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Position statement of the ZKBS on the classification of genetic engineering operations for the production and use of higher organisms using recombinant gene drive systems

Rationale

In a publication issued in the spring of 2015 (Gantz and Bier 2015), Gantz and Bier described the development of a “mutagenic chain reaction” as a method of converting specific heterozygous mutations into homozygous mutations in populations of sexually reproducing organisms. Based on the example of fruit flies (*Drosophila melanogaster*), they demonstrated that such a system, also referred to as gene drive, can rapidly spread in a laboratory strain. Other recent publications (Gantz et al. 2015; Hammond et al. 2016) describe the use of gene drive systems in *Anopheles* mosquitoes with the goal to modify natural populations. In view of the extensive discussions and enquiries concerning the risk potential of related genetic engineering operations in genetic engineering facilities, the ZKBS has found it necessary to describe the function of gene drive systems and to provide advice on the safety assessment of such operations.

1. General information

Gene drive systems are understood to be genetic elements or gene constructs that expedite their own spread in populations of sexually reproducing organisms by being inherited to more than 50% of the offspring. When a gene drive system is only present in one of the two genomes of a diploid cell, it can copy itself into the homologous site in the other genome through molecular mechanisms. This process can take place in a zygote or later on in the germ line.

Because of their non-Mendelian inheritance, gene drive systems substantially differ from recombinant nucleic acid segments in genomes that are inherited conventionally. Gene drive systems can spread relatively rapidly in a population, even if they are associated with a selective disadvantage.

The existence of genetic elements that increase the probability of their inheritance in natural populations has long since been known (homing endonucleases, Chevalier and Stoddard 2001; replicative transposition Shapiro 1979; sex-ratio meiotic drive, segregation distortion Zimmering et al. 1970). Experimental further development has made it possible to produce recombinant gene drive systems and optimise their efficiency.

2. Description of natural and recombinant gene drive systems

2.1. Homing endonucleases

Homing endonucleases (HE) are naturally occurring proteins that are characterised by their capability of binding specifically to relatively long DNA target sequences (14-40 nucleotide pairs, Np) and introducing an endonucleolytic double-strand break (DSB). The gene for the HE is naturally flanked by the two target sequence segments that form as a result of the endonucleolytic cleavage in the target sequence. The DSB only takes place in the target sequence which does not contain the sequence coding for the HE. Subsequently, the DSB is

repaired through homologous recombination, leading to duplication of the DNA segment that contains the sequence coding for the HE. Such a nuclease (I-*Sce*I) was first described in yeast. Today, more than 250 other such enzymes are known, predominantly from bacteriophages, fungi and algae (Chevalier and Stoddard 2001; Belfort and Roberts 1997).

2.2. Recombinant gene drive systems

Recombinant gene drive systems can be constructed to function in the same way as HE. This can be achieved by integrating an endonuclease-encoding gene into the genome of an organism in such a way that it is flanked by the segments of the target sequences of the endonuclease. The target sequence of the endonuclease should be so specific as to allow only one cleavage in the genome of the organism. In order for a gene drive system to bring its self-duplicating activity to bear, the target sequence of the endonuclease must be present in the organisms of the target population. This can be achieved by inserting the target sequence of an endonuclease (e.g. HE) into the population in advance (Windbichler et al. 2011) or by adapting the target sequence of an endonuclease to a nucleic acid segment that is present in the genome of the target organism.

Once the endonuclease is expressed, a DSB takes place in the target sequence. A copy of the endonuclease gene can then be integrated at the cleavage site through homologous recombination, as described above. As a result, the target sequence is converted into a gene drive system. At the same time, the target sequence is interrupted, preventing another cleavage. This way, heterozygous gene drive genotypes in diploid organisms are converted into homozygous gene drive genotypes. As a consequence, the “gene drive allele” is passed on to all offspring, which are, in turn, converted from a heterozygous into a homozygous state. This makes it possible to successively convert all wild-type alleles that contain the target sequence into gene drive alleles, eliminating them in the population.

Theoretically, all nucleases that have a sufficiently long recognition sequence (20-30 Np) to specifically induce a DSB at only one target sequence in the genome of the respective organism can serve as endonucleases. For example, zinc finger nucleases, TALENs, homing endonucleases and CRISPR/Cas9 systems can be used. The CRISPR/Cas9 system (Hsu et al. 2014), which has recently been discovered and technically optimised for genome editing, is of special significance, because it allows for the specific recognition sequence of the nuclease to be adapted to nearly any sequence in the target organism with little effort. This is why the first recombinant gene drive systems with a potential effect in wild populations have also been implemented on the basis of the CRISPR/Cas9 system (Gantz and Bier 2015).

A gene drive system can include additional genes which are then spread along with the gene for the endonuclease. These genes, which are also referred to as cargo genes, can support the gene drive system or possess independent characteristics (Gantz et al. 2015).

The use of gene drive systems may have various effects on the metabolism of target organisms. If the target sequence of the employed endonuclease is located in a genome segment with a regulatory function or in a functional gene, its function may be affected by the copying process. Furthermore, a cargo gene inserted along with the gene drive system may have an influence on metabolism. However, it is equally conceivable that a gene drive system only spreads the endonuclease gene without having a further influence on the recipient organism (e.g. if the target sequence is located in a non-coding DNA segment).

3. Safety assessment

According to Sec. 3 GenTG [Genetic Engineering Act], organisms with recombinant gene drive systems constitute genetically modified organisms (GMO). The nucleotide segments and genes of recombinant gene drive systems are arranged in a specific way and can therefore be clearly identified as such. The unintentional or unnoticed production or use of genetically engineered gene drive systems is not expected.

As is the case with other genetic engineering operations, the safety assessment of genetic engineering operations with gene drive systems is based on the assessment criteria set forth in Sec. 7 in conjunction with Appendix I GenTSV [Genetic Engineering Safety Regulations]. These comprise information on the employed donor and recipient organisms (GenTSV Appendix I No. 1.), the genetic modification of the respective GMO (GenTSV Appendix I Nr. 2.1), health-related aspects (GenTSV Appendix I No. 2.2) and environmental aspects (Appendix I No. 2.3). According to the present state of knowledge, the gene drive function itself is not associated with any effects on health-related aspects.

The special challenge in the safety assessment of operations with gene drive systems is the enhanced inheritance of the gene construct within a population that contains the target sequence and is therefore receptive to the gene drive system.

Within closed systems, the gene drive function plays no safety-relevant role.

However, the self-duplicating activity of the gene drive system gains substantial significance in the assessment of the possible consequences of an accidental escape of such GMOs from a closed system. In this case, sexual interaction between the carrier of the gene drive system and a wild population may occur, resulting in the altered mode of inheritance taking effect. Basically, it must be assumed that the spread of a gene drive allele in a susceptible population is significantly higher than that of a comparable allele without a gene drive effect.

Hence, in addition to the assessment of the recipient organism (GenTSV Appendix I No. 1, in particular d), j), q), t), v), and x)), the assessment of all genetic elements of the recombinant construct (GenTSV Appendix I No. 2.1, in particular d), h), and j)) and the receiving environment (GenTSV Appendix I No. 2.3, in particular a), d), and e)) is of special significance in the event of accidental escape.

In this respect, it is relevant whether the GMO with a gene drive system can get in contact with interfertile organisms of a wild population in the event of accidental escape. Particular attention must be paid to the occurrence of populations in the immediate vicinity of the genetic engineering facility. In addition, it must be checked whether the GMO can get in contact with interfertile populations found further away as a result of its own mobility, or persistence and transport.

Once sexual interaction with wild populations can be ruled out with a high degree of certainty, the consequences of an accidental escape would not differ from those of an escape of a conventional recombinant gene construct.

Sexual interaction with individuals of an interfertile, naturally occurring population is not necessarily associated with the spread of the gene drive system. If, for example, the genome of the population does not contain any specific target sequences for the gene drive system, a gene construct with a gene drive effect does not differ from a gene construct without a gene drive effect. The same applies when using a gene drive system in a laboratory population if the specific nuclease target sequence that has been inserted into the genome of the laboratory population is not present in naturally occurring populations.

Another example of the gene drive system not necessarily spreading after sexual interaction is the functional expression of the inserted nuclease. If this requires specific inductors or effectors (e.g. nutritional supplements not found in nature) due to the targeted use of promoters or other regulatory elements, the gene drive system is also not assumed to spread within the population.

The enhanced inheritance of gene drive systems requires free and random mating in the population. However, the breeding of many domesticated species, for example livestock, involves the targeted mating of selected individuals. The risk of undesired spread of gene drive systems in domesticated populations is therefore estimated to be significantly lower than in wild populations.

4. Classification

As a precautionary measure to counterbalance potential environmental risks posed by the accidental escape of a gene drive system from a genetic engineering facility, the production and handling of such systems is subject to containment level **2**. Based on the currently available data, genetic engineering operations involving the production and use of gene drive systems cannot be regarded as comparable within the meaning of the GenTG; rather, they require a case-by-case assessment by the ZKBS.

In the case-by-case assessment, it has to be evaluated whether and to what extent the gene drive system can be transmitted to wild populations in the event of unintentional release. The extent and rate of spread of a gene drive system in interfertile, naturally occurring populations depends on a number of factors that need to be identified and assessed on a case-by-case basis.

Literature

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