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Review:

Synthetic Biology and Dual Use

Daisuke Kiga

Department of Computational Intelligence and Systems Science, Tokyo Institute of Technology
J2-1806, 4259 Nagatsuta-cho, Midori-ku, Yokohama-shi, Kanagawa 226-8503, Japan
E-mail: kiga@dis.titech.ac.jp
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Synthetic biology is science or technology concerning layers of life such as individuals, organs, cells. In this field, components of a layer are combined to construct a system in the upper layer. This paper focuses on studies, at a layer related to gene recombinant experiments, modifying genes and combining multiple genes. By introducing research accomplishments in synthetic biology such as gene networks and synthesis of the whole genome, this paper explains how synthetic biology is an extension of conventional gene engineering and the field of interdisciplinary open innovation. The risks of synthetic biology and risk reduction methods are also introduced.

Keywords: synthetic biology, DNA synthesis, reconstruction, risk reduction, breeding

1. Introduction: Synthetic Biology as an Extension of Breeding and Gene Engineering

Wild flora and fauna have been bred from ancient times for producing and improving their domesticated counterparts – animals and grain with characteristics desired by breeders (**Figs. 1(a)** and **(b)**). In the process of breeding, variants of organisms emerged by natural-occurring mutations or DNA recombination through sexual reproduction. From those variants, specific individuals with the desired features were selected. In the sexual reproduction of domesticated ones, individuals with specific characters were mated. Breeding thus included design process. Exploration efficiency of searching variants with desired characteristics was also improved by preparation of a large number of variants which are artificially increased its mutation rate by addition of medication or irradiation of radioactive ray.

Conventional genetic recombination experiment, which extends mating using biotechnology, inserts foreign genes into a host organism (**Fig. 1(c)**). Discussion arose about whether increased variations due to new gene combinations of the host genome and foreign genes threatened human society and about what benefits this technology offers. Guidelines for genetic recombination experiments were established after a moratorium and the Asilomar Conference in February 1975. As one result, inserting a human insulin gene into nonpathogenic Escherichia coli hosts made it possible to mass-produce insulin, generating medical treatment benefits [1]. In contrast, however, in the case of genetically modified plants for increasing yield by producers and nursery companies, which required inserting genes of pest-killing toxins or herbicide tolerance proteins into host crops, raised widespread objections from consumers about the risks involved.

Once a technology for site-directed mutation of DNA, as opposed to random mutation, was established, this DNA manipulation technology allowed protein engineering to modify amino acid sequences based on with 3D protein structure information. This enabled researchers to assign designed characteristics to proteins more effectively than random mutation (**Fig. 1(d)**). An enzyme governing chemical reactions in organisms accommodates a substrate molecule and catalyzes reactions against the substrate similar to combining a lock and a key, an enzyme had a 3D structure for binding to the specific key substrate molecule. Applying protein engineering to enzymes changes the form of locks and allows reactions to new substrate molecules. Protein engineering also broadens application area of enzymes. Natural enzymes have such limitations as being active only under certain reaction conditions, e.g., a certain temperature range. The application of protein engineering provides fat-degradation enzymes which work under low-temperature conditions or conditions including bleach. These enzymes are added to detergent and utilized.

In many cases, however, desired functions cannot be obtained by site-directed mutations in protein engineering, so researchers developed evolutionary molecular engineering (**Fig. 1(e)**) as a sort of "molecule breeding." Expressions used to explain breeding are directly applicable to this method. In evolutionary molecular engineering, variants of genes are prepared by artificial mutations or artificial DNA recombination. From those variants, specific gene variants with desired characteristics were selected. By artificial DNA recombination of genes, the genes with the desired characteristics were mated. Through this process, evolutionary molecular engineering is used to explore variations more effectively than could be done through conventional breeding. This is because breeding must deal with "losing" individuals with no mutation in intended genes, whereas mutations may occur in genes not intended to mutate; mutations are introduced to the whole genome as the sum of genes in the case of breeding. If mutations are introduced to individuals in breeding so that a mutation appears in the intended gene

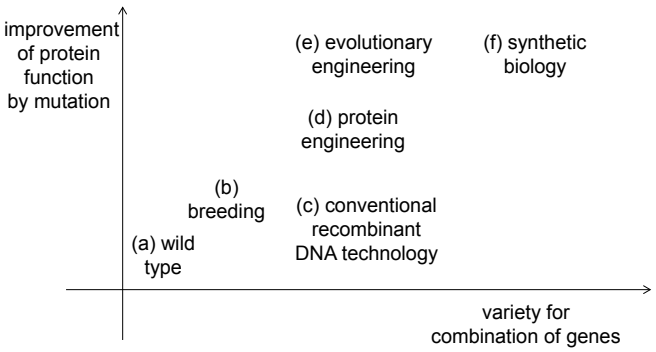

Fig. 1. Biotechnology development. Synthetic biology is an extension of breeding and conventional gene engineering.

of almost all individuals, mutations would be also occurred in most of the other genes in the genome. This method would be impractical, however, because an organism could not survive when it has mutations in most of the genes. In the case of evolutionary molecular engineering, meanwhile, mutations are concentrated in specific genes. This gives evolutionary engineering the advantages of producing mutants with desired characteristics more easily, in the current situation of a limited number of mutant individuals to be evaluated. The promotion of evolutionary cycles of selection enabled by introducing further mutations into obtained mutant genes can, moreover, generate mutants with more desired characteristics. Another strong point of the evolutionary engineering is that this technology has made it possible to combine single genes of 3 or more mutant individuals [2], whereas natural mating creates a new variation by combining single genes of 2 mutant individuals

For developments in preparation for new variations in DNA sequence, synthetic biology seeks design strategy for mutations and mating (**Fig. 1(f)**). The development of variations by combining multiple genes in synthetic biology is considered as an extension of breeding and conventional gene engineering. When individual gene products are compared to machine tools, gene-recombinant cells containing multiple foreign genes are utilized as if they were factories where multiple machine tools cooperate to produce items. The introduction of mutations is also important for adjusting individual machines to the system of a factory as a whole. We will discuss, in the same line in this paper, synthetic biology and part of conventional gene engineering both of which insert multiple foreign genes, though conventional one had problems in research efficiency. We will then introduce a new technology for synthesizing long-chain DNA as a basal technology which provides materials with any variations of combined genes. Extension of this technology created genome DNA that codes information on the whole virus or cell. The intended virus or cell has then reconstituted from public information by introducing this artificial genomic DNA to a different cell. Finally, we will summarize the risks of synthetic biology and safety measures using synthetic biology.

2. Reconstitution of Organisms from Public Information

Because processing of biological polymers such as DNA, RNA, and protein has become increasingly easier, experiments on system reconstitution by biological polymers in test tubes or cells have been frequently performed based on public information. This trend includes the scientific significance for understanding biological systems and the engineering significance for constituting and utilizing modified biological systems. When technology for reconstituting the same systems as natural ones is established, reconstituting experiments are also meaningful in the engineering field because modified biological systems could also be easily constituted by the same method.

Both of analytical approach that investigates individual functions of components separately and constitutive approach that fulfills original functions by combining individual components appropriately are mutually and inextricably linked in understanding of systems, in biology as well as in other scientific fields. In conventional 1970s biochemistry, the reconstitution of biological polymer complexes in transcription and translation promoted life science. On a par with this, reconstitution experiments done on sub-systems of life in the 21st century hold a prominent position in the life science. Specifically, it is highly significant that reconstitution was achieved for a system formed by interactions between different biological polymers in a solution. Protein synthesis systems, for example, are the most significant basic function of cells and complex systems in which more than 100 types of biological polymers cooperate in a solution. Takuya Ueda at University of Tokyo reconstituted protein synthesis reactions in test tubes by combining individually purified biological polymers [3]. Reconstitution experiments are progressing regarding the biological clock adversely affected by jet lag, for example. It was thought that reaction networks for biological clocks were, in many cases, included feedback systems involving complex protein synthesis. By adequately mixing 3 different proteins, however, Takao Kondo of Nagoya University showed that the reaction of the biological clock can be reconstituted in test tubes even in the absence of protein synthesis. Research on reconstituting mammalian phosphorylation signaling system in yeast cells is also progressing.

Reconstituting a partial system of a whole life system in test tubes is not alone: synthetizing entire virus with infection ability has also been studied without using "live" parent viruses. Two instance of this synthesis include a study synthetizing virus by reaction in test tubes without using host cells and a study reconstituting virus with infection ability from virus DNA synthetized based on electronic information. A group at RIKEN led by Shigeyuki Yokoyama and Hiroaki Imataka (currently at the University of Hyogo) recently modified the cell-free preparation method of virus particles infective in mammal cultured cells. Their group added RNA that codes Encephalomyocarditis virus genes to extract of cultured human cells [4]. Early instances of virus preparation us-

ing similar cell-free systems include the synthesis of the polio virus in 1991 by Echard Wimmer et al. of New York University [5]. They further indicated that a virus can be reconstituted, even in the absence of the original virus particle, simply by using public information on 7500-letters virus-genome sequences of the polio virus [6]. This study described synthesis of long-chain DNA that codes the virus by combining short-chain DNA synthetized by using organic chemistry. A similar example of virus synthesis, in 2003, taken from public information is the case of a virus infective in bacteria (5386 letters), supported by U.S. public funds. In addition, regarding the 1918 Spanish influenza virus, Yoshihiro Kawaoka at University of Tokyo focused on the fact that virus gene sequence information was obtained from dead tissue even though the virus itself no longer survives, synthetizing virus RNA from the sequences information. He also showed that highly virulent virus was obtained by introducing RNA into appropriately modified cells [7]. The Kawaoka and Foucher teams applied similar technology and showed that viruses mutated to become able to show respiratory droplet transmission between mammals, in mutants of the highly pathogenic avian influenza virus [8, 9].

A study by a Venter team in 2010 reconstituted an entire cell from electronic information [10]. They reconstituted, based on genome information, the Mycoplasma mycoides genome DNA in yeast by combining DNA outsourced to a custom-gene-synthesis company. Because the company processed DNA by combining chemically synthetized DNA, artificial genome DNA was processed without using genome DNA molecule of original organisms as a template, unlike in genome replication in a cell that requires template DNA molecule. By introducing the reconstituted genome DNA into closely related Mycoplasma, M. capricolum, the organism was successfully "hacked" by the M. mycoides synthetic genome DNA in the following process. Two genome DNA molecules coexist temporarily in the host cell to which the foreign genome is introduced. Because the growth rate of cells in the coexisting state is slow, 2 types of cells missing one of the genomes, namely 2 types of cells with the host genome and cells with the synthetic genome, are generated when culture is continued. Only cells with the synthetic genome are selected because the synthetic genome includes antibiotic-resistant genes. In these cells, almost all of the cytoplasm is initially occupied by the proteins generated by the host genome and resultant compounds, but they are gradually replaced by materials generated by the introduced genome, finally producing cells that have the origin of the sequence information of the synthetic genome, as designed. This study is significant in creating a modified single-celled organism with genome sequence specified by researchers. Towards this creation concerning million-letter genome DNA, gradual progress in conventional technology on DNA manipulation results in the 2 key technologies: technology for synthetizing long-chain DNA and that for introducing such long-chain genome DNA into closely related single-celled organisms. In that study, this gradual progress exceeds the threshold value, 1 million letters, in the length of DNA. As a result, revolutionary engineered organism emerged: DNA-dependent organisms are created even in the absence of transmission of genome DNA molecules form its parents.

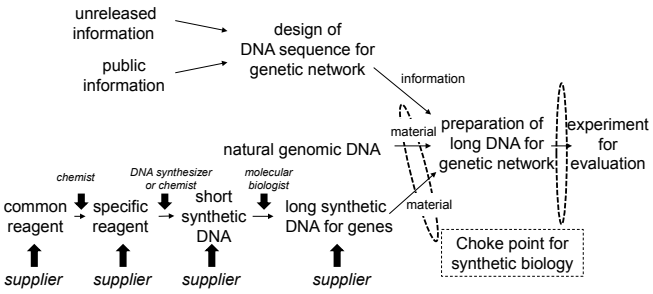

Fig. 2. Flow and choke points in synthetic biology. In synthetic biology that integrates information and materials, choke points (dots-lined ellipses) exist on the conduct of biological experiment and the supply of long-chain DNA as experimental material. Supply of long-chain DNA is detailed later. DNA and its materials can be bought from supplier companies (bold up-arrows). Instead, technician or machine having professional technics indicated as bold down-arrows is needed on each reaction step.

3. System Modeling and Database: Elements of Open Innovation in Synthetic Biology

For construction of engineered biosystem in cells, most synthetic biology researches design new combination of genes described in genome interpretation information on various organisms, and prepare DNA molecule which has the sequence of the combination of the genes (**Fig. 2**). As a result of introducing this DNA molecule into microbes, it becomes possible to utilize cells as factories combining proteins that are coded by individual genes. The following section introduces the importance of open innovation, in the process of synthetic biological research, that bridges biology and other scientific fields.

When a new network is being constructed by combining multiple genes each of which has protein-coding sequence and regulatory sequence for production control of the protein, not only selecting combinations of proteins but programming regulatory sequences is important. A cell may be compared to a factory equipped with multiple machine tools. The adjustment of operation rates for individual machine tools is required in a factory depending on the supply and demand of materials. If all machine tools in a factory operate at full capacity, intermediate products would pile up or become insufficient in a certain step, resulting in inefficiency of the whole system. It is vital to keep the amount of proteins in cells within an appropriate range by controlling protein productions to have proteins work properly when constructing a new network in cells. For natural life that consists of multiple genes, control information regarding protein productions is described in the genome as the integral of the gene network so that changes in external environments are addressed. Here, when constructing an artificial net-

work by accumulating genes from various organisms, the Universal genetic code allows the same DNA nucleotide sequence in a host organism to produce a protein with the same protein amino-acid sequence with the donor organism. A new production control information network for the engineered biosystem, however, must be created to address changes in external environments and to keep an appropriate amount of each protein.

In creating an appropriate network for protein production control, biological findings are not sufficient and a design scheme based on control engineering findings becomes important. Production control information, as well as amino acid sequence information on proteins, is coded on sequence information described in 4 types of letters of ACGT on DNA. Production control includes information on conditions under which a production switch is on or off, and that on the maximum extent of production. Because possible DNA sequence varieties are too many to create a production control network for proteins in a hit-or-miss fashion by a brute force manner, it is essential to design artificial networks by combining control engineering and biology findings. An early synthetic biology study was praised for its new explanation of behaviors of a designed biological network, by combined viewpoints of biological experiments and mathematical model of the network, where mutual suppression showed either bistability or monostability depending on a small difference in a parameter defined by DNA sequence [11].

When a gene network is designed with production control programmed, the use of prediction results of simulation based on mathematical models is important. The mechanisms of embryo development and iPS transformation are explained by an analogy in which balls roll on a "landscape" with valleys branching downwards [12]. The author recently constructed a gene network that programs gene expression states in live cells as if balls rolled on the landscape, where position of each ball, a cell, indicated the gene expression state of the cell and communication intensity state between cells [13]. In this movement, temporal change in cell-cell communication intensity between cells, corresponding to slope inclination of the landscape, is the key for the rolling movement of the balls. In order to adjust the movement, computer simulation can arbitrarily specify individual parameters, such as the cell-cell communication strength and protein production rates. On contrary, properties in a biological polymer network in a living cell cannot be arbitrarily set at specific values though the properties can be set in certain extents. Significant processes for the completion of our landscape study were therefore to select feasible networks by computer simulation before biological experiments. In this case, feasible networks meant those with a wider range between minimum and maximum values of parameters for the designed movements of the balls. The importance of mathematical models is also advocated in design of artificial networks producing compounds in bioindustry.

Establishment of a database containing properties for combining sequences that specify protein sequences and production controls is also important for synthetic biology. Information for combining electronic circuit parts such as transistors is shown on catalog sheets. In contrast, a database established in genome projects and other omics studies indeed includes data for understanding individual genes and their interaction but often lacks data for constructing a network by artificially combining individual genes. Parts Registry[1] is published as a database specializing in synthetic biology. The database, in a Wiki format, unfortunately contains information with less reliability, while DNA sequences that were validated by a third party are increasing. Such database improvement associated with increased reliability is progressing and utilized in a student competition in synthetic biology described below.

4. Radical Modification of Biological Polymer System

Synthetic biology is also applied to research for modifying common frameworks of lives. Modification of the genetic coding system, which converts genetic information on DNA to functional information on a protein, is explained as an example. Nucleotide sequence information described in 4 letters of ACGT in chain molecule DNA is converted to amino acid sequence information on a protein molecule that is synthetized by combining 20 types of amino acids in a chainlike fashion. Each protein exerts its specific function by forming specific cubic structure corresponding to its amino acid sequence. Groups led by Shigeyuki Yokoyama and Peter Schultz made it possible to use 21 types of amino acid at the same time by adding biologic polymers with modified functions to protein synthesis systems in test tubes and cells [14]. Hiroaki Suga at University of Tokyo is deploying research focusing on the use of multiple amino acids other than 20 types of natural ones at the same time [15]. In turn, the author's group recently created protein synthesis systems reducing types of amino acids [16]. This research is attracting attention as new approaches answering the question "Why do all forms of life use 20 amino acids in common?" by realizing "possible forms of lives," but they are beyond the focus of this paper. Refer to the literatures for detailed information on these approaches [17].

Once synthesis of biological polymers with arbitrary sequence becomes possible, it is natural that a study concept arises for fulfilling new functions using different combinations of biopolymers other than combinations in natural lives. One extreme example is the study of DNA computers that solves sample problems in information science such as Hamilton path problem, a search to draw a picture with a single stroke, using DNA [18, 19]. This study, started in the 1990s, supported by DARPA of the U.S. and a large grant in aid for scientific research from Japan. Trends in such studies continue in this century focusing on the idea that biological polymers are composing life, which includes autonomous reaction systems. Thus, by new combinations of biological polymers, recent constructions of artificial autonomous systems accomplished

1. http://partsregistry.org/Main_Page [accessed May 10, 2013]

multiple steps of reactions which proceed autonomously after preparation of reaction solution [20]. Trends of construction of autonomous biosystems are not limited to the pure information sciences. Researchers are trying to exploit the high affinity of biological polymers with natural living-systems including human being. The creation of micrometer-scale drug delivery systems, i.e., micromachines, is being planned to produce drugs at appropriate timing when a lesion is detected in an organism.

5. Commissioned/Custom Synthesis of Long-Chain DNA and Functional Evaluation of Genes: Choke Point of Technology Management and Development

Although synthetic biology is progressing smoothly in the direction of easily producing networks combining genes by integration of scientific technologies in various fields, the difficulties in participating in this progress would be synthesis of long-chain DNA that codes the gene network and functional evaluation of the network (**Fig. 2**). Even if DNA sequences to be combined are obtained from a published database, the actions of prototype systems combining these sequences are predicted by modeling, and system parameters are tuned to design DNA sequences likely to be activated, so the artificial network cannot be introduced into cells without obtaining long-chain DNA with their sequences. Similarly, without experimental equipment evaluating such functions, researchers cannot obtain data required for publishing papers, receiving patents, and developing products.

DNA synthesis combining nucleotides is achieved not only by biochemical reactions based on enzymes called DNA polymerase as in cells but by organic synthesis. The biochemical reaction is known as replication because DNA is required for use as the template to be copied. For the enzyme that accurately copies a genome consisting of several thousand genes, it is easy to copy a single gene although more than 1000 letters must be combined to copy a gene. Until recently, obtaining genes thus requires to extract the genome DNA of organisms or to share genome DNA extracted by other researchers. In sharing, material transfer agreements were commonly entered into between the organizations that researchers were affiliated with. In contrast, DNA processing by organic synthesis produces arbitrary DNA or RNA molecule sequences because template DNA is not necessary. With this advantage, the genetic code table was determined, in 1960s, by synthetizing RNA with various sequences combining 3 nucleotides. By mechanizing repetitive operations in polymerization reactions, it was also made possible to synthetize DNA molecules with sequences input to a computer. Some biological laboratories had this kind of machines in the early 1990s, but outsourcing DNA synthesis became a standard practice except in laboratories that chemically synthetize special nucleotides other than ACGT. This was the result of the emergence of commissioned/custom synthesis companies and lower costs due to price competition. The processing of long-chain DNA combining more than 200 nucleotides is difficult, however, in the case of organic synthesis. This is because, even when the yield rate of organic synthesis is 99% per nucleotide, the rate for 100 nucleotide length is $37\%(= 0.99^{100})$. Similarly, that for a 1000 nucleotide length is less than 0.01%. In addition, the probability of containing incorrect nucleotides is approximately 1% to 0.1% per nucleotide due to the contamination of solution or protective group required for chemical synthesis not having been removed. This error rate is significantly higher than that of DNA synthesis by biochemical reactions. Thus complementary pros and cons in DNA preparation are seen between enzyme reactions and organic chemical reactions.

In the last decade, researchers have easily obtained long-chain DNA having more than 1000 nucleotides with arbitrary sequences by outsourcing to commissioned/custom synthesis companies because of technical progress and price competition among commissioned synthesis companies. Such long-chain DNA encoding a gene is created by biochemically combining short DNAs prepared by organic synthesis reactions. In 1972, the first artificial gene, is shown be active on organisms; the gene encodes tRNA whose length is shorter than that of a proteins. In addition, researches have been reported about the synthetizing of protein genes by combining chemically synthetized DNA. With progress in commissioned synthesis of long-chain DNAs, a 1000 nucleotide length gene that codes proteins is currently supplied for less than US$500 dollars. In this competition on gene synthesis, important technological developments are the simultaneous synthesis of various kinds of DNA in small amounts and the removal of DNA molecules when base substitution has occurred.

In synthetic biology combining design and biological experiments, the "choke point" of study resides in the fact that actual materials must be dealt with. In other words, biological experiments are conducted in 2 study steps out of 3 as follows:

(1) design sequences for artificial gene circuits;

(2) obtain long-chain DNA molecules with the sequences;

(3) evaluate functions of the artificial gene circuits by introducing the obtained long-chain DNA into cells.

The restriction on step (3) is that a few personnel with expertise in individual objective materials are necessary. The restriction on step (2) is significant so this step should be focused on when dual use is considered. Long-chain DNA molecules corresponding to genes and gene circuits are obtained by outsourcing to commission synthesis companies, whereas creation by individual personnel and laboratories is ineffective (**Fig. 2**). The system, operated by major commissioned companies, for rejecting the commissioned synthesis of genes thought risky against human society is therefore a means for ensuring the safety of synthetic biology. Nonetheless, synthesis of a gene from public information without committing to the restriction of necessary outsourcing is ineffective but possible if a large

number of short DNA species can be obtained as parts of the gene. Such short DNAs can be synthetized, without outsourcing, using an automatic DNA synthesis machine or manual synthesis by a large group of trained personnel. While specialized techniques are required for organic manual synthesis, it will be easy to obtain short DNA using the automatic machines which can currently be gained through net auction. Obtaining special reagents for DNA synthesis is also an additional restriction in both manual and automated cases, but this restriction is relatively insignificant for the manual synthesis case. This is because such personnel are able to prepare the special reagents using simple reagents, whilst the special reagents must be purchased from reagent makers in the case of DNA synthesis by the machine run by a molecular biologist.

This choke point in gene synthesis should be focused on as a measure against dual use emerged due to accumulation of knowledge and technologies of synthetic biology. Potential problems caused by synthetic biology are divided into unintended accidents and malicious creation. Malicious creation by personnel who do not have "live" parent pathogens is probably re-synthesis of viruses or small-scale modification of bacteria and viruses because the number of genes currently accumulated in artificial gene network design is still 30 or fewer. It is very difficult to regulate attempts by the malicious using existing laws or guidelines regarding recombinant DNA experiments corresponding to step (3). Thus attempts for long-chain DNA synthesis required for the malicious creation should be screened or searched.

6. Risk Reduction Using Synthetic Biology Techniques

To address unexpected accidents that might occur when studying synthetic biology, synthetic biology technologies as such may be a measure for reducing risks. Because long-chain DNA with arbitrary sequences are processed using chemically synthetized DNA, not only sequences necessary for gene network functions but information such as an operating manual of the functions or creator names can be written as sequences readable by others. In fact, information including web addresses is written in artificial bacteria created by Venter et al. Should recombinant organisms be leaked, creators would be asked for measures against using such information.

Taking this concept one step further, if design information for gene networks with problems is made available by indicating project numbers of recombinant experiments applied beforehand, the problem would be handled immediately. Application forms are archived based on laws or regulations by recombinant DNA committees for each institution or department, so the numbers should indicated in long-chain DNA, as a first step of safety improvement. For recombinant DNA experiments conducted both by public research institutes such as universities and by private companies, archives of experiment application forms are desired assuming disclosure by a public information entrustment system when accidents occur as a result of the experiment.

Modification of genetic codes is also a future measure for reducing the possibility of unexpected accidents. Unexpected activities of a recombinant organism that unexpectedly could change its genome are a threat. These unexpected changes take two forms. One is that by mutation in cells. The other is that by the horizontal transfer of genes from other organisms, which is known to be common in the wild microbes and can make more considerable changes in cell characteristics than the mutation. As well as the horizontal gene transfer, genetic engineering currently easily introduces genes derived from other organisms into cells and makes them produce proteins from the genes. This is based on the fact that the genetic code for producing proteins from DNA is universal. On contrary, when DNA from other organisms is introduced to modified cells with different genetic codes, functioning proteins cannot be produced [16], and thus the probability of unexpected changes decreases. The possibility of accidents by artificial genetic network is thus reduced when researchers use combination of a cell with a modified genetic code and DNA corresponding to the modified code.

7. Risk Reduction by Interaction with Society: International Competition of Undergraduate Students

To handle malicious creation using synthetic biology techniques, it is important that there are persons both in the biological field and in other fields of society, in addition to deals based on technologies and laws, who know what can be done using current technologies. A student competition is introduced here as a measure for enlightenment and human resource development. International genetically engineered machine competition (iGEM) is an international synthetic biology competition played by undergraduate students. Although this iGEM competition has similarities to robot contests, iGEM does not mean the battle between recombinant organisms in Colosseum; this would not be permitted by regulations. In iGEM competition, experimental results based on ideas of students from around the world are gathered in Boston to determine who is best. Typical seasonal activities include organizing teams in spring, undertaking preliminary research on various themes focusing on summer vacation, conducting experiments during summer vacation, and preparing for presentations in September. The number of team members varies with the school, but 10 may be good because a student in a too large team tends to lose his/her active participation. Teachers provide advice on project progress so ideas from students are expressed in experiments, and have the students observe various regulations of experiments.

A wide variety of projects are based on innovative ideas of students. Many application-oriented projects have been planned, e.g., creation of sweeper bacteria which absorb heavy metal and aggregate by themselves to be collected, optimization of reaction by arranging an enzyme group on a scaffold, design of repair bacteria for cracks detected in

concrete, and the development of virus particles for use in drug delivery systems.

Not only for biology field but for other fields of society, education in iGEM competition for students provides human resource who pay attention to dual use of synthetic biology. Not a small number of students participated in synthetic biology iGEM competition, which accepts interdisciplinary undergraduate student teams, get employed in special fields other than biology in Japan and overseas. Such future communication among iGEM ex-participants in different fields would effectively increase security by indirect ways against biological threats. Cooperation between those in biological fields and those in security organizations is also important. The FBI of the US participated in iGEM competition as a sponsor and held a seminar on awareness and responsibility of a person who knows well synthetic biology. Another measure to increase the number of people who know what can be done using synthetic biology is that those who involved in synthetic biology transmit current situations in the field to society through media such as science cafes. In iGEM, transmission to society is also an item evaluated in competition, and several interesting activities are observed. In the science café planned by iGEM participants and the author, for example, general public participants were divided into groups of 4-6 and each group presented their imaginary product developed by synthetic biology. Responding to this presentation, other teams commented on risks and ethical problems. The team presented improved plans based on comments on their project. This all meant that a simulated research cycle was experienced. Through these processes, participants shared the recognition that criticisms in synthetic biology are divided into those that researchers can address and those they cannot. As a result, if constructive criticism leading to modifications in research for reduced risk are given to researchers from society in actual research, progress in science and technology can be expected based on cooperation between society and researchers.

In conclusion, means are needed for informing the public of what is possible and the risks now existing in synthetic biology. The author hopes that this paper will helpful in this purpose.

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References:

[1] L. Villa-Komaroff et al., "A bacterial clone synthesizing proinsulin," Proc. Natl. Acad. Sci. USA, Vol.75, pp. 3727-3731, 1978.
[2] W. P. Stemmer, "Rapid evolution of a protein in vitro by DNA shuffling," Nature, Vol.370, pp. 389-391, doi:10.1038/370389a0, 1994.
[3] Y. Shimizu et al., "Cell-free translation reconstituted with purified components," Nat Biotechnol, Vol.19, pp. 751-755, doi:10.1038/90802, 90802 [pii], 2001.
[4] T. Kobayashi, S. Mikami, S. Yokoyama, and H. Imataka, "An improved cell-free system for picornavirus synthesis," J Virol Methods, Vol.142, pp. 182-188, doi: S0166-0934(07)00052-3 [pii], 10.1016/j.jviromet.2007.01.026, 2007.
[5] A. Molla, A. V. Paul, and E. Wimmer, "Cell-free, de novo synthesis of poliovirus," Science, Vol.254, pp. 1647-1651, 1991.
[6] J. Cello, A. V. Paul, and E. Wimmer, "Chemical synthesis of poliovirus cDNA: generation of infectious virus in the absence of natural template," Science, Vol.297, pp. 1016-1018, doi:10.1126/science.1072266, 1072266 [pii], 2002.
[7] D. Kobasa et al., "Aberrant innate immune response in lethal infection of macaques with the 1918 influenza virus," Nature, Vol.445, pp. 319-323, doi: nature05495 [pii], 10.1038/nature05495, 2007.
[8] M. Imai et al., "Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets," Nature, Vol.486, pp. 420-428, doi:10.1038/nature10831, nature10831 [pii], 2012.
[9] S. Herfst et al., "Airborne Transmission of Influenza A/H5N1 Virus Between Ferrets," Science, Vol.336, pp. 1534-1541, doi:10.1126/science.1213362, 2012.
[10] D. G. Gibson et al., "Creation of a bacterial cell controlled by a chemically synthesized genome," Science, Vol.329, pp. 52-56, doi:science.1190719 [pii], 10.1126/science.1190719, 2010.
[11] T.S. Gardner, C.R. Cantor, and J.J. Collins, "Construction of a genetic toggle switch in Escherichia coli," Nature, Vol.403, pp. 339-342, 2000.
[12] S. Yamanaka, "Elite and stochastic models for induced pluripotent stem cell generation," Nature, Vol.460, pp. 49-52, doi:10.1038/nature08180, nature08180 [pii], 2009.
[13] R. Sekine et al., "Tunable synthetic phenotypic diversification on Waddington's landscape through autonomous signaling," Proc Natl Acad Sci USA, doi: 10.1073/pnas.1105901108, 2011.
[14] D. Kiga et al., "An engineered Escherichia coli tyrosyl-tRNA synthetase for site-specific incorporation of an unnatural amino acid into proteins in eukaryotic translation and its application in a wheat germ cell-free system," Proc Natl Acad Sci USA, Vol.99, pp. 9715-9720, 2002.
[15] Y. Goto, T. Katoh, and H. Suga, "Flexizymes for genetic code reprogramming," Nat Protoc, Vol.6, pp. 779-790, doi:10.1038/nprot.2011.331, nprot.2011.331 [pii], 2011.
[16] A. Kawahara-Kobayashi et al., "Simplification of the genetic code: restricted diversity of genetically encoded amino acids," Nucleic Acids Res, Vol.40, 10576-10584, doi:10.1093/nar/gks786, 2012.
[17] K. Amikura and D. Kiga, "RSC Advance."
[18] L. M. Adleman, "Molecular computation of solutions to combinatorial problems," Science, Vol.266, pp. 1021-1024, 1994.
[19] K. Sakamoto et al., "Molecular computation by DNA hairpin formation," Science, Vol.288, pp. 1223-1226, doi: 10.1126/science.288.5469.1223, 2000.
[20] M. Takinoue, D. Kiga, K. Shohda, and A. Suyama, "Experiments and simulation models of a basic computation element of an autonomous molecular computing system," Phys Rev E Stat Nonlin Soft Matter Phys, Vol.78, 041921, 2008.
[21] H. G. Khorana et al., "Studies on polynucleotides. 103. Total synthesis of the structural gene for an alanine transfer ribonucleic acid from yeast," J Mol Biol, Vol.72, pp. 209-217, 1972.

Name:
Daisuke Kiga

Affiliation:
Department of Computational Intelligence and Systems Science, Tokyo Institute of Technology

Address:
J2-1806, 4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8503, Japan

Brief Career:

1994 B.S. in Biophysics and Biochemistry from the Univ. of Tokyo
1999 Ph.D. in Biophysics and Biochemistry from the Univ. of Tokyo
2005- Assoc. Prof., Tokyo Institute of Technology

Selected Publications:

• R. Sekine et al., "Tunable synthetic phenotypic diversification on Waddington's landscape through autonomous signaling," P Natl Acad Sci USA, Vol.108, pp. 17969-17973.
• A. Kawahara-Kobayashi et al., "Simplification of the genetic code: restricted diversity of genetically encoded amino acids," Nucleic Acids Res, Vol.40, pp. 10576-10584.
• Instructor of iGEM team in Tokyo Tech (2006-)

Academic Societies & Scientific Organizations:

• Japanese Society for Cell Synthesis Research (JSCSR)
• The Biophysical Society of Japan (BSJ)
• The Society for the Study of the Origin and Evolution of Life Japan