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Synthetic biology and its regulation in the European Union

Research Paper

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The term synthetic biology is used increasingly, but without a clear definition. Most of the recent research carried out in this field is genetic engineering, as defined by current GMO-legislation in the EU. Synthetic biology has developed its own language. *In vitro* synthesis of DNA also carries the label synthetic biology. It is important to analyze whether present and future activities of synthetic biology are within the scope of existing EU-legislation.

Introduction

The term synthetic biology is used increasingly, but without a clear definition. Most of the recent research carried out in this field is genetic engineering, as defined by current GMO-legislation in the EU. This legislation regulates activities by which organisms are genetically modified and by which the resulting genetically modified organisms (GMOs) are used in any other way, including marketing the GMOs or their products.

Synthetic biology has developed its own language. For example, the recipient organism is called a chassis and the introduced modifying DNA is called a bio brick. *In vitro* synthesis of DNA also carries the label synthetic biology. New breeding methods applying different molecular methods have been developed since the introduction of the EU legislation on GMOs. This raised the question whether they are within or outside the scope of the GMO-legislation. Similarly, it is important to analyze whether present and future activities of synthetic biology are within the scope of existing EU-legislation.

Genetically modified organism

In the EU the *Council Recommendation concerning the registration of work involving recombinant deoxyribonucleic acid (DNA) (82/472/EEC)* was established in 1982. National guidelines and the Council Recommendation provided the basis for the subsequent development in 1990 of two Directives: one was Directive 90/219/EEC on the contained use of genetically modified micro-organisms (GMMs); the other was Directive 90/220/EEC on the deliberate release of genetically modified organisms (GMOs).

For the interpretation of the current EU legislation on GMOs it is necessary to bear in mind its history. According to the Directives, a *genetically modified (micro-)organism (GMM or GMO) means a (micro-) organism, with the exception of human being, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination* (Article 2, Directive 2009/41/EU and Directive. 2001/18). Interpreting these definitions frequently provokes the unanswered question whether the technique by which the GMM or GMO is produced or the presence of a modified nucleic acid is crucial for the resulting organism to be called a GMM or GMO.

Within the terms of this definition

- genetic modification refers to the use of the techniques listed in Annex I A Part 1 of Directive 2001/18/EC and Annex I Part A of Directive 2009/41/EU;
- the techniques listed in Annex I A Part 2 of Directive 2001/18/EC and Annex I Part B of Directive 2009/41/EU are not considered to result in genetic modification.

GMMs derived by cell fusion are fully exempted. GMMs derived by self-cloning are only exempted under certain conditions from current Directive 2009/41/EU on the contained use of GMMs but not from Directive 2001/18/EC on the deliberate release of GMOs. Since the ancestors of both Directives were drafted in parallel by the same committee and since these differences have been

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retained unchanged by the amendments of the ancestors of both Directives, it is a clear indication that self-cloning and cell fusion were deliberately excluded from the scope of Directive 2009/41/EU but not from the scope of Directive 2001/18/EC.

For the purpose of the Directives (micro-)organism means any biological entity capable of replication or of transferring genetic material, including cellular and non-cellular micro-organisms such as viruses, viroids and animal and plant cells in culture.

Synthetic biology

The term synthetic biology is not clearly defined. Some groups have concluded that synthetic biology presents a self-defining community of researchers from a variety of disciplines who are articulating themselves around the term synthetic biology and related terms such as synthetic genomics [1]. They have reviewed several descriptions and classifications of synthetic biology. The question whether synthetic biology is something new or a mere extension of genetic engineering and therefore covered by the EU-legislation regulating GMOs needs to be considered [2,3]. If the existing legislation is applicable, for how long will it be sufficient?

New techniques of genetic modification have evolved since the introduction of the legislation in 1990. The EU Commission set up a specialized Working Group in December 2008 to consider new biotechnological techniques being applied in plant breeding or the modification of other organisms [4]. The Working Group has examined a range of new techniques to assess whether they should be considered to lead to GMOs or GMMs as defined under Directive 2001/18/EC or Directive 2009/41/EU, respectively. They are implemented in the EU-member states by national legislation.

The following techniques were identified as the starting point for the consideration:

1. Zinc Finger Nuclease Technology (ZFN) (comprising ZFN-1, ZFN-2 and ZFN-3 as defined in the report)
2. Oligonucleotide Directed Mutagenesis
3. Cisgenesis (comprising Cisgenesis and Intragenesis)
4. RNA-dependent DNA methylation via RNAi/siRNA
5. Grafting
6. Reverse Breeding
7. Agro-infiltration
8. Synthetic Biology

The EU Commission will consider both the results of the Working Group and the analysis of their final report from December 2011 (European Commission, unpublished data) by the Competent Authorities of the member states. In the final report the majority of the Working Group came to clear recommendations concerning which of the organisms resulting from the new techniques considered are within the scope of the definition of a GMO or GMM, respectively. The final report does not indicate the need for amending the Directives in order to cover new techniques considered to result in genetic modifications that can be identified as such.

A modification of a single base pair within the genome of a given organism can be detected, but such detection does not indicate whether this modification occurred just by chance or whether it has been introduced intentionally. The Task Force on Detecting and Identifying Crops Produced with the New Plant-Breeding Techniques expressed its opinion that a genetic modification must comprise at least 20 nucleotide pairs (NPs) in order to allow identification of the resulting organism based on the modification [5]. Statistically, a specific sequence of 20 NPs within a nucleotide sequence with a random distribution of the NPs occurs once in 4^{20} NPs (1.1×10^{12} NPs). Hence, any specific sequence of less than 20 NPs is to be expected to arise by chance in large genomes such as that of maize (its haploid genome comprises 2.5×10^{9} NPs) with a certain degree of probability. A deliberate alteration of less than 20 NPs cannot be distinguished with sufficient certainty from an incidental occurrence of this sequence, so although specific sequences of less than 20 NPs can be detected, they are not suitable for determining their origin. They cannot be differentiated from genetic modifications arising from conventional mutagenesis or natural mutation (incidental occurrence) [6]. A mutation that is induced by mutagenesis techniques does not constitute a genetic modification according to Annex 1 B (1) of Directive 2001/18/EC and Annex II Part A No. 1 of Directive 2009/41/EU.

The generation of synthetic biological processes that start with a natural product that is then modified chemically to generate a biological process that does not occur naturally is also considered as synthetic biology. Depending on the nature of the process and the chemicals involved, certain provisions of the worker protection legislation may apply. The European Framework Directive on Safety and Health at Work (Directive 89/391/EEC) ensures minimum safety and health requirements. In addition there are several sector-specific directives related to worker protection at the EU level.

The most relevant for the sector including synthetic biology is Directive 2000/54/EC on the Protection of Workers from Risks Related to Exposure of Biological Agents at Work. The term biological agent refers mainly to micro-organisms (bacteria, fungi and viruses), but also includes GMMs, cell cultures and human endoparasites. The list of biological agents provides indications of allergenic potential and toxic effects. Measures proposed include containment categories for laboratory work and industrial processes. The Directive also lays down requirements for notification of selected activities to national authorities. The requirements are minimum requirements and have been implemented into national legislation.

The following were members of the New Techniques Working Group:

Austria: Alois Haslinger, Dietmar Vybiral; Belgium: Didier Breyer, Philippe Herman, Katia Pauwels; Bulgaria: Genoveva Nacheva; Czech Republic: Milan Bartos, Jaroslava Ovesna; Denmark: Jan Pedersen; Estonia: Hannes Kollist; Finland: Kirsi Törmäkangas, Matti Sarvas; France: Olivier Le Gall, Jean-Christophe Pages; Germany: Detlef Bartsch, Hans-Jörg Buhk, Wilfried Wackernagel; Republic of Ireland: Tom McLoughlin, Bernadette Murray, Donal Grant; Italy: Elena Sturchio, Latvia: Isaak Rashal; Lithuania: Odeta Pivoriene; The Netherlands: Boet Glandorf, Hanneke Bresser; Norway: Eirik Biering, Casper Linnestad, Tove Loken; Portugal: Teresa Borges, Clara Fernandes, João Lavinha; Romania: Călina Petruţa Cornea; Slovakia: Zdenka Balatova, Piet van der Meer; Slovenia: Borut Bohanec, Marko Dolinar; Spain: D. Rafael Pérez Mellado; Sweden: Katarina Eskils, Marie Nyman; United Kingdom: Louise Ball, Michael Paton.

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