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Monitoring of Synthetic Biology in Germany
1st Interim report of the Central Committee on Biological Safety

Introduction

Synthetic Biology is a research field that is associated with high expectations, but also with some concerns among the public. In 2009, the German Research Foundation (DFG), the Leopoldina (National Academy of Sciences) and the acatech (German Academy of Science and Engineering) issued a position statement that presented this research field and expressed recommendations concerning research funding and biological safety measures. They suggested that the Central Committee on Biological Safety (ZKBS) monitor the current scientific developments in a competent and critical manner and assess them as regards biological safety. In a letter to the DFG, Federal Minister Ilse Aigner (Federal Ministry of Food, Agriculture and Consumer Protection) entrusted the ZKBS with this monitoring and announced in an exchange with the ZKBS that the safety-relevant aspects of Synthetic Biology, as the ZKBS' field of activity, will be embodied in the Genetic Engineering Act (GenTG) as part of an amendment.

Synthetic Biology aims to design biological units, such as enzymes, genetic circuits or cells, in a way they do not occur in nature. Synthetic Biology employs elements of engineering by planning and modelling biological elements from scratch on a large scale and using them in biological systems (Keasling 2008).

The main difference to conventional genetic engineering consists in the further development of molecular biological methods enabling significantly more extensive manipulations, the large-scale use of bioinformatics enabling a modelled approach and the efforts to enhance the predictability of these manipulations via standardised components. This involves collaboration between a number of different scientific subfields, such as mathematics, physics, informatics, molecular biology and chemistry.

Since the beginning of this century, Synthetic Biology has seen dynamic development. Since that point in time, the number of publications in this field has risen by more than 10% annually (Oldham *et al.*, 2012). This development requires extensive scientific progress. Progress in the field of DNA synthesis that makes it possible to synthesise ever larger nucleic acid segments at an ever more favourable price is of central significance (Carlson, 2009). Although the *in-vitro* production of nucleic acid segments using "conventional methods" has been possible for some time, it was laborious and expensive. Nowadays, it is offered mainly by companies and has become faster, easier, less expensive and available to "anyone". This has made the genome-wide modification of DNA considerably easier, permitting, for example, the production of the genome of *Mycoplasma mycoides* ssp. *capri* by combining small DNA fragments to create a genome consisting of approx. 1 million base pairs, including the introduction of genome-wide modifications, such as genetic "watermarks" and selection markers (Gibson *et al.*, 2010).

The progress made in the field of systems biology is also very important. Systems biology is concerned with the investigation of cellular regulatory processes in their entirety and the identification and analysis of, for example, transcriptomes, proteomes and metabolomes, making it possible to create models of all metabolic processes in organisms. This allows for predicting changes in process parameters that occur as a result of the implementation of new metabolic pathways. For example, this includes modelling metabolic changes in cyanobacteria that are to be used to produce biofuel (Steuer *et al.*, 2012), analysing the *metabolic flux balance* during

the formation of barley endosperm (Grafaehrendt-Belau *et al.*, 2009) or examining the proteome of *Staphylococcus aureus* to be able to make predictions concerning the cyto- and pathophysiology of the pathogenic bacterium (Becher *et al.*, 2009). The “Focus Area of Plant Genomics and Systems Biology Potsdam” investigates, among other things, the connection between biomass production and photosynthesis in the green alga species *Chlamydomonas reinhardtii* (Winck *et al.*, 2011).

The present report briefly introduces the research field and provides an overview of the research activities in Germany. As the basis for this report, Synthetic Biology events, publications in scientific journals and the GEPRIS database of research projects funded by the DFG [German Research Foundation] were evaluated. The report is to give a representative overview of the research activities in the field of Synthetic Biology in Germany and is aimed at the Federal Ministry of Food, Agriculture and Consumer Protection as well as all other interested individuals.

Furthermore, the presented subfields of Synthetic Biology are examined to determine whether they involve any risks to biological safety and whether the research projects are covered by the scope of the GenTG [Genetic Engineering Act].

Presentation and safety-relevant classification of research fields

Research institutes in Germany are engaged in the following subfields of Synthetic Biology:

1. Design and synthesis of genes and genomes
2. Design of tailor-made metabolic pathways
3. Xenobiology
4. Production of minimal organisms and creation of artificial cells
5. Construction of genetic circuits

The individual subfields are presented below.

1. Design of genes and genomes

The rational, systematic design of genes and genomes *in silico* is indispensable in numerous fields of biology and medicine. These principles are applied, among other fields, in the development of vaccines against viral infections and involve optimisations, for example, to *codon usage* and the CpG content of genes (Kindsmüller and Wagner, 2011).

As already mentioned in the introduction, gene synthesis is a key technology for Synthetic Biology. To achieve the best possible expression of the *in vitro* synthesised genes in the target organisms, the nucleotide sequence needs to be modified. These modifications include the optimisation of codon usage, the avoidance of restriction sites and repetitive sequences, the influencing of the secondary structure and stability of the mRNA or the insertion of watermarks to identify the genetic modifications. The University of Regensburg develops algorithms *in silico* to facilitate the optimisation of nucleotide synthesis (Raab *et al.*, 2010; Liss *et al.*, 2012).

Assessment of the ZKBS:

The progress made in DNA synthesis technology has made the generation of targeted mutations considerably easier. The *de novo* design of genomes is currently not yet possible; genomes produced *in vitro* are strongly based on natural models, making it possible to assess their risk potential by comparing them with the “donor organism” of the nucleotide sequence

(see also the Position Statement of the ZKBS on the Risk Assessment of *M. mycoides* JCVIsyn1.0, file ref. 6790-05-01-94 of September 2010).

The extended possibilities in the synthesis of genes and genomes *per se* do not lead to an increase in the risk potential. The possible consequences of genome-wide modifications should nevertheless be assessed on a case-by-case basis.

The introduction of genetic modifications into genomes is covered by the GenTG, irrespective of the scope of the modifications. By contrast, the *in-vitro* synthesis of nucleic acid segments is not governed by the GenTG as long as these nucleic acid segments are not introduced into the genome of living organisms.

2. Design of tailor-made metabolic pathways

Tailor-made metabolic pathways have been developed in Germany for decades in white, red and green biotechnology to synthesise desired products in biological systems. Synthetic Biology provides this *metabolic engineering* with a multitude of bioinformatic tools that allow for developing and optimising metabolic pathways *in silico*, in addition to the possibilities of large-scale gene synthesis. As a result, *metabolic engineering* becomes more rational and more successful. The Centre for Synthetic Microbiology (SYNMIKRO) in Marburg or the Jülich Research Centre, for example, optimises the synthesis of interesting biotechnological products, such as amino acids or hydrogen, or studies the degradation of alternative substrates, such as methane or methanol (Becker *et al.*, 2011; Becker and Wittmann 2012; Blombach *et al.*, 2011; Chen *et al.*, 2010; Goldet *et al.*, 2008; Friedrich *et al.*, 2011; Marienhagen and Bott, 2012; Polen *et al.*, 2012).

Assessment of the ZKBS:

Over the last decade, the technical progress in the field of advanced biotechnology and Synthetic Biology has considerably extended the possibilities of designing tailor-made metabolic pathways, making it possible to specifically influence metabolic pathways, both by influencing gene regulation and by introducing heterologous genes. The design options have changed in quantity, but not in quality. Consequently, they are not associated with any fundamental challenges for the risk assessment of the genetically modified organisms or any risks to biological safety. The improvement of existing or the development of novel metabolic pathways by modifying genes or transferring genes of other organisms is fully covered by the GenTG.

3. Xenobiology

Xenobiology aims to also incorporate those building blocks into biological molecules that are not found in nature. Several groups in Germany are working on incorporating non-canonical amino acids into proteins. To this end, various approaches are pursued: The first approach uses organisms that are incapable of synthesising a specific amino acid, i.e. are auxotrophic for this amino acid. These organisms are dependent on the addition of the “missing” amino acid for their growth. If this amino acid is replaced by a structurally similar amino acid to change the characteristics of a target protein in the desired manner, the non-canonical instead of the original amino acid is incorporated into the proteins during the translation. A second strategy uses what are called suppressor tRNAs that recognise one of the three stop codons. The suppressor tRNAs are charged with the desired amino acid and lead to the incorporation of the amino acid instead of chain termination. This second strategy requires an additional tRNA recognising the stop codon and an aminoacyl tRNA synthetase that charges the suppressor tRNA with the desired amino acid.

The incorporation of non-canonical amino acids can be used to produce proteins with altered characteristics, which can be used in a wide variety of applications. For example, at Berlin University of Technology (TU), the University of Göttingen and TU Munich, this includes synthesising peptide antibiotics, referred to as lantibiotics, with altered spectrums of activity (Oldach *et al.*, 2012), introducing non-canonical amino acids into proteins to examine the protein-protein interaction during chromatin condensation (Neumann *et al.*, 2010), producing proteins with altered fluorescence spectrums (Kuhn *et al.*, 2012), enhancing the stability of the peptide hormone erythropoietin and developing antiviral drugs with non-canonical amino acids.

Assessment of the ZKBS:

In the ZKBS' opinion, the modification of individual amino acids in proteins is not associated with any additional risks to biological safety. Amino acids in proteins can also be specifically modified using conventional genetic engineering methods.

Additionally, the expression of the proteins can only be achieved under defined conditions in the laboratory. Given that non-canonical amino acids are available in the environment to a much lesser extent, the probability of these proteins being expressed in the event of a possible escape of these organisms is extremely low.

Proteins with non-canonical amino acids that are incorporated by suppressor tRNAs are only expressed if the organisms possess the appropriate tRNA-/aminoacyl tRNA synthetase system, which is another reason why the expression of the proteins is limited to these organisms.

Hence, these approaches of xenobiology are rather associated with an increase in biological safety by limiting the expression of the proteins to the laboratory or to specific organisms equipped for this purpose.

Even if the described efforts were to lead to the expression of proteins with non-canonical amino acids that are not found in nature, they are nevertheless covered by the GenTG. The genome is modified by using nucleic acid recombination techniques to express novel tRNAs or aminoacyl tRNA synthetases. The organisms produced this way are thus covered by the scope of the GenTG.

4. Production of minimal organisms and artificial cells

One of the goals of Synthetic Biology is the development of what are referred to as minimal cells that are to have as simple a structure as possible and serve as the basis for a wide variety of applications. There are two different approaches: the *top down* and the *bottom up* approach. In the *top down* approach, the genome of a parent organism is downsized to such an extent that only the essential genes required for the survival of the organism are left. By contrast, the *bottom up* approach involves the combination of biological systems out of building blocks, which can therefore differ greatly from existing biological systems. In the field of the *top down* approach, a method has been developed that allows for the genome of the bacterium *Pseudomonas putida* to be downsized repeatedly in a random fashion until the genome is reduced to the minimum set of genes (Leprince *et al.*, 2012).

The production of synthetic cells is complex. Synthetic cells must have various capabilities to constitute an independent biological system:

- The biological units must be capable of changing their size to allow growth,
- Directed transport processes via the cell membrane must be possible to maintain metabolism,

- They must be capable of organising themselves in compartments for certain metabolic processes,
- They must be capable of maintaining an ion gradient,
- They must be capable of maintaining metabolism,
- Metabolism and growth must be coordinated by chemical information (such as the DNA) to be capable of replication,
- The cell division must be coordinated to distribute the genetic information to the daughter cells,
- In addition, these biological systems should be capable of adapting to changing environmental conditions, if necessary.

Present research activities are aimed at developing biological modules that convey one of the above-mentioned capabilities, the long-term goal being to create a minimal cell by combining all necessary modules. In Germany, only individual, limited aspects are currently being addressed, for example at Ludwig-Maximilian University and the Max Planck Institute (MPI) of Biochemistry in Munich, such as the investigation of bacterial cell division systems and cytoskeleton proteins (Halatek and Frey, 2012; Vogel and Schwille, 2012; Schwille, 2011), the investigation of bacterial cell polarity (Lenz and Søgard-Andersen, 2012) and ATP synthases that can be used for the energy supply of artificial cells (Matthies *et al*., 2011).

Assessment of the ZKBS:

Minimal organisms created through targeted downsizing of their genome exhibit reduced adaptability to the environment, resulting in a general reduction in *fitness* and, if applicable, also their pathogenicity. Most of these organisms can only survive under defined conditions, which is why an increased risk to biological safety is not apparent in this case. The risk potential can be well estimated by comparing the organisms with the parent organisms, as is also stipulated in the GenTG for the risk assessment of genetically modified organisms.

The risk assessment of organisms designed from scratch without taking a natural model as a basis turns out to be more difficult, because in this case the risk potential cannot be concluded from the known risk potential of the parent organism. These organisms require their own assessment criteria and, if necessary, safety measures. As shown in the analysis of research approaches in Germany, only individual elements for artificial organisms are currently being investigated. The threshold to the creation of replicating artificial organisms that cannot be compared with a natural model has not (yet) been crossed, which is why this research field is not associated with any risks to biological safety at the present state of research.

Whereas the minimal organisms created using the *top down* approach are covered by the scope of the GenTG to the extent that the modifications in the genome can be traced back to genetic engineering operations, this is not the case with organisms designed from scratch using the *bottom up* approach. The scope of the GenTG covers organisms “the genetic material of which has been altered in a way that does not occur naturally by crossing or natural recombination (Sec. 3 GenTG).

The GenTG applies to known organisms the genome of which is modified and thus does not cover organisms the genome of which has been constructed without taking a natural model as a basis. However, as mentioned above, the currently pursued research approaches do not yet lead to organisms capable of reproduction, but only focus on individual aspects, such as the provision of a functional cytoskeleton or the development of cell division systems.

5. Construction of genetic circuits

The terminology in this research field is based on computer language, comprising terms such as “programmable components”, “genetic toggle switches”, “logic gates” or “Boolean logic”. As in informatics, circuits the components of which interact with each other in a predictable manner and respond to defined *inputs* with specific *outputs* are also to be created in living systems. Components such as regulators, activators or repressors from different organisms are freely combined with each other. The areas of application include the production of biological sensors that respond to environmental stimuli, such as UV light or chemicals, or measure the concentration of metabolic products or hormones in the body and thereupon produce the desired enzymes.

At the University of Freiburg, synthetic signalling pathways are being developed in mammalian cells that respond to light or chemical signal molecules and produce, for example, insulin (Hörner and Weber, 2012; Karlsson and Weber, 2012). In addition, the University of Potsdam investigates light-regulated peptides that can be used in genetic signalling networks (Mason *et al.*, 2008; Zhang *et al.*, 2012). Genetic circuits are also being tested outside living systems to reduce the interactions with other cellular constituents so that the systems can be better characterised (Franco *et al.*, 2011) or modelled *in silico* (Fritz *et al.*, 2009).

Regulatory RNA structures that can be used in genetic circuits as sensors and switches are also interesting. This approach makes use of the RNA’s capability of forming complex secondary structures and binding to chemical structures or proteins as a so-called aptamer. The binding of signal molecules can change the conformation of the RNA structures. For this reason, RNA molecules that form secondary structures can be used, for example, to mask the ribosomal binding site in dependence on the binding of a messenger substance in the 5’ end of genes, allowing for protein expression to take place in dependence on the messenger substance (referred to as *riboswitches*), or to determine the activity of interesting enzymes for diagnostic purposes (Rühl *et al.*, 2012). In addition, the University of Frankfurt, the University of Konstanz and the University of Heidelberg examine the tetracycline-regulated expression of genes via aptamers in *Saccharomyces cerevisiae* (Süß *et al.*, 2012), the catalytic activity of so-called ribozymes and the affinity of aptamers to their ligands and develop *riboswitches* (Hartig, 2010; Klauser *et al.*, 2012) that can also be regulated by light (Singer and Jäschke, 2010).

One goal of the efforts in this research field is to freely combine the individual constituents of different signalling pathways with each other. These constituents can be classified according to their function and included in a publicly accessible database (www.biobricks.org) as so-called *biobricks*, making it possible for other researchers to also use these modules for new genetic circuits. For this reason, efforts are made to characterise the individual modules *in silico* and *in vivo* as accurately as possible so that they can be well combined with each other.

Assessment of the ZKBS:

The creation of genetic circuits involves the combination of accurately defined, usually well characterised genetic elements. These genetic circuits are often introduced into model organisms that have long since been known in research while using so-called biological safety measures.

Therefore, an additional risk to biological safety is not apparent in field of genetic circuits. In these efforts, genetic elements are introduced into the genome of organisms in new combinations to create genetically modified organisms that are fully covered by the scope of the GenTG.

Conclusion

As pointed out in the introduction and in the overview, subfields of Synthetic Biology are being investigated at numerous institutes in Germany by pursuing a wide variety of innovative approaches. At the same time, the consequences of Synthetic Biology are being addressed, examining various ethical aspects and technology assessment, for example by the Office of Technology Assessment at the German Bundestag, the “Innovation Analysis and Technology Assessment of Synthetic Biology” project of the University of Bremen or the “Engineering Life” project funded by the Federal Ministry of Education and Research, which investigates the ethical, philosophical and theological aspects of Synthetic Biology as well as the opportunities, risks and legal aspects of this field of development. In September 2011, the ZKBS organised the “Status Quo Synthetic Biology” workshop attended by representatives of various research institutions. It turned out that, with the exception of DNA synthesis, the presented approaches pursued in Synthetic Biology in Germany are covered by the GenTG.

However, this would not apply to novel living systems, such as artificial cells without a natural model, for which no binding assessment criteria exist or to which the assessment criteria set forth in the GenTG are not applicable.

The analysis of the current research approaches in Germany reveals that these are covered by the GenTG. Furthermore, the research approaches often employ measures that increase the biological safety of the organisms. These include the use of donor and recipient systems classified as biological safety measures or of well characterised genetic modules as well as the possibilities of limiting the exchange of genetic information in xenobiology.

Individual subfields of artificial cell research, such as the investigation of bacterial cell division systems, take place *in vitro*, i.e. outside living systems, and are therefore not covered by the GenTG. These experiments involve no specific risk potential, since they do not employ viable organisms. At present, the production of self-replicating biological systems is not yet possible.

In summary, it can be said that the research approaches currently pursued in Synthetic Biology in Germany involve no specific risk potential for biological safety which goes beyond that associated with “conventional” genetic engineering experiments and which cannot be countered by consistently applying the GenTG. At the present state of research, all research approaches are covered by the GenTG with the exception of nucleic acid synthesis.

A reference analysis of the international state of research in the various research fields of Synthetic Biology and their relevance for biological safety is currently ongoing.

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Stellungnahme der ZKBS zur Risikobewertung von *Mycoplasma mycoides* subsp. *capri*, *Mycoplasma capricolum* subsp. *capricolum* und *Mycoplasma mycoides* JCVI-syn1.0 als Spender- und Empfängerorganismen für gentechnische Arbeiten nach § 5 Absatz 1 GenTSV. http://www.bvl.bund.de/SharedDocs/Downloads/06_Gentechnik/ZKBS/01_Allgemeine_Stellungnahmen_deutsch/02_Bakterien/Mycoplasmen.pdf?__blob=publicationFile&v=2.

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