Genetic Engineering:

The

“New Breeding Technologies”

their risks and unpredictabilities

Dr. Ricarda A. Steinbrecher
ENSSER (European Network of Scientists
for Social and Environmental Responsibility)
Brussels, 2 March 2016

r.steinbrecher@econexus.info

Intro

Over the last 5-10 years there have been rapid developments in genetic engineering techniques (genetic modification).

Along with these has come the **increasing ability to make deeper and more complex changes** in the genetic makeup and metabolic pathways of living organisms. This has led to the emergence of **two new fields of genetic engineering** that overlap with each other: **synthetic biology** and the so-called **New Breeding Techniques (NBTs).**

NBTs are mix of old and new.

NBT classifications

1) Genome editing techniques

- ODM
- Nucleases (Zinkfinger Nucleases (ZfN) Talens, CRISPR/Cas)

2) current/traditional GE:

a) with random integration (& transformation induced mutations)

- Cisgenics & intragenics
- Grafting onto GM rootstock
- Floral Dip

b) GM process but no intended GM sequence in end product

- Reverse Breeding (removed in breeding process)
- Agroinfiltration (not intended to integrate)

3) Genetic Engineering techniques that alter the 'outside' of the DNA to alter gene regulation (ie gene silencing).

(Epigenetic changes)

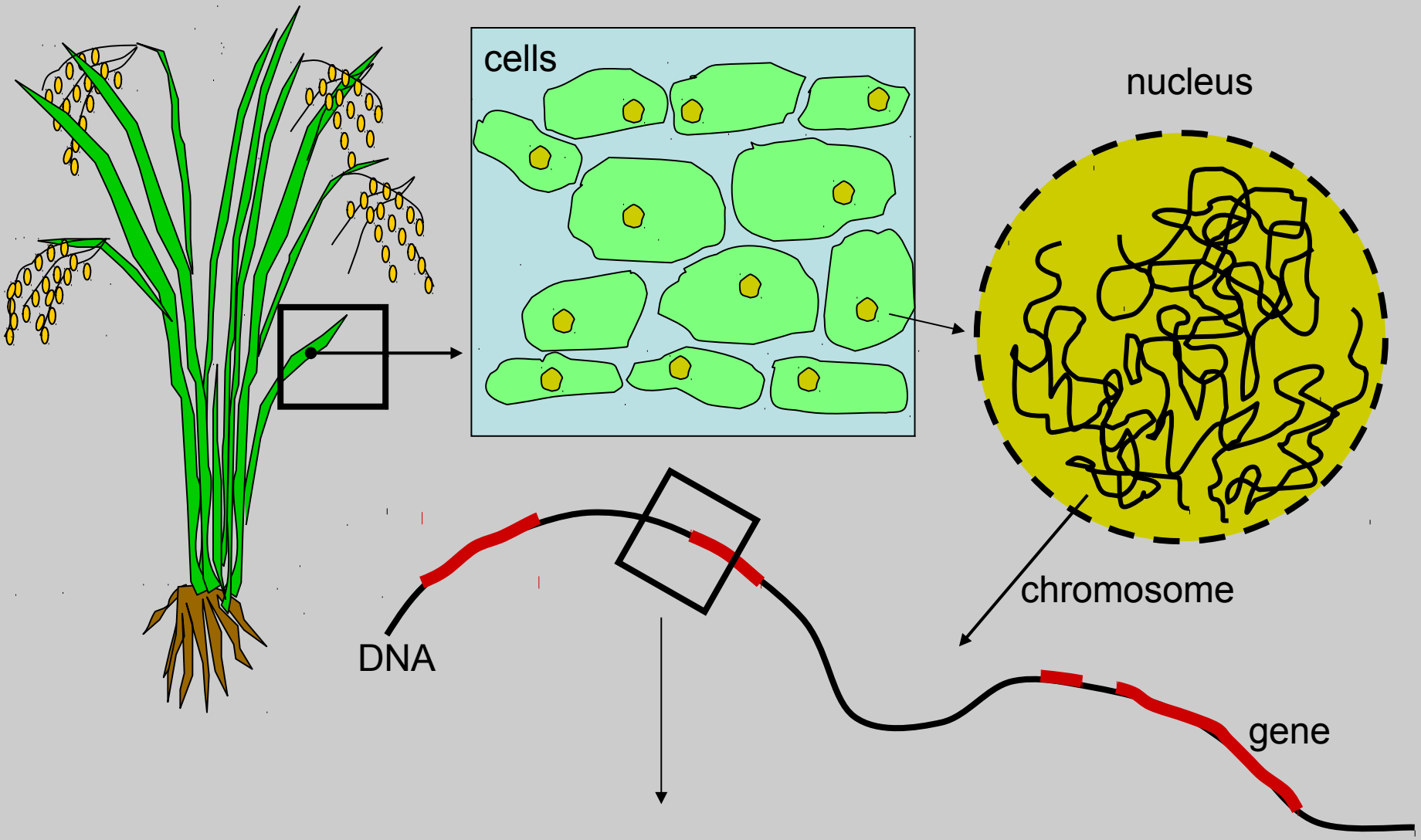
- RNA dependent DNA Methylation (RdDM)

Risks and uncertainties – old and new

In conclusion we will find: the seven new genetic engineering techniques referred to as NBTs **each bring their own set of risks and uncertainties**. Whilst many of these are the same as with older GM **techniques there are also serious additional concerns, such as the potential environmental and health impacts of RNA dependent DNA methylation (RdDM).** Equally a **new degree of uncertainty and risk of unintended effects** arise from the use of gene editing techniques (ZFN and ODM as well as CRISPR and TALENs).

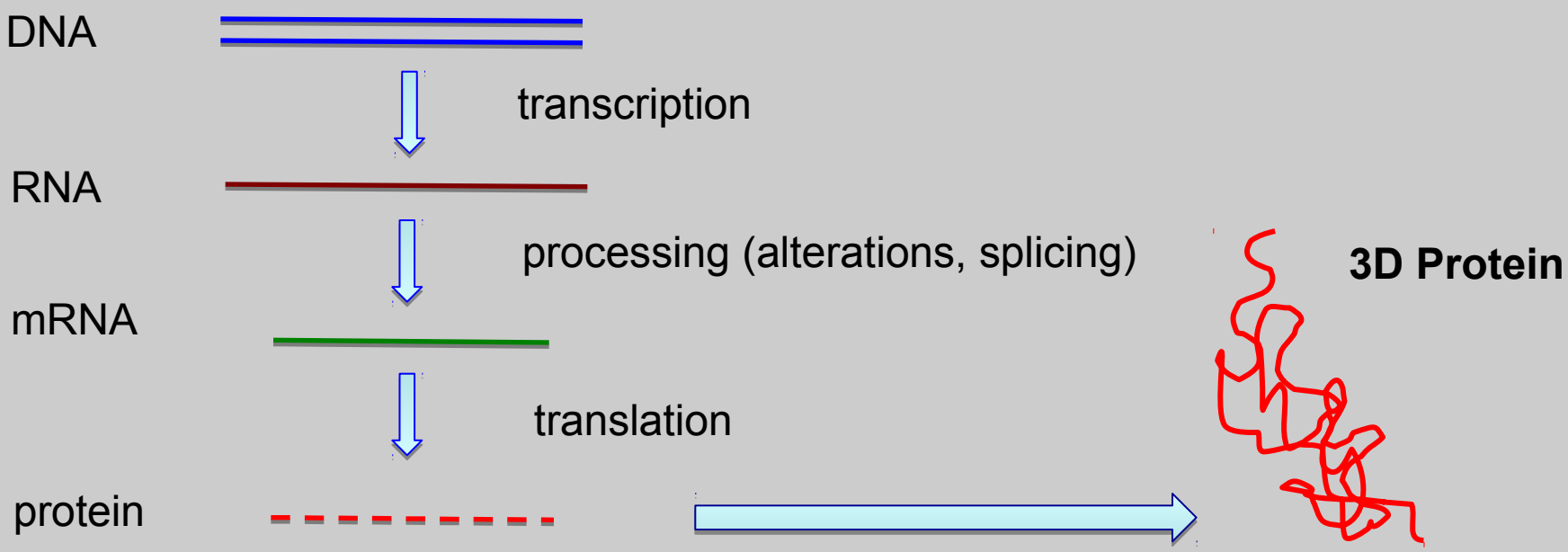
MAS: NOT genetic engineering or GMO

(trait hardly ever single gene)

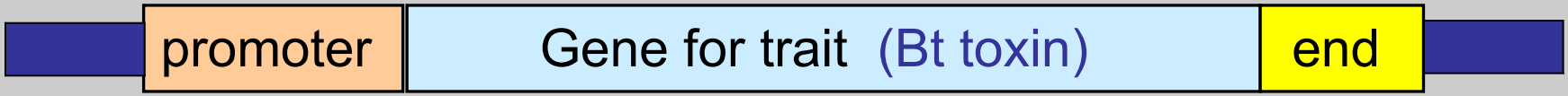

cells
nucleus
chromosome
DNA
gene
A
T
A
T
C
G
T
A
C
G
G
C
T
A
Basepairs: A-T & C-G (*nucleotides*)

Genes and Genetics: The Fundamentals
"The New Genetics"

In many cases, more than one RNA/protein is produced from a given gene.



Gene & gene construct



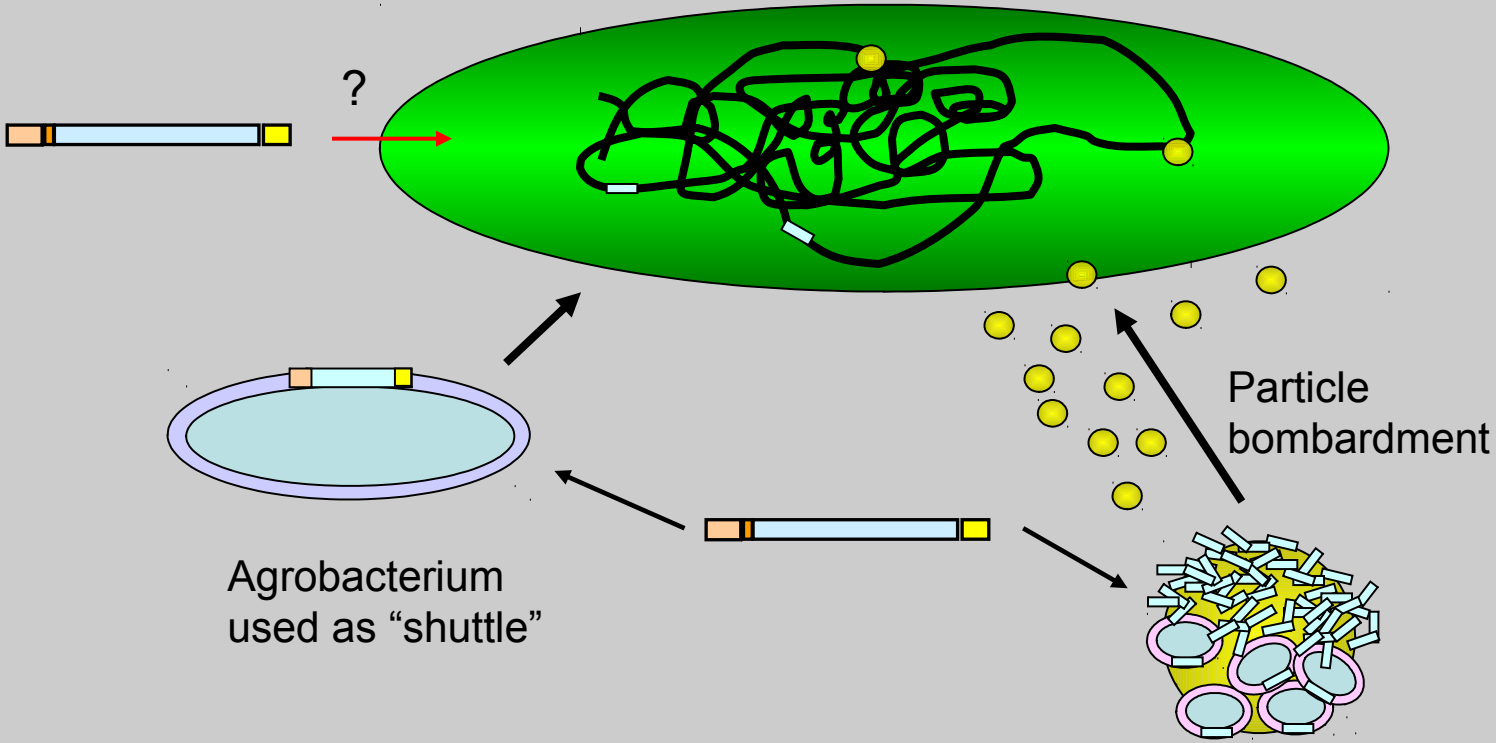
Regulatory sequence: on/off switch - often CaMV (virus)

Coding sequence of a gene - e.g. *pat* or *bar* gene for herbicide resistance from soil bacteria →

Regulatory sequence: Termination signal - e.g. from pea

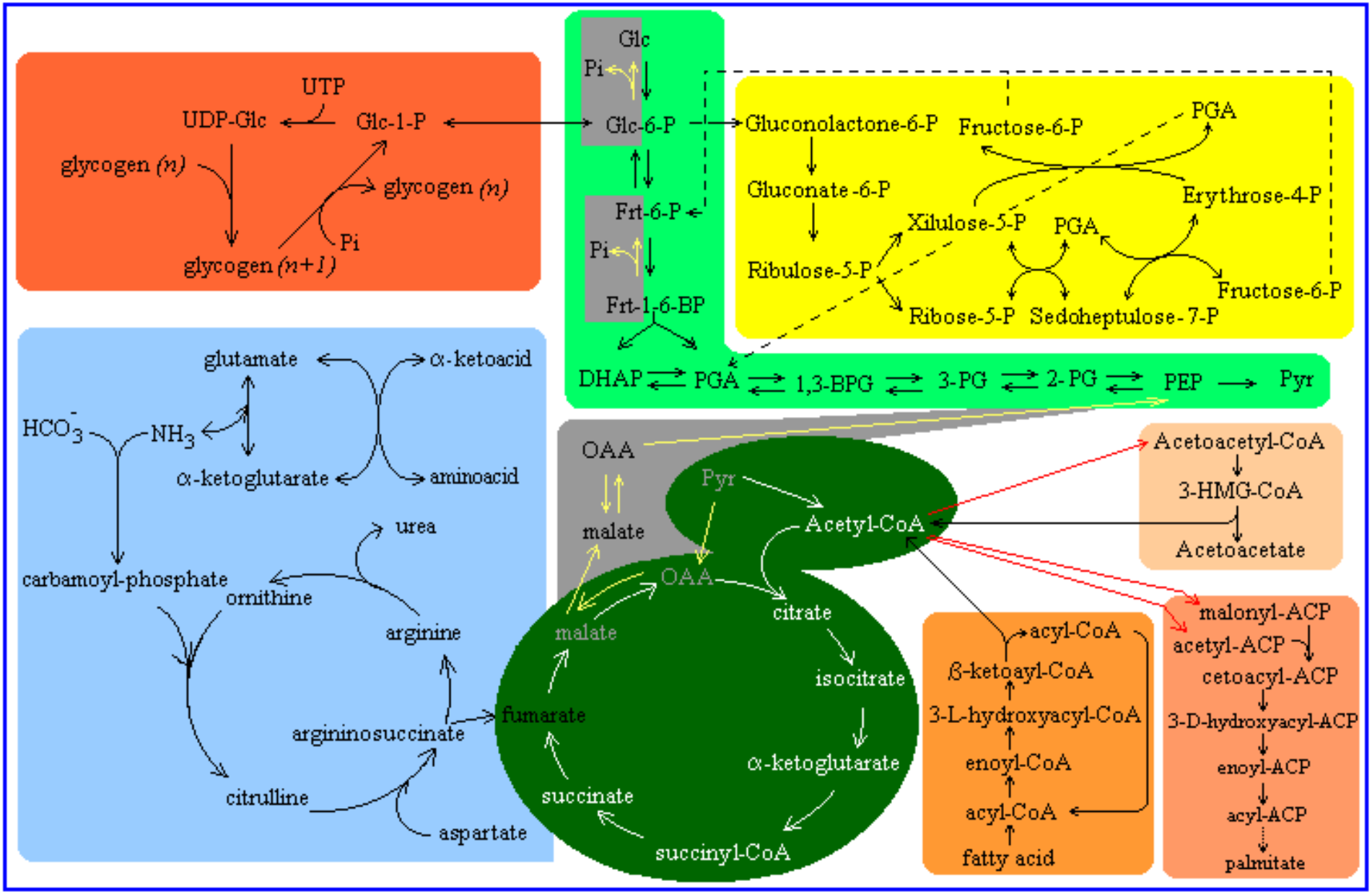
Plasmid backbone DNA, superfluous genetic material

Transformation



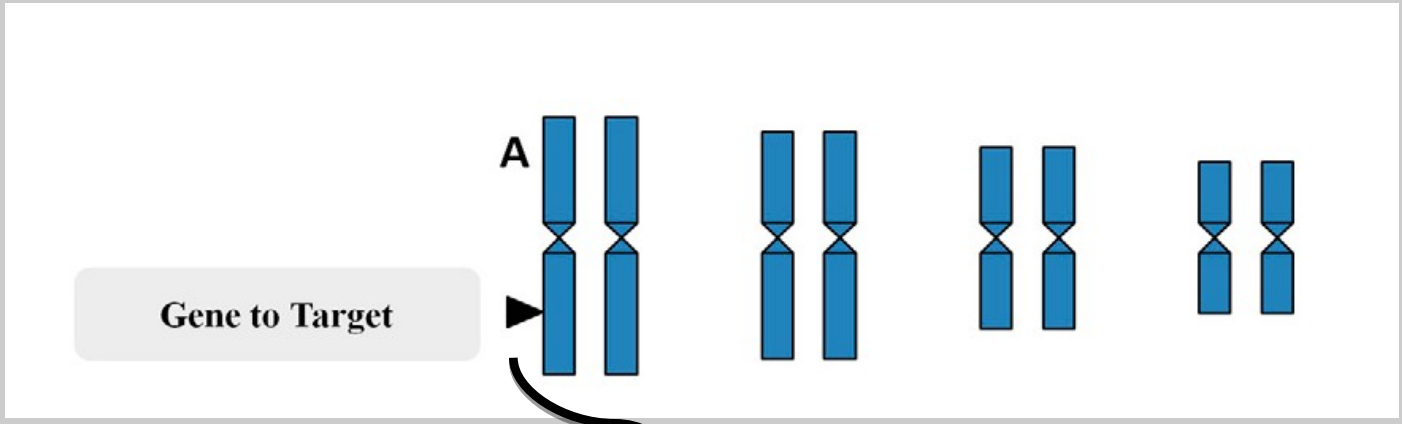
No control of where the gene will insert itself:

- Random integration
- Imprecision (incl. superfluous DNA)
- 100 -1000s of Mutations (Sala et al. 2000, Wang et al. 1996, Labra et al 2001)



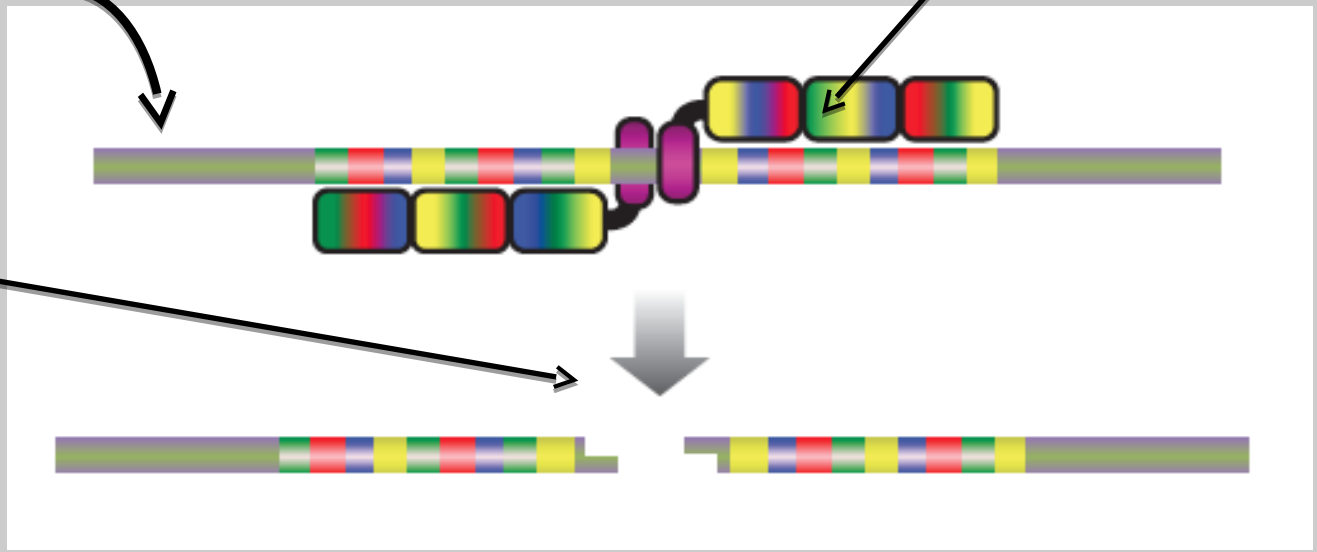
ct: glycolysis - glucose oxidation in order to obtain ATP
cb: citiric acid cycle (Kreb's cycle): acetyl-CoA oxidation in order to obtain GTP and valuable intermediates. bl: AA degradation

Genome editing



Special enzyme

Targeted ‘break’ of both strands of DNA in a chromsome



slide with thanks to Jack Heinemann

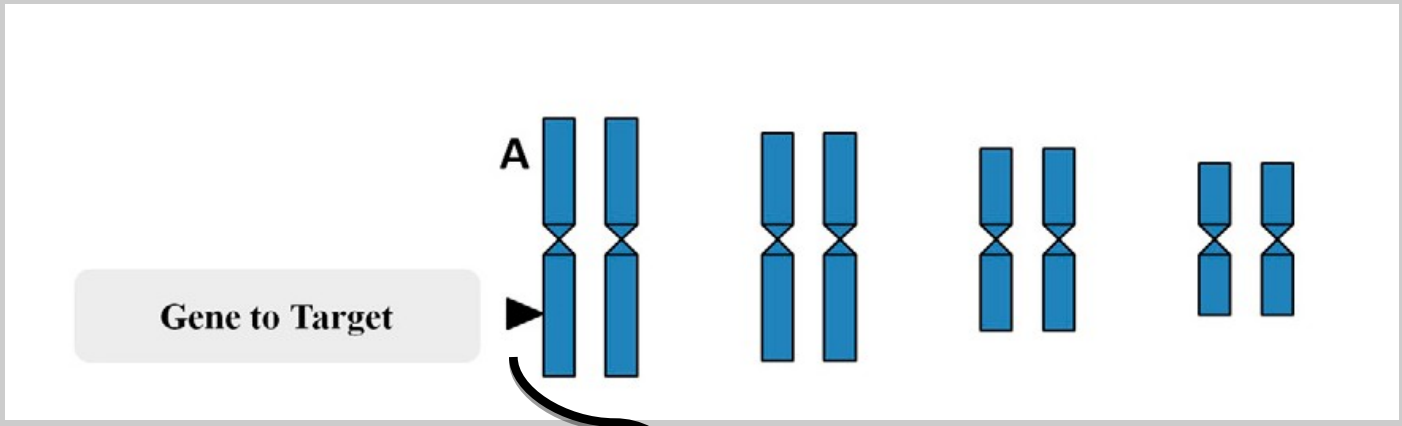
Special enzymes (nucleases)

The special enzymes are called:

1. CRISPR/Cas
2. ZFN (zinc finger nucleases)
3. Tales/Talens (transcription activator–like effector nucleases)
4. Meganucleases

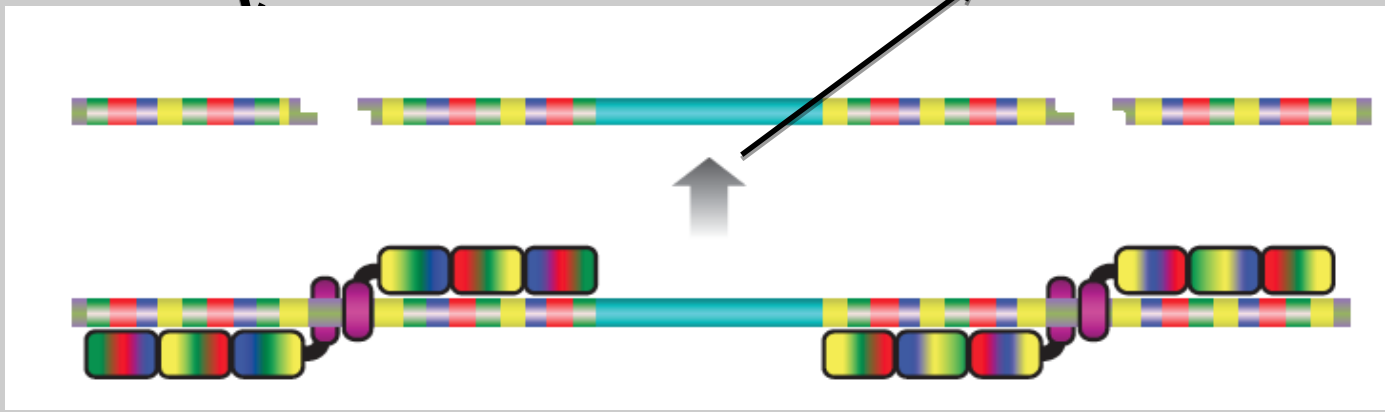
nucleases = “molecular scissors”

Genome editing



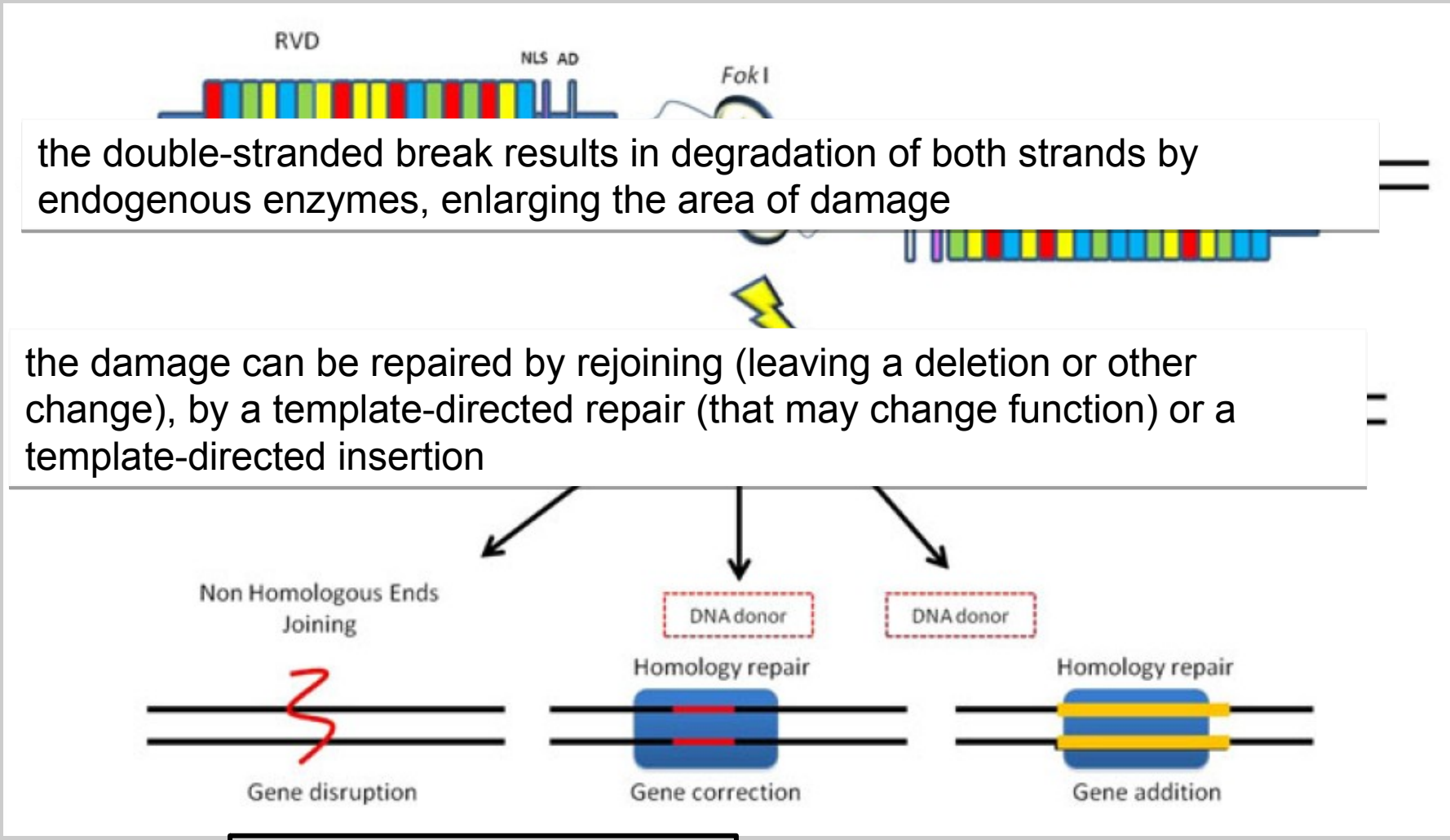
Material ‘deleted’

Two separate breaks result in large deletion of material



J

alternative endings



multiple use: deep changes

slide with thanks to Jack Heinemann

Unpredictabilities and risks

- **Off-target effects** (due to non-specific binding to non-target DNA): off-target mutations in the genome. These mutations can

a) if in the coding sequence, result in changes of function of proteins, or **b)** if in regulatory sequences, result in changes in the expression of genes, such as increased presence of plant toxins, or absence of proteins important for nutrition, plant defence or disease resistance, increased presence of allergens.

- Integration of added DNA or oligonucelotides into the genome

- Impacts of the genetic engineering processes (mutations)

Steinbrecher, R (2015). Genetic Engineering in Plants and the “New Breeding Techniques” (NBTs) - Inherent risks and the need to regulate. www.econexus.info

The idea of Precision

The idea of precision is based on that one knows what one is doing – here this is not the case.

Knowledge & precision at the level of nucleotides is only the bottom layer.

What is missing is the contextualisation into

- the genome
- the epigenetic landscape
- the organism
- the ecosystems
- the socio-economic conditions that differ around the world

Precision around nucleotides gives a false sense of predictability and safety – there is no data to support such extrapolations.

thank you