory and application restrictions is diminished; in addition, to avoid inspection during transportation, sequences can be synthesized at different locations and assembled into an intact functional DNA unit via genome editing or DNA fragment assembly techniques. In 2017, it was reported that synthesized horsepox virus was successfully constructed from overlapping DNA fragments ordered through the mail. Horsepox virus has a close evolutionary relationship with variola virus.[57] The above activities have raised concerns among many virus experts, who have stated the need to strengthen the dual-use research supervision of biology, especially for research conducted in the private sector. Some European antiterrorism experts have warned that terrorist groups may be able to construct biological weapons in a kitchen. Gilles de Kerchove, the E.U.'s counterterrorism coordinator, has warned that synthetic-biology-related risks may be magnified when drones can be employed by terrorists to spread such viruses.[58]

The emergence of CRISPR/Cas9, a new genome-editing technology, has had tremendous effects on the synthetic-biology field. This technology not only improves the accuracy and efficiency of editing of pathogens', animals', plants', and human genomes but also yields traceless modification of genomes in a short period. Therefore, the technology can be utilized to enhance the pathogenicity, virulence, or transmission of toxins or bacteria or to disrupt the essential genes in humans, animals, and plants. In addition, several recent studies indicate that CRISPR/Cas9 has "off-target" effects, which could result in undefined health consequences.[59,60] Furthermore, easy and low-cost operation increases the risks of intentional abuse. In a recent report submitted to the U.S. Senate by the U.S. Intelligence Agency in 2016 and 2017, the CRISPR/Cas9 genome-editing technology was suggested to have a potential as a weapon of mass destruction.[61]

To foster a culture of mindful, responsible work in synthetic biology, the iGEM Safety & Security Program was initiated in

2018, which is supported by grants from the Open Philanthropy Project. The mission of this program includes: *i*) ensuring that the projects of participant teams do not pose risks to participants, their communities, or the environment; *ii*) considering related advancing technologies, regulation and policies, and changing guidelines. To achieve this goal, iGEM rewrote the safety forms and White List to explain the current understanding of risks and built new risk assessment tools to help participants identify risks in their projects, and these tools are regularly improved to better suit safety & security practices.

3.3. Ethical concerns

When Synthia, a human-made cell, was created in 2010, a global debate on ethics related to synthetic biology began. Opponents criticized the work as destroying people's basic beliefs about life and charging that the spread of artificial organisms into nature may cause environmental and health disasters. U.S. President Barack Obama also expressed concerns about this research and asked the Presidential Commission for the Study of Bioethical Issues to review the synthetic-biology field and identify appropriate ethical boundaries to ensure that Americans reap the benefits of synthetic biology and to minimize identified risks. A report entitled "The Ethics of Synthetic Biology and Emerging Technologies" was released to the public in 2010. In the report, experts concluded that the research at this stage still relied on an existing natural host, rather than creating life from inorganic chemicals alone. Complete human-made life remains only a remote possibility even in the foreseeable future. In the reports, five ethical principles were provided to ensure the development of synthetic biology in an ethically responsible manner: *i*) public beneficence, *ii*) responsible stewardship, iii) intellectual freedom and responsibility, *iv*) democratic deliberation, and *v*) justice and fairness. Finally, recommendations were proposed based on these guiding principles to ensure the advances of synthetic biology improve human health and public welfare and to identify and mitigate risks as synthetic biology matures.

In June 2016, a group of leading synthetic biologists announced that they will launch a Human Genome Project-Write (HGP-Write) federation, which will develop the relevant synthetic-biology technology required to chemically synthesize the human genome. The technology, once established, will be applied to address many challenges, such as human organ transplantation, ultrasafe cells resistant to natural viruses, and the development of new therapeutic cell lines with resistance to cancer.[16] The news again caused public discomfort and significant debates on the ethics of cutting-edge biological research. Several ethical concerns were voiced: Will research involve implantation of DNA into human embryonic cells? How should regulators deal with fairness in light of the high cost of the technology? Will the technology become a privilege for the rich? The misuse of outcomes of the HGP-Write project may increase public fear of technology. Prenatal genetic testing and selective abortions have led to concerns in many countries regarding unintended consequences of the HGP-Write project. Finally, although scientists have claimed that the project is not-for-profit, private investment may be involved; will the achievements of this project be monopolized by a few powerful companies, and only in developed countries?[62] The scientists later explained these misunderstandings, noting that the project was aimed at improving the capability for large-scale DNA synthesis, which can be applied to industrial biotechnology or agriculture: synthesizing animals', plants', and microbes' genomes, rather than creating humans or ushering in a new era of eugenics. At its second annual meeting in May 2017, the federation agreed that all ethical, social, and legal issues must be carefully studied in parallel with technology development, and a working group on ethical, social, and legal implications was formed to conduct an open discussion and ensure ethical boundaries of the project.

4. Laboratory level precautionary measures for synthetic-biology-related biosafety and biosecurity issues

To address biosafety issues related to synthetic microbes, such as horizontal gene transfer and emergence of superbugs, technical efforts have been made to develop laboratory level precautionary measures to restrict the release and survival of synthetic microbes in environments.

4.1. Genetic safeguards

Genetic safeguards are efficient biocontainment strategies against an accidental release of genetically engineered microbes into the environment. A robust genetic safeguard can restrict cell growth in a defined environment containing certain types of synthetic small molecules. The design principles of biocontainment include toxin expression, auxotrophy, and essential gene regulation.[63]

The early design of biocontainment systems typically uses toxin gene expression cassettes. Molin et al. reported the first conditional suicide system for bacteria, in which a toxin gene, *hok*, was employed to kill a variety of bacteria when expressed. A combination of condition-regulated promoters and toxin gene cassettes will stop the growth of cells when released from the designed environments.[64] Another toxin-controlled system is based on toxin/antitoxin pairs of bacteria, where the basic mechanism involves neutralization of a toxin by antitoxin at either the transcriptional or translational level. In addition, genetic safeguards can be built based on auxotrophy mechanisms. For instance, a synthetic auxotrophic *E. coli* strain dependent on the unnatural amino acid 3-iodo-L-tyrosine has been constructed, in which the amino acid 3-iodo-L-tyrosine is necessary for the production of an antidote protein against the toxic enzyme colicin E3. When 3-iodo-L-tyrosine is absent from the environment, the antidote protein is not produced, and the toxic enzyme colicin E3 can kill the host cells.[65] A biological containment system based on essential-gene expression has also been developed in *Salmonella enterica*. The engineered *S. enterica* was first designed for arabinose-dependent growth, and the transcription of arabinose-regulated genes is shut down due to a lack of arabinose when the bacteria enter host cells; in addition, the downregulation of these genes will activate the synthesis of antisense mRNA of targeted essential genes; this action eventually will cause cell lysis of bacteria.

Although these strategies can kill cells if they are accidentally released into the environment, the drawbacks are obvious too. For example, any gene mutation will cause functional loss of the toxin; auxotroph biocontainment systems will not function when the targeted essential genes are complemented by cross-feeding with related microbes in the same environment. In addition, the expression leakage of essential genes can occur if the promoter is not strictly controlled. Therefore, more recent approaches involve construction of multilayer safeguards via a combination of different mechanisms to limit the escape frequency to 10^{-8} engineered microbes, as recommended by the National Institutes of Health.[66] As one example, Gallagher et al. recently presented a functional safeguard consisting of overlapping auxotrophy, transcriptional-translational riboregulation of essential genes, repressor supplementation, and engineered addiction.[63] The engineered riboregulator can tightly control the expression of essential target genes, and the engineered addiction can lead to nuclease expression for cleavage of the host genome in a medium containing supplied synthetic molecules. Taken together, these findings indicate that the multi-

layer safeguards have limited the escape frequency to below 1.3×10^{-12}.

4.2. A genetic firewall

Xenobiology is an important branch of synthetic biology and is aimed at designing and synthesizing xenonucleic acids or at engineering a protein with noncanonical amino acids.[67] Chemically synthetized biomaterial components, such as xenonucleotides or noncanonical amino acids, do not exist in nature; therefore, synthetic organisms dependent on such artificial molecules will not survive outside their designed environments. The strategy can effectively eliminate the risks related to genetic information exchange and preclude horizontal gene transfer between synthetic and existing natural organisms. Moreover, genetic materials released by dead synthetic cells cannot be incorporated into a natural organism because they cannot be recognized by a natural DNA polymerase.

Artificial analog base pairs can be designed by means of interchangeable hydrogen bond donors and acceptors in natural bases or via modification of purine and pyrimidine rings of natural base pairs.[68] It has been reported that a new unnatural base pair, dNaM–d5SICS, was utilized and incorporated into *E. coli* DNA such that the synthetic bacterium is orthogonal to natural *E. coli* because the artificial base pair dNaM–d5SICS cannot be recognized by a natural DNA polymerase. When the synthetic bacteria escape into the environment, they quickly die due to a lack of artificial nucleotides dNaM and d5SICS in natural environments.[18] Another strategy is the use of noncanonical amino acids as building blocks. Due to the flexibility of aminoacyl-tRNA synthases and tRNAs, the amino acid analogs can be incorporated into proteins.[67] In addition, a strategy focusing on stop codon suppression has been proposed because three stop codons are responsible for terminating protein biosynthesis in organisms. When the function of a stop codon is suppressed or altered by introducing a new engineered aminoacyl-tRNA synthetase::tRNAs pairs into the cells, it will force the cell to use several noncanonical amino acid codes as stop codons, such as L-4,4′-biophenylalanine. A redesigned essential enzyme named "bipA aminoacyl-tRNA synthetase (bipARS)/tRNA-bipA system" has been constructed in *E. coli* strain C321.ΔA, and the UAG stop codon was assigned to a nonstandard amino acid.[69] The resulting *E. coli* strain cannot survive without the nonstandard amino acid (L-4,4′-biophenylalanine). Moreover, studies showed that the engineered organisms are more resistant to evolutionary escape through mutagenesis and horizontal gene transfer. For example, if the UAG stop codon is reintroduced into the *E. coli* strain C321.ΔA, not all essential genes or mechanisms can be naturally restored, due to competition between incorporation of a nonstandard amino acid and protein termination.[69]

4.3. DNA watermarks or barcodes

Detection and identification of contaminating synthetic DNA or organisms is important if they are intentionally or accidentally released into the environment. In this regard, DNA watermarks or barcodes, i.e., unique synthetic DNA sequences embedded in multiple loci of synthetic genomes, provide valuable means for isolating or identifying and tracking synthetic organisms. It has been proposed that an efficient watermarking system should have the following five features: *i*) the watermark does not influence the phenotype of the synthetic organism; *ii*) the watermark is resistant to gene mutation; *iii*) the watermark can be identified and recovered by private or governmental authorizing entities for strain management; *iv*) each laboratory has a different unique DNA watermark; and *v*) the DNA watermark is resistant to a malicious attack.[70] Several DNA watermarks have been independently devised for DNA coding regions, regulatory sequences, and noncoding DNA sequences to encrypt information by the DNA-Crypt algorithm.[71] The watermarks or barcodes not only help with tracking and identifying synthetic organisms but can also provide proprietary protection of the engineered strains.

5. Regulatory policy related to synthetic biology

Although no biosafety and biosecurity incidents related to synthetic biology have been reported, regulation or governance at the levels of individual scientists, institutions, nations, and the global community should be considered to prevent future crises. A key challenge is how to define ethics and management boundaries without restriction of the rapid development of synthetic biotechnology.

5.1. Code of conduct for scientists

Scientists are at the frontier of the synthetic-biology innovation and should also be the first line of defense against misuse or abuse of the synthetic-biology technology. One important area with consensus in the synthetic-biology community and governments is self-discipline and the responsibilities of scientists conducting synthetic biology research. For example, awareness of the possible dangerous consequences associated with the research, reporting or terminating the research when any sign of danger presents itself, and barring publication of results possibly related to nefarious applications.[72] Kuhlau et al. proposed that life scientists engaged in dual-use research should not only be responsible for preventing acts of misuse but also be concerned with preventing foreseeable harmful effects.[73] Besides, many countries have drawn up guidelines to ensure the responsible conduct of researchers. Examples of such codes include the Australian Code for the Responsible Conduct of Research, Code of Conduct for Scientists: revised Science Council of Japan, and Self-discipline of Moral Behavior of Scientific and Technical Workers implemented by China Association for Science and Technology. At the international level, the delegations of China and Pakistan jointly proposed a "Model Code of Conduct for Biological Scientists" to the Eighth Review Conference of BWC in 2016. The "Model Code of Conduct for Biological Scientists" provides several recommendations and principles from different perspectives for all relevant personnel engaged in biological research. Three of the proposed recommendations are related to the duty and responsibility of synthetic biologists: *i*) life scientists must carefully grasp the current controversial direction of research and judge the possible ethical and moral risks of biotechnology, striving to benefit all people with scientific research and minimize possible harm; *ii*) life scientists should perform a full risk assessment and feasibility certification of the possible health and social threats caused by the biological research process and achievements; and *iii*) prevention and emergency plans for risk management and control are needed for effective supervision of scientific research.

5.2. Governance at the national levels

In addition to ethical considerations, the formulation of governance or regulations at the national level should be considered as well. As early as 1999, concerns about biosafety and biosecurity issues related to synthetic biology were voiced. Cho et al. proposed that monitoring and regulation of knowledge relevant to the construction of biological weapons must be seriously considered at the national and international levels.[74] Meanwhile, strict governmental control should be discussed, or individual scientists or

the scientific community should be given authority over management of dual-use synthetic biology research. Miller and Selgelid suggested that neither strategy is sufficient and proposed a balanced solution: mixed regulation via institutional and government controls—or a governance system that relies on an independent authority—should be adopted.[75]

Government regulations on synthetic biology are similar in the European Union and U.S.A. In 2012, an international scientific workshop comprising the French High Council for Biotechnology, the German Central Committee on Biological Safety, the Netherlands Commission on Genetic Modification, and the Belgian Scientific Institute of Public Health examined the possible challenges associated with risk assessment of synthetic biology. This group concluded that in the short term, microorganisms or entities constructed by synthetic biology techniques are difficult to conceive from existing organisms and therefore are unlikely to cause additional risks, even if released into the environment. In addition, they concluded that current synthetic biology still uses techniques that fall within the scope of Directives 2009/41/EC and 2001/18/EC, whose governance includes the use and deliberate release of genetically modified organisms into the environment, respectively.[76] Similarly, the National Research Council of the U.S.A. concluded that synthetic biology should not be treated as a special genetic-engineering technique and does not create unique hazards as compared to other methods of genetic modification.[77] In contrast, the International Scientific Workshop of the European Union proposed that the chemically modified products of xenobiology may fall within a new, specific regulatory framework; long-term, new regulations will be needed because synthetic biology can generate organisms that are fundamentally different from naturally occurring life forms, and this situation may lead to potential vulnerabilities. In 2013, the National Institutes of Health published a new version of "*NIH Guideline for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*," to which the term "synthetic nucleic acid molecules" was added. The updated guideline states the need for the appropriate biocontainment of DNA molecules regardless of whether they are synthetic or recombined through conventional genetic manipulation. Notably, the decisions of both the U.S. and E.U. are based on early progress of synthetic biology when the potential risks are not fully understood. More recently, a study entitled "Synthetic biology and the U.S. Biotechnology Regulatory System: Challenges and Options" conducted by the J. Craig Venter Institute identified two major challenges in synthetic biology for the current U.S. regulatory system in the long run: *i*) synthetic biology will increase the amount of genetically engineered organisms outside the Animal and Plant Health Inspection Service's authority to review. Currently, the Animal and Plant Health Inspection Service's oversight depends on whether plant pests or some component of a plant pest is used for engineering the plant, but synthetic biology provides new solutions for genetically modified organisms; *ii*) synthetic biology will lead to a larger number of genetically engineered microbes intended for commercial use, including many organisms that are designed for possible environmental exposure. These entities may overwhelm Environmental Protection Agency Biotechnology programs. In addition, risk assessment of these genetically engineered organisms will be a greater challenge as they become increasingly complicated. The authority of these agencies will be inadequate or constrained for dealing with engineered organisms.

5.3. Efforts by international societies

The international community has expressed deep concern about the possible use of the synthetic-biology technology for bioterrorism and bioweapon purposes. During the eighth BWC convention in 2016, it was proposed that with the rapid development of synthetic biotechnology, the scope and destructive degree of biological weapons have expanded. For example, synthetic biology can be used to produce "material damage factors," including accelerated corrosion of rubber and metal parts, degradation of fuel or food supply, and destruction of equipment, with potential devastating risks for civil and military use. In 2018, the National Academies of Science, Engineering, and Medicine of the U.S.A. published a report entitled "Biodefense in the age of synthetic biology." The report concluded that synthetic biology has expanded the landscape of potential defense concerns; as a result, ongoing strategies for chemical and biological defense should continue, and approaches should be pursued to account for the broader capabilities enabled by synthetic biology. In addition, a framework for assessing synthetic-biology capabilities was proposed. Based on this framework, the three greatest concerns are thought to be related to national biosecurity, namely recreation of known pathogenic viruses, making existing bacteria more dangerous, and production of harmful biochemicals via *in situ* synthesis.

6. Conclusions

In the past decade, rapid progress has been made in synthetic biology, which has made significant contributions to basic life science research, human health, environmental protection, and economic growth. As synthetic biology becomes less expensive, easier to use, and more accessible, any unintentional misuse or deliberate abuse of dual-use synthetic biology will have serious consequences for the economy and security at both the national and international levels. Potential biosafety, biosecurity, and ethical risks should be carefully assessed. Most work in synthetic biology is currently done by scientists or amateur biology groups; therefore, codes of conduct for scientists or amateur biology groups should be proposed and implemented. Meanwhile, current supervision systems and biosafety and bioethics measures for dual-use synthetic biology must be reinforced. Other than ethical and regulatory measures, laboratory efforts should certainly be strengthened to deal with specific problems relevant to the progress of synthetic biology in the future. To address ethics concerns, which in most cases have been caused by misunderstanding of the technology, a public dialog on synthetic biology held by scientists and social experts will be helpful. To minimize the biosafety and biosecurity issues of synthetic biology, technical and ethical measures, such as genetic safeguards and firewall, and ethical codes of conduct for biological scientists, have been developed by the scientific community. Further investigation and discussion of relevant regulations or oversight rules will be necessary in the future. Finally, because biosafety, biosecurity, and ethical issues are not limited by the national boundaries, extensive discussion and exchanges of ideas should be encouraged at the levels of the scientific community, international organizations, and countries, with a goal to formalize a suitable governance system at the international level to prevent the misuse and abuse of dual-use synthetic biology.

Conflict of interest

We claim that there is no conflict of interest associated with the paper entitled "Synthetic biology: recent progress, biosafety and biosecurity concerns and possible solution".

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