General position statement of the ZKBS on the use of zinc finger nuclease-1 technique (ZFN-1)

Background

The European legislation on contained use of genetically modified microorganisms (Directive 2009/41/EC) and the deliberate release of genetically modified organisms (Directive 2001/18/EC) together with the German national legislation (GenTG) date back to the year 1990 regarding the assessment of the biotechnologies used for genetic modification. However, the respective techniques have further developed over the last 20 years. Therefore, in some cases it is questionable whether they still fall under the scope of the GMO legislation. This has led to the establishment of a working group consisting of representatives of the European Commission and of the European Competent Authorities. The members of the New Technique Working Group (NTWG) examined on eight so called “new techniques” including zinc finger nuclease technique whether they are covered by the legislation or not. Final positions on the assessment of the new techniques have not yet been achieved. Enquiries of Federal authorities have now prompted the ZKBS to provide a position statement on the assessment of zinc finger nuclease-1 technique based on the current status of the discussions.

Zinc finger nuclease-1 technique (ZFN-1)

Zinc finger nucleases are protein chimeras composed of two covalently linked, functional domains: the zinc finger domain and the nuclease domain. Zinc fingers comprise a class of phylogenetically highly conserved protein structures which are especially important for the regulation of DNA transcription. Zinc fingers used for (the) ZFN technology coordinate one zinc ion by positioning of two cystein and two histidin residues. The polypeptide chain takes on a loop structure allowing a sequence specific interaction with DNA. Depending on the amino acid sequence zinc fingers recognize three consecutive nucleotides. In most cases however, a specific interaction occurs with only two of them.

The nuclease domain is derived from the FokI restriction endonuclease of Planoncrobium okeanokoites (formerly: Flavobacterium okeanokoites). This enzyme is part of the bacteriophage defence system of the bacterium and is characterized by the ability to cleave DNA.

Zinc finger nucleases are generated by arrangement of several individual zinc finger domains to allow recognition of target sequences of up to 12 nucleotides on one DNA strand [1]. The use of two zinc finger nucleases allows recognition of neighbouring target sequences on opposite DNA strands. The two FokI cleavage domains can induce a double-strand break (DSB) between the ZFN binding motives [2, 3]. The ZFN-1 uses the cell’s natural repair
process of non-homologous end-joining [4]. No repair template is provided by the cell, so that cleaved DNA strands are joined randomly. This process can lead to exchanged base pairs at the repair site, short deletions or insertions. These modifications are often associated with a shift of the reading frame. The ZFN-1 usually aims at the knock out of the targeted gene in cell’s genome [5].

Generally, the ZFN protein is delivered into the cell as genetic information via (DNA) recombinant vectors (viral or bacterial). Additionally, the ZFN can be directly transferred to the cell as mRNA [5, 6] or as protein.

The generation of DNA recognizing nucleases has undergone rapid development. Based on the transcription activator like effectors (TALE) expressed by a phytopathogenic bacterium of the genus Xanthomonas spp., custom designed TALE nucleases allow specific binding to a target nucleotide DNA sequence. The DSB is again generated by the FokI nuclease domain [8, 9]. Furthermore, modified mega nucleases of genetic elements (homing endonucleases) are employed for the targeted introduction of DSB [10].

Assessment

If chromatographically purified pairs of ZFN proteins are directly introduced into a cell, they specifically bind to target nucleotide sequences of chromosomal DNA resulting in a DSB. The DSB is repaired with the help of the cellular DNA repair machinery. This often results in modifications of the nucleotide sequence that potentially lead to the knock out of the (respective) gene. While ZFN proteins are only transiently present in the cell, the alterations of the nucleotide sequence in the genome will be passed on to the daughter cells. The genetic material of the cell is specifically changed by external influences, namely the mutagen ZFN. The described technique complies with mutagenesis in terms of § 3 No. 3b GenTG. According to these terms mutagenesis is not a procedure resulting in genetic modification. Thus, the ZKBS does not regard the resulting organisms as organisms modified by gene technology.

If the ZFN protein is introduced into the cell as an isolated protein-coding RNA hereditary material is not transferred. The RNA is transiently present in the cell; there is neither replication nor reverse transcription nor integration into the genome of the cell. This RNA is merely translated. The introduction of mRNA into eukaryotic cells has been classified as a natural process by the ZKBS [11, 12]. According to § 3 No. 3b GenTG natural processes are procedures that do not result in genetic modification. The protein translated from the transferred mRNA is again a ZFN. As described above this mutagen is able to specifically bind to target nucleotide sequences and generates a DSB. Alteration(s) occur in the chromosome, however the process is not regarded as a genetically modifying procedure according to § 3 No. 3a GenTG. The ZFN-1 technology is rather a mutagenesis according to § 3 No. 3b GenTG. To conclude, the ZKBS does not regard the resulting organisms as organisms modified by gene technology.

Literature


