UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

BIOTRIN

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MTA ATK Martonvásár Hungary

Genetic improvement of microbes, plants and animals in the beginning of the twenty first century, a key to the agricultural innovation

International conference on

New Breeding Techniques (NBT) - Hope for Agriculture and Food Chain

Prague September 13, 2018

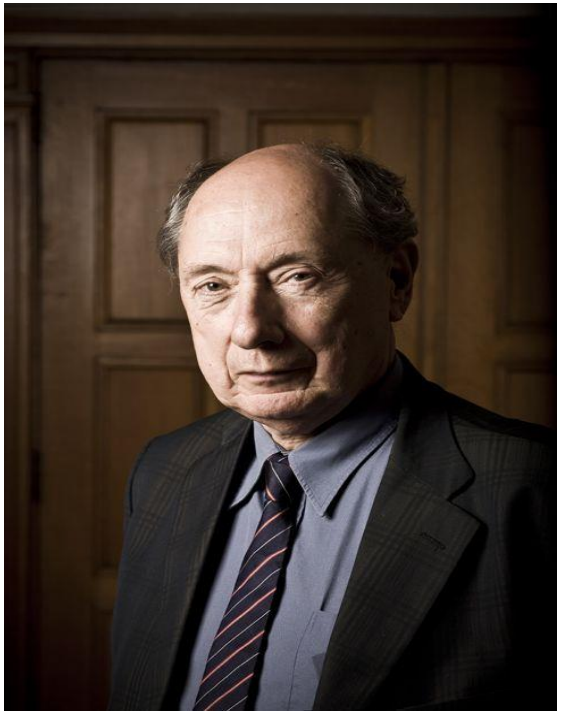
EuropaBio™
The European Association for Bioindustries

UNITED STATES OF AMERICA EMBASSY

Since more and more primary structure of the genetic material of different organisms has been described, our understanding on the different genomes rapidly evolved.

The first transgenic organism constructed by Paul Berg more than four decades ago, and the revolution of the molecular biology made it possible to extend that technology for almost all living organisms.

In the case of higher plants the first transgenic tobaccos were produced in the same time in two independent laboratories in the US lead by Mary Dell Chilton and in Belgium headed by Marc Van Montague and Jeff Schell.



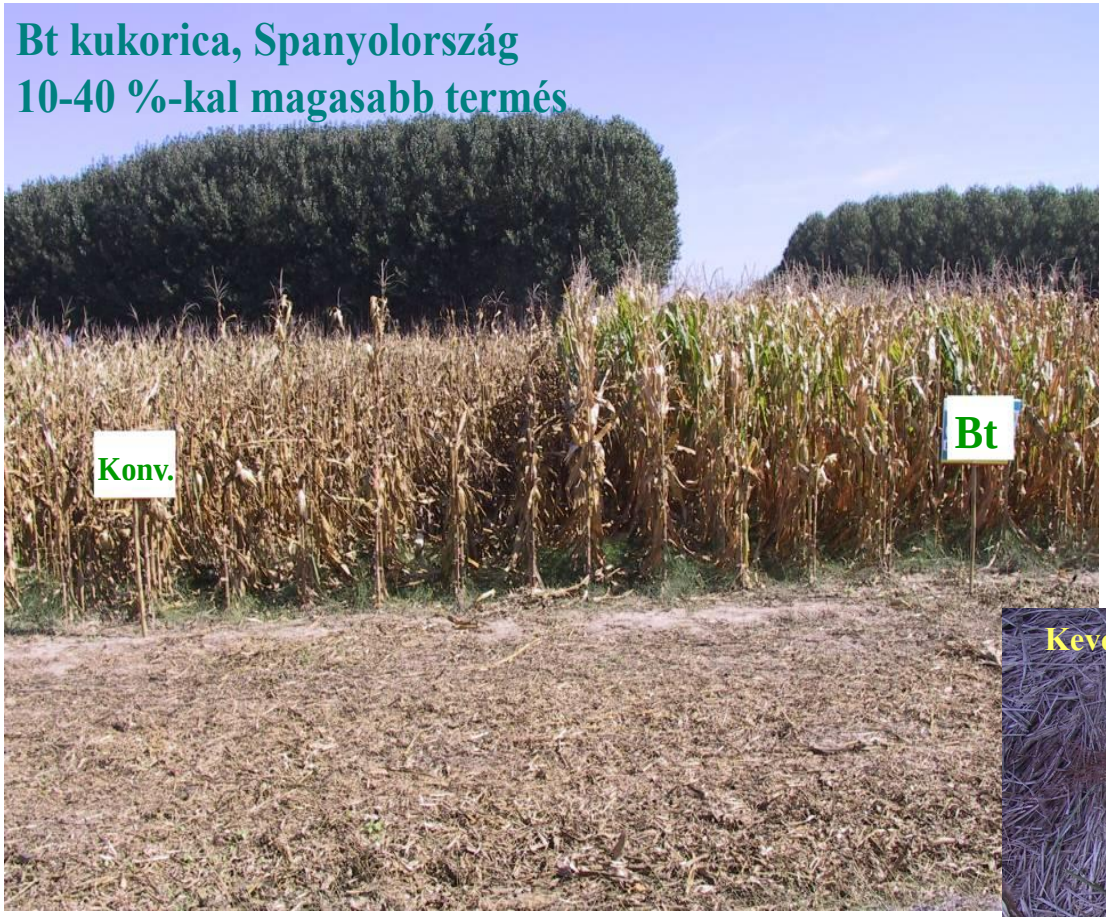
The series of insect resistant, herbicide tolerant and virus resistant plants commercial production started in 1996.

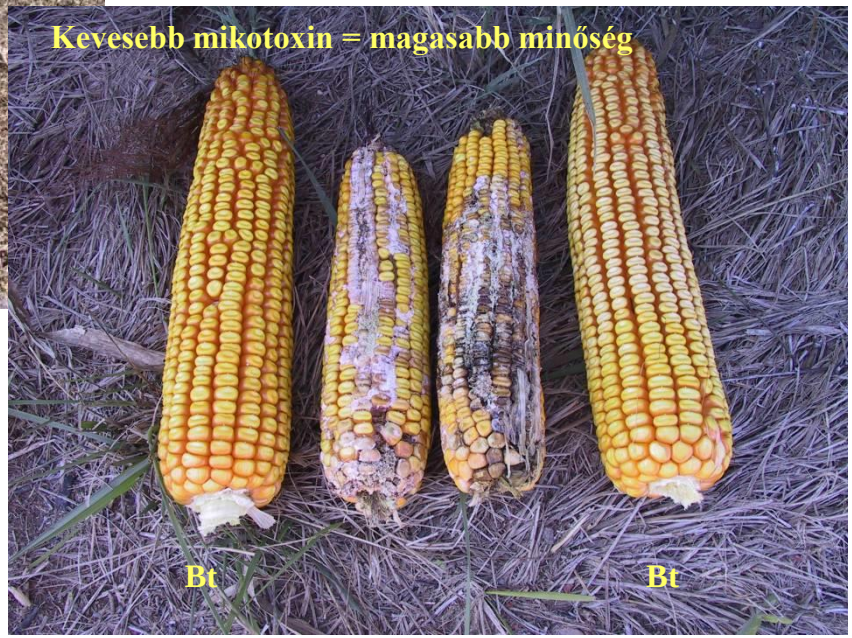
Today their cultivation exceeded yearly 214 thousands of hectares in the world this year.

The introduction of these crops is in the forefront of the debate among different stakeholders, and almost completely blocked in the European Union countries.

Papaya ringspot virus resistant papya planting in Hawaii

Some examples





European corn borer resistant GM corn in Spain

Diabrotica corn rootworm resistant corn hybrids

PVY resistant GM potatos

JRC Scientific and Technical Reports 2011

New Breeding techniques
State-of-art and prospects for economical development

Maria Lusser, Claudia Parisi, Daniel Planand Emilio Rodrigez-Cerezo

New plant breeding techniques

Cisgenesis and intragenesis

RNA-dependent DNA methylation

Grafting on GM rootstock

Reverse breeding

Oligonucleotide directed mutagenesis

Zinc finger nuclease EXZACT™

Synthetic genomics

TALEN

CRISPR

Cisgenesis and intragenesis

- **Restriction of transgensis to DNA fragments from the species itself or from a cross-compatible species. In the case of cisgenesis, the inserted genes associated introns and regulatory elements are contiguous and unchanged. In the case of intragenesis, the inserted DNA can be a new combination of DNA fragments from the species itself or from a cross-compatible species.**

RNA-dependent DNA methylation (RdDM)

- RdDM allows breeders to produce plants that does not contain foreign DNA sequences and in which no changes or mutations are made in the nucleotide sequences but in which gene expression is modified due to epigenetics. RdDM induces the transcriptional gene silencing (TGS) of targeted genes, via the methylation of promoter sequences. In order to obtain targeted RdDM, gene encoding RNAs which are homologous to promoter regions delivered to the plant cell by suitable methods of transformation. These genes give rise to dsRNAs which, alter processing specific enzymes, induce methylation of the target promoter sequences thereby inhibiting the transcription of the target gene.

Grafting on GM rootstock

- Non GM scion is grafted onto a GM rootstock, leaves, stem, flowers, seeds and fruits would not carry the genetic modification with respect to changes in genomic DNA sequences. Nematode resistant GM grape rootstock prevents Nematode transmitted virus infection of grape.

Reverse breeding

- Reverse breeding is a method in which the order of events leading to the production of a hybrid plant variety is reversed. It facilitates the production of homozygous parental lines that, once hybridized, reconstitute the genetic composition of an elite heterozygous plant, without the need for back-crossing and selection. The reverse breeding technique makes use of transgenesis to suppress meiotic recombination. In subsequent steps, only non transgenic plants are selected. Therefore, the offspring of the parental lines would genotypically reproduce the elite heterozygous plant and would not carry any additional genetic change.

Oligonucleotide directed mutagenesis, ODM

- ODM is another tool for targeted mutagenesis in plant breeding. ODM is based on the use of oligonucleotides for the induction of targeted mutations in the plant genome, usually of one or a few adjacent molecules. Oligonucleotides target the homologous sequence in the genome and create one or more mismatched base pairs corresponding to the non complementary nucleotides. The cell's own gene repair mechanism is believed to regconise these mismatches and induce their correction. The oligonucleotides are expected to be degraded in the cell but the induced mutations will be stably inherited.

Zinc finger nuclease ZFN technology

- **ZFNs are proteins which have been custom designed to cut at specific deoxyribonucleic acid sequneces.**
- **They consist a „zinc finger” domain recognizing specific DNA sequences in the genome.**
- **Three actions, ADD, INSERT,DELETE.**

The major concerns of the opponents based on that fact that the genetic material contains foreign genes originating from other organisms which new combinations may not be formed in the nature.

However the latest new methods of genome editing made it possible that even a single nucleotide addition from different organism not produced by these techniques.

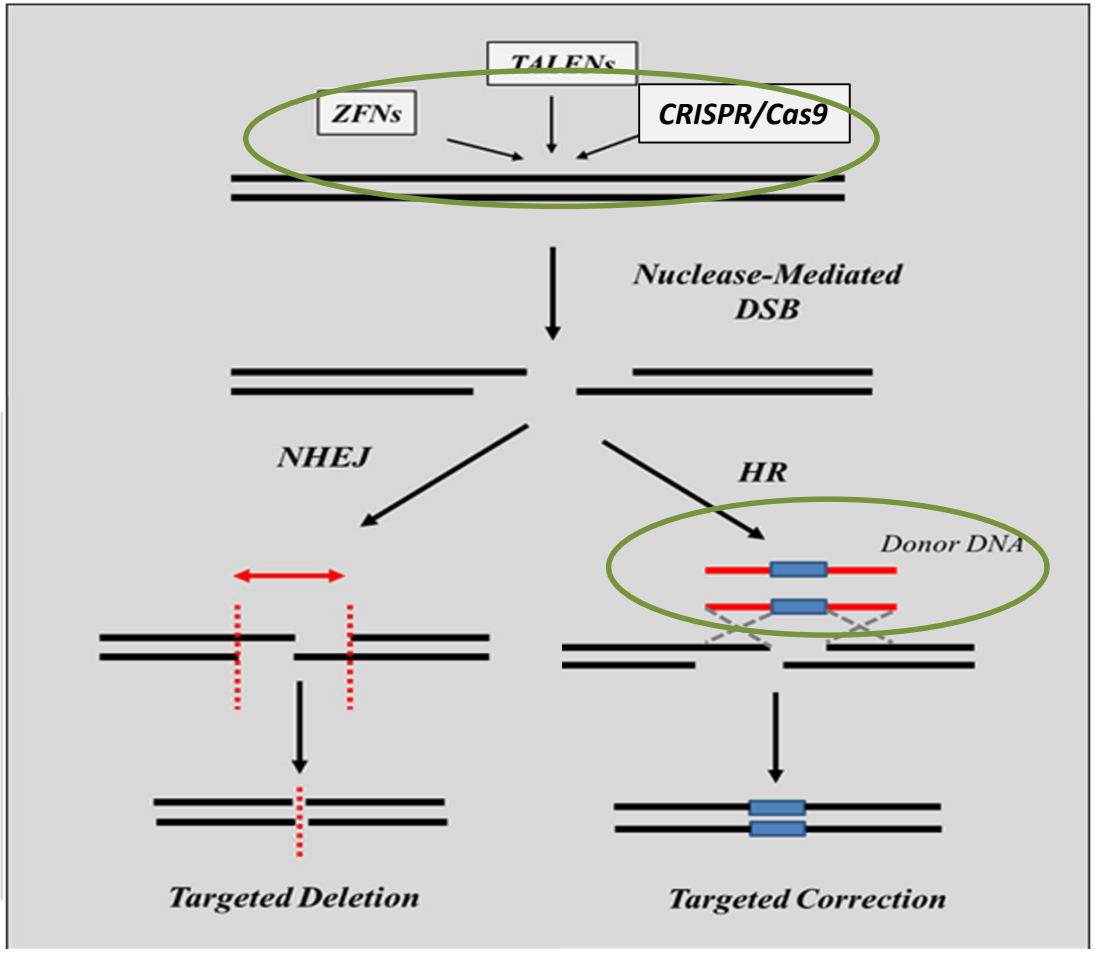
These mutants cannot be distinguished from naturally evolving mutants.

These four techniques the oligo nucleotide directed mutagenesis, the zinc finger nucleases, the TALE nucleases and the CRISPR/Cas9 systems are efficient technologies

These four molecular scissors are either directed by DNA, RNA or Proteins all just produced mutations on already existing genes in the organisms, just activating the silent gene of the organisms or blocking them.

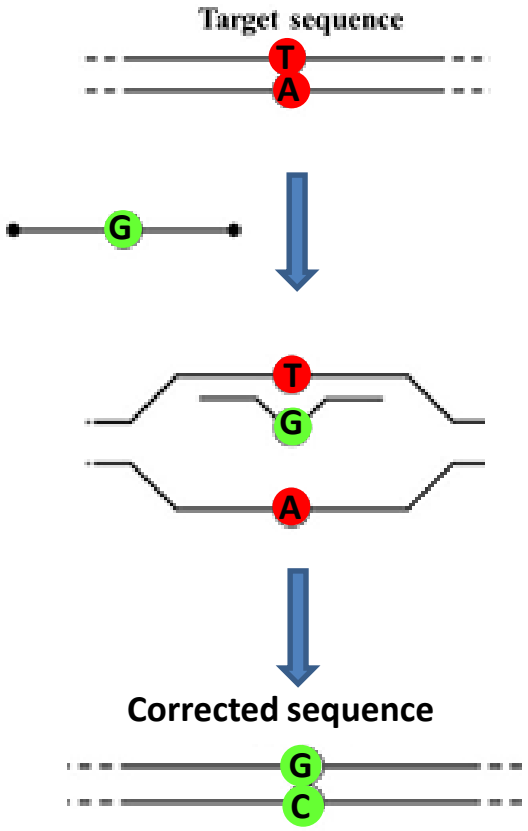
It is also possible with these techniques that a useful traits form the same species can be incorporate into a commercial varieties.

Specific gene editing methods



oligonucleotides

Oligonucleotide Targeted Nucleotide Exchange (OTNE)



Advantages:

- Not requiring transgene introduction
- Simple design
- Cheap synthesis

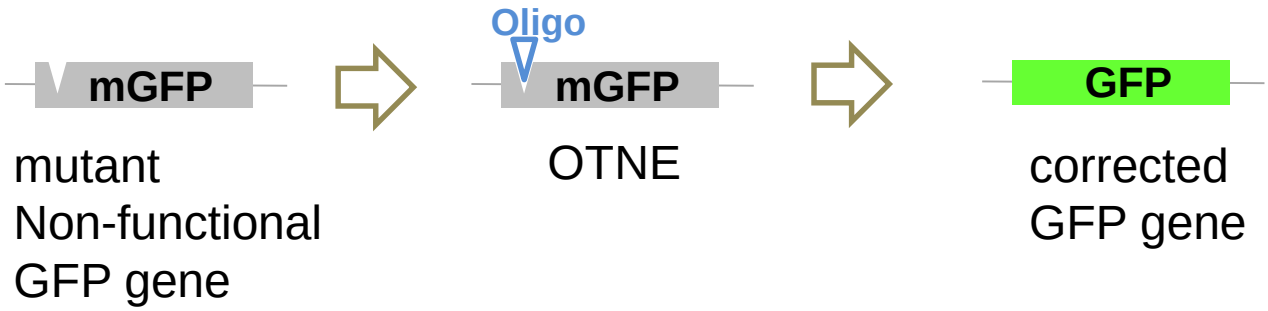
Major limitations:

- low frequency
- Identification of the non-selectable mutations

- successful generation of herbicide tolerant variants of different crop species.

Review by Breyer *et al.* (2009)

Oligonucleotide Targeted Nucleotide Exchange (OTNE)



Mutant GFP sequence	5'. . AG GGC **TAG** GAG CTG TTC AC . . 3'
Truncated GFP protein	. . K G * E L P T . .
Wild type GFP sequence	5'. . AG GGC **GAG** GAG CTG TTC AC . . 3'
Wild type GFP protein	. . K G **E** E L P T . .

Correcting oligonucleotide:
5'-CCACC ATG GTG AGC AAG GGC **G**AG GAG CTG TTC ACC GGG GTG-3'
(Dong *et al.*, 2006)

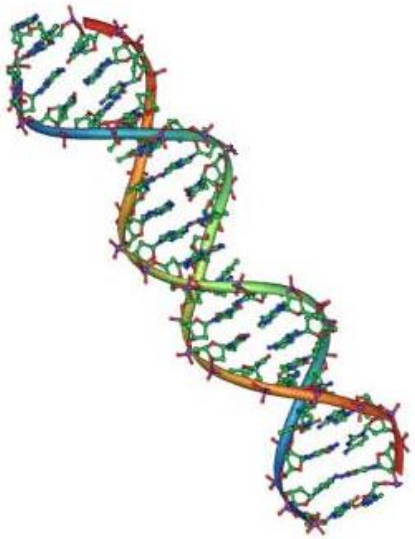

EXZACT™
Precision Technology
Dow AgroSciences

EXZACT™ is a Toolkit for Precise Genome Modification

Genome Modification: several modalities

- Targeted mutagenesis of an endogenous gene
- Targeted gene addition at an endogenous locus
- Targeted genome editing at an endogenous locus

- Based on proprietary zinc-finger engineering.
- Precisely targets any DNA sequence.
- Accurately modify the genome
- Mode of action based upon natural DNA repair processes and promoter activity.



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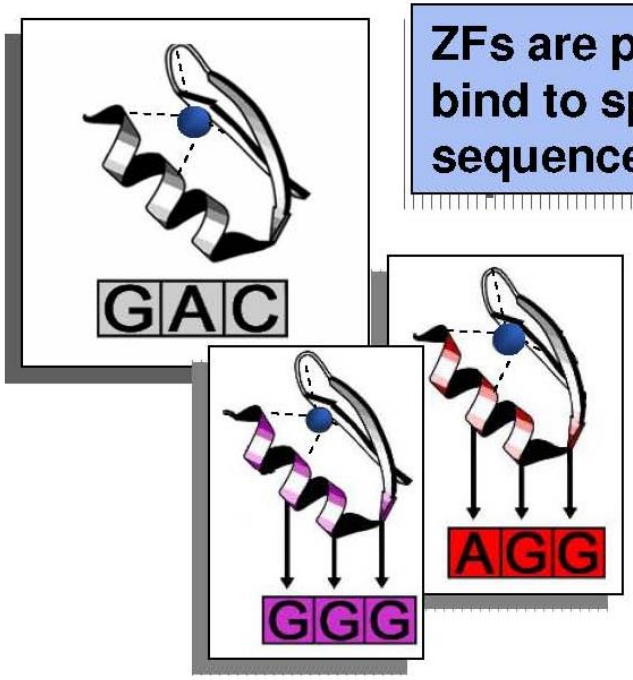
EXZACT™ Precision Technology ZFP Design in a Nutshell



ZFs are protein domains which bind to specific 3 base pair DNA sequences.

Altering the amino acid sequence in the ZF changes its DNA binding specificity.

A series of zinc finger protein domains are custom engineered to bind to a target DNA sequence.

The addition of a functional domain provides specific DNA modification features.

Functional Domain

Cleavage
Activation
Repression

6-finger protein creates an 18 base pair recognition module

Dow AgroSciences

EXZACT™ Precision Technology Represents a New Way to Develop Biotech Products

Targeted Mutagenesis / non-GMO

- **End-product is indistinguishable from mutation breeding**
- **Desired mutations at intended, specific sequences**
- **More efficient screening and breeding**
- **No foreign DNA will be present in the genome** *(considered a conventional variety)*

Targeted Trait Insertion / GMO

- **Target trait/gene to a specific genetic locus**
- **Insert multiple traits/genes at one locus**
- **More efficient generation of desired GMO events**
- **Target DNA to location of current de-regulated event or 'safe' locus**
- **GMO events with no disruption of native gene function**

Dow AgroSciences

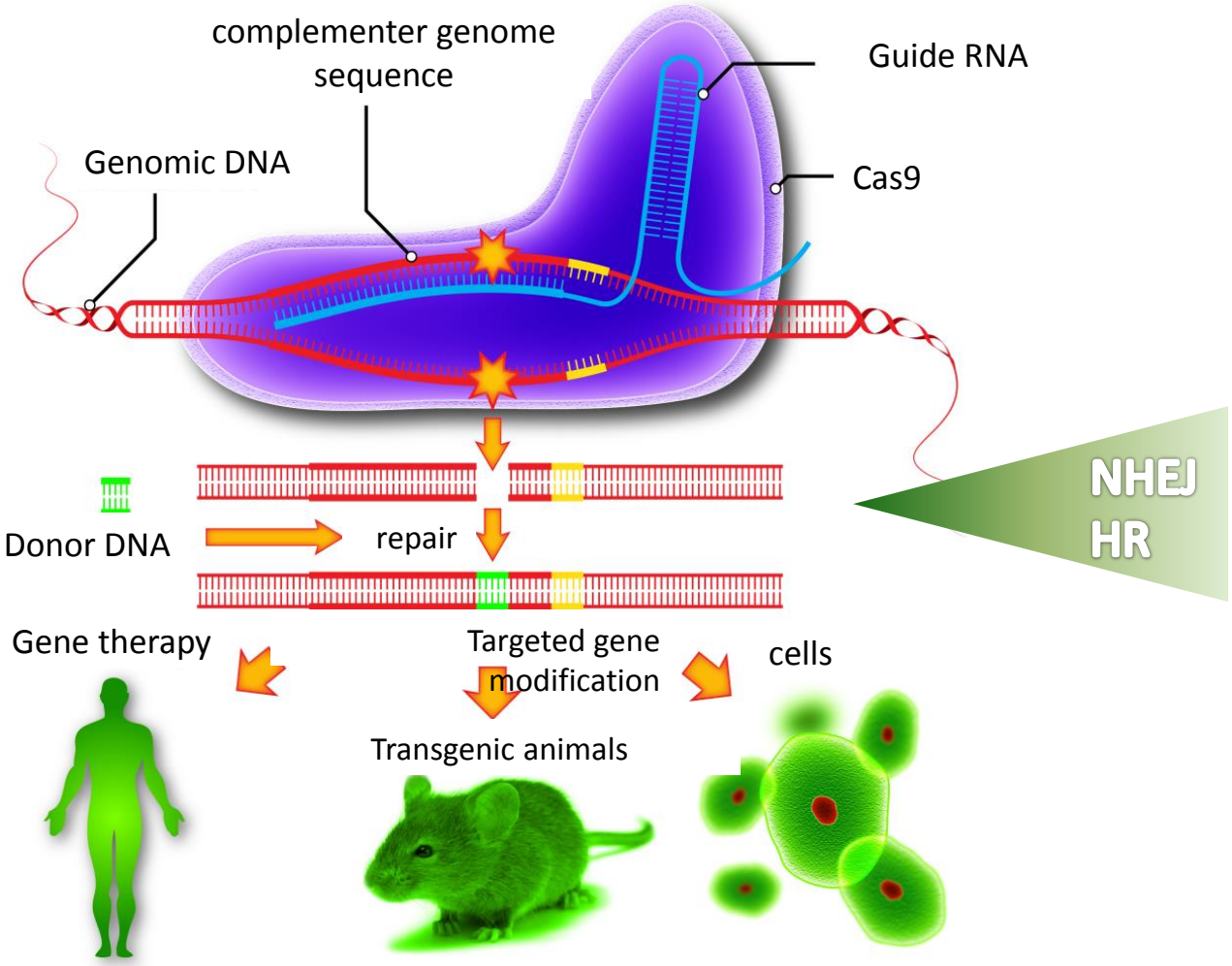
CRISPR developers

Emanuelle Charpentier

Feng Zhang

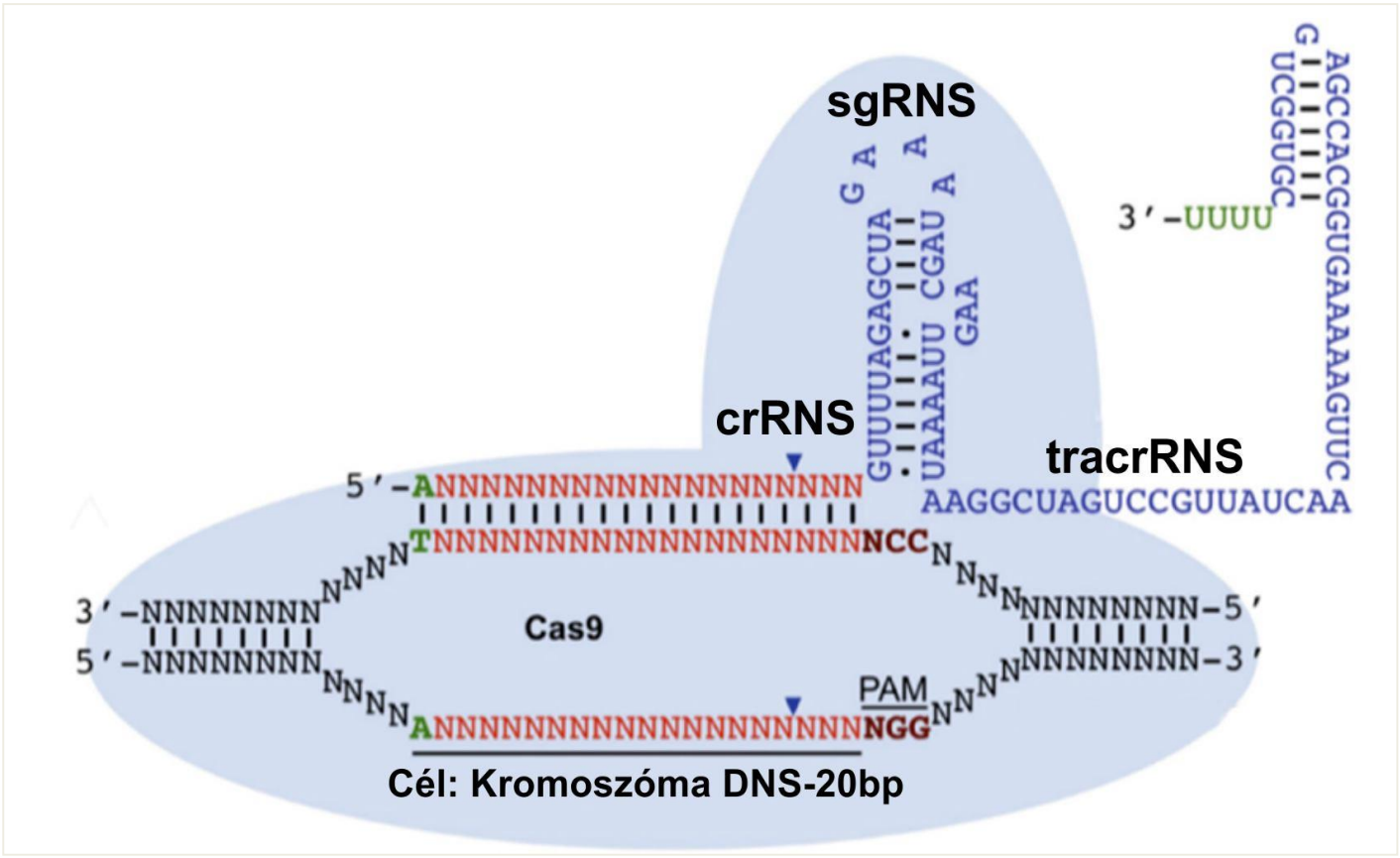
Jennifer Doudna

CRISPR/Cas9
Clustered Regularly Interspaced Short Palindromic Repeats



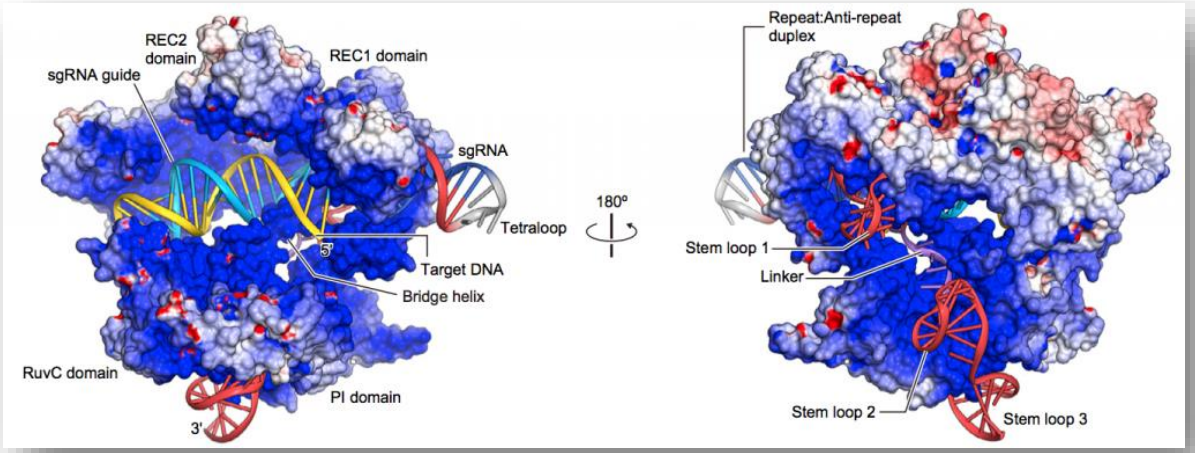
CRISPR/Cas9

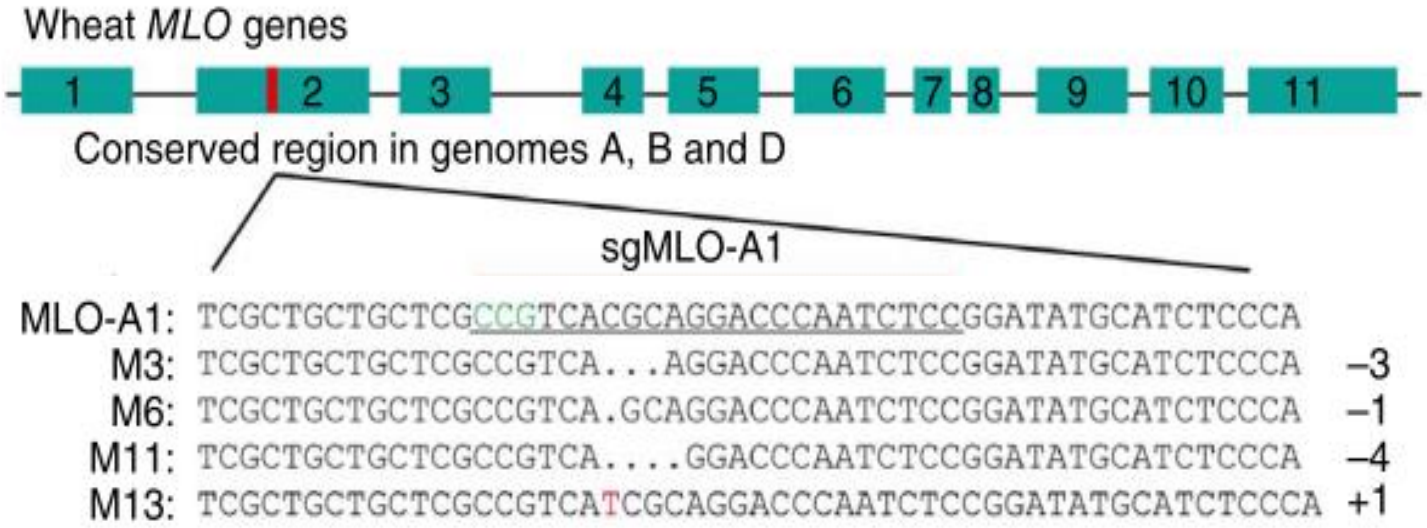
Clustered Regularly Interspaced Short Palindromic Repeats



Cas9

- *Streptococcus pyogenes* Cas9 *(SpCas9)*
- NLS signal
- Purified mRNA from Sigma *(500 ng/ul, Sigma Ald.)*
- Concentration
  - **150 ng/ul**
  - 55 ng/ul





Powdery mildew resistant wheat

Genome edited

```
586    DTTPLAGLRLSQVRSMAFFGQVKCMP
612  SIADYRLLRVLILCFWADQEKTSYD
637  LTSISELLQLRYLKITGNITVKL
660  PEKIQGLQHLQTLEADARATAVLLDIVHTQ
690  CLLHLRLVLLDLLPHCHRYIFTSI
714  PKWTGKLNNLRILNIAVMQIS
735  QDDLDTLKGLGSLTALSLLVRTAPAQRIVAANEGFGSLKYFMFVCTAPCMTFVEGAMPSVQRLNLRFNANEFKQY
810  DSKETGLEHLVALAEISARIGGTDDDESNKTEVESALRTAIRKHPTPSTLMVDIQWVDWIFGAEGRDLDEDLAQQDDH
888  GYGFFILFPGYNLQGL
904  LSFFLSLPWLLSLPAMHLQPDLMIV
                   S
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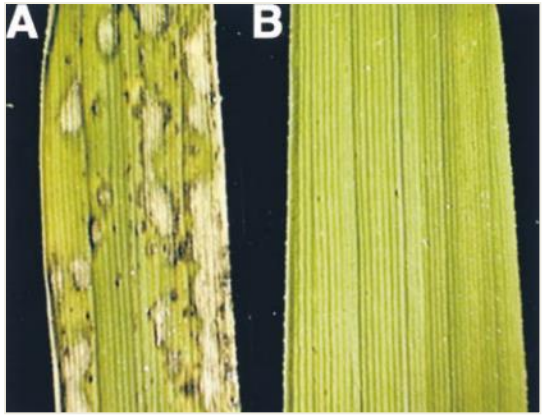
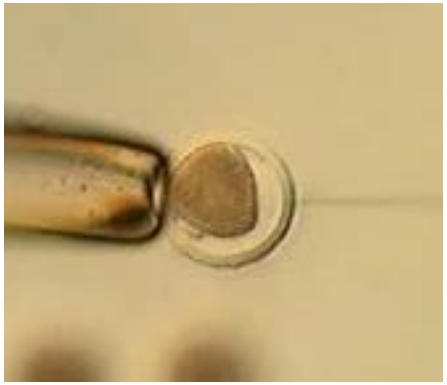
Gene edited blast resistant rice



Methods to produce transgenic rabbits



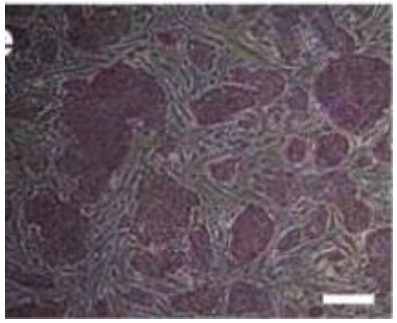
Microinjection

lentiviral gene transfer

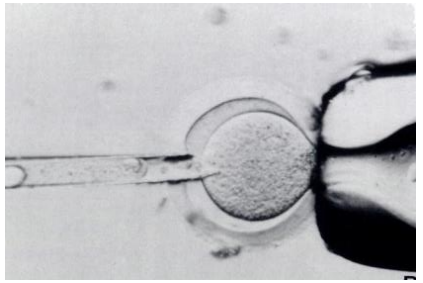
Only for additive transgenesis

transposon transgenesis

Rabbit ES or iPS cells- not working

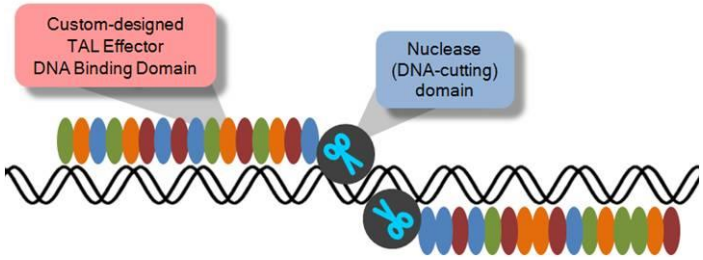


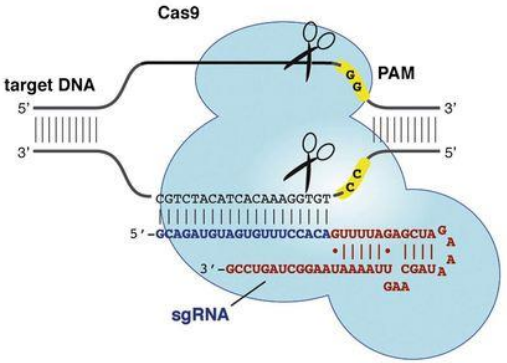
Rabbit nucleat transfer- very low efficiency



New techniques are necessary for targeted transgenesis in rabbits and other farm animals

„Designer nuclease technology”





•Artificial systems, based on natural protein or protein-RNA systems

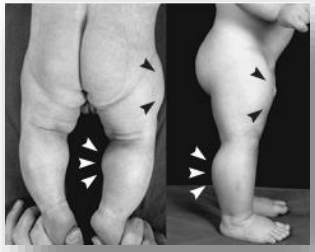
•Always contain a DNA binding domain and a cleavege part

Molecular scissors

Zinc finger nuclease
Talen nuclease
RNA mediated Crispr System

Aim: Target Myostatin (GDF-8) in rabbit

- Negative regulator of muscle growing
- Inhibiting myoblast terminal differentiation and proliferation
- Protect myoblasts from apoptosis
- Lack of the protein causes hyperplasia/hypertrophia
- Fatty acid composition can be altered



Janus face of genome editing

Major driving force and difference between GMO introduction and introducing genome edition in practical agriculture

GMO

genom editing

Agricultural innovation policy in plant breeding

Old World

§

New World

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A way forward in Hungary

Lecture series at Universities, Road show

Publications in popular science magasins, ***Hungarian Agriculture*** (weekly)
Green Biotechnology (quarterly. published by
the Association for innovation agricultural biotechnology)

Text book publication on precision breeding

Statement on genome editing by the Hungarian Academy of Sciences

Conference organisations

Lecture series at different Universities
Road show

Lecture series at different Universities
Road show

Lecture series at different Universities
Road show

11 January, 2018

Precision genome editing for a livable world – official declaration of the Hungarian Academy of Sciences

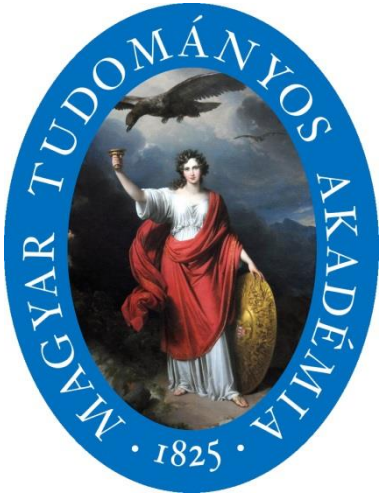
The Presidium of the Hungarian Academy of Sciences has unanimously accepted the resolution drafted by the Academy's three life sciences sections (Section of Biological Sciences, Section of Medical Sciences, Section of Agricultural Sciences) concerning the most important scientific method today, genome editing, which opens up enormous possibilities for medical science and agriculture.

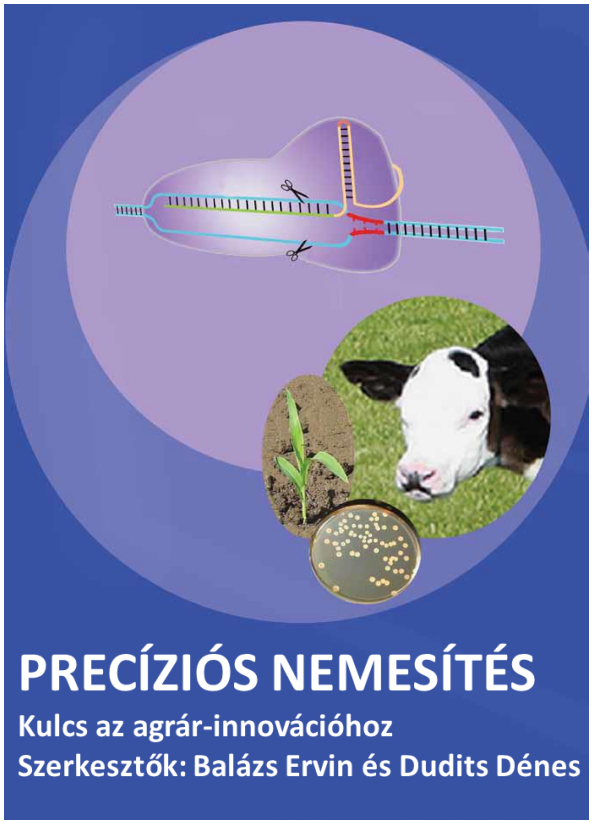
The past years have witnessed revolutionary changes in the life sciences, primarily in genetics, owing to the emergence and the exceptionally rapid spread of genome editing. The different techniques of genome editing enable us to freely modify the genome of a living being with much more precision than before, much like modifying a text in Word.
The case of genome editing very clearly shows that legislation is unable to keep up with the pace of technological development. The delay in legislation results in an innovational disadvantage. While the United States and China have already started creating rules for this new technology with great economic potential, the European Union has yet to take steps. It is a realistic danger that this uncertainty will cause Hungary and the European Union to lag behind in international research and innovation competition.

Some months ago, EASAC (European Academies Science Advisory Council) issued a resolution urging the creation of more sensible rules that address the real potential and risks of genome editing. Now the Hungarian Academy of Sciences has also passed a resolution on this problem.

The cornerstone of the Academy's declaration is that it states– in accordance with other European academies – that genome editing, as a method for precision breeding, may fundamentally differ from the creation of genetically modified organisms (GMOs).
In the case of genome editing, it is possible to enhance the characteristics of living organisms without inserting genes from unrelated species. With this technology, DNA is modified in a similar manner to natural processes, with much higher precision than with previous methods, and thus risks are reduced considerably. It is scientifically supported that following EASAC's recommendations, genome editing should not fall within the scope of the legislation on GMOs; therefore, the use of genetically-edited organisms would not be in violation of the Hungarian constitution.

Genome editing is a new, quickly spreading breeding technique, which already has several uses in the field of agriculture, including the creation of disease or virus-resistant breeding stocks. Concerning medical uses, if proper ethical rules are followed, genetic diseases caused by a single mutation could be cured (the number of such known diseases is around 800). Genome editing could make it possible to effectively create new proteins to be used in human medicine or alimentation that have not been produced by bacteria or yeast fungi.
The MTA's resolution declares that the public must be informed about the possibilities and risks of new genome editing techniques – especially those of the most frequent CRISPR/Cas9 technology – and public discussion is also called for. Information on genome editing should also be included in school curricula.





Precision breeding a key to the agricultural innovation

Edited by Ervin Balázs and Dénes Dudits

Precision breeding a key to the agricultural innovation

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Content

International conferences

MTA ATK

Centre for Agricultural Research, Hungarian Academy of Sciences

New Breeding Techniques

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regulate or not to regulate

Date:

26 - 27 September 2016

Venue:

Hungarian Academy of Sciences

1051 Budapest, Széchenyi István sqr. 9.

Large auditorium / Nagyterem

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Q and A