Reliability of CRISPR/Cas

FGU Fachstelle Gentechnik und Umwelt

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FGU Fachstelle Gentechnik und Umwelt

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Clearing House in regards to Genome Editing methods

Screening of scientific publications around these topics with respect to the Precautionary Principle

Observing regulatory development

Risk Assessment

Advisory board

GeN Gen-ethisches Netzwerk e.V.

BUND FRIENDS OF THE EARTH GERMANY

SOS save our seeds

TEST BIOTECH

AbL Arbeitsgemeinschaft bäuerliche Landwirtschaft e.V.

BUND Naturschutz in Bayern e.V.

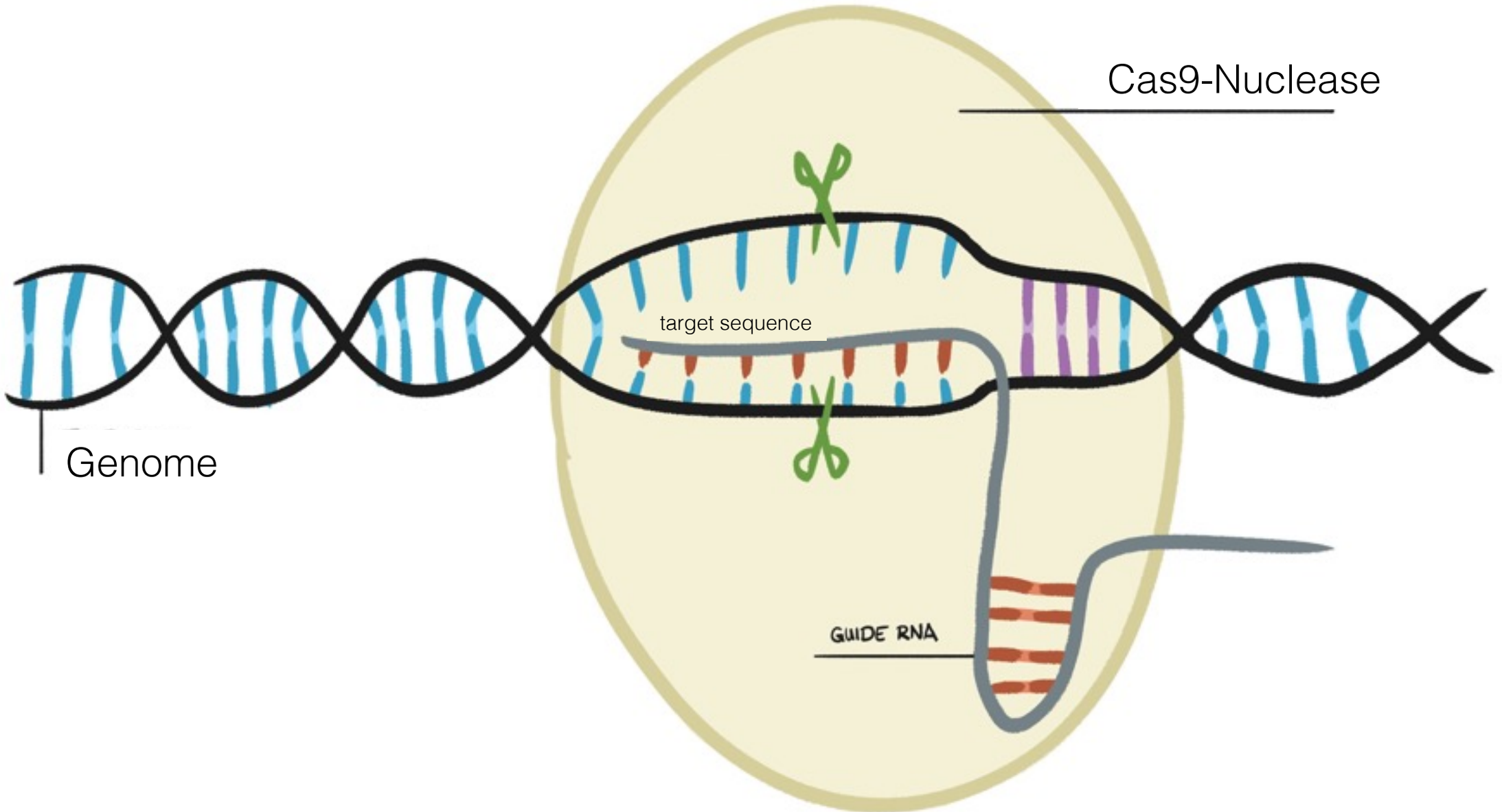
IG Saatgut Interessengemeinschaft für gentechnikfreie Saatgutarbeit

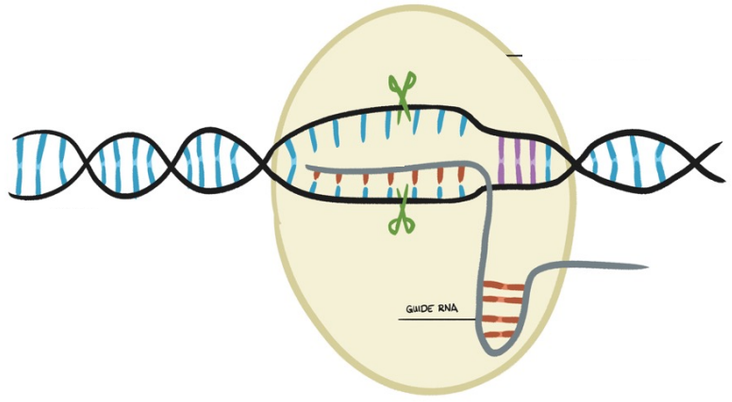
Research project funded by the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety & Federal Agency for Nature Conservation

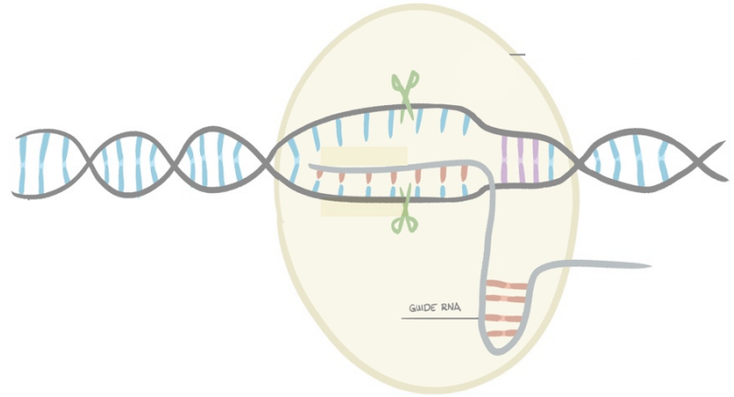
BfN Bundesamt für Naturschutz

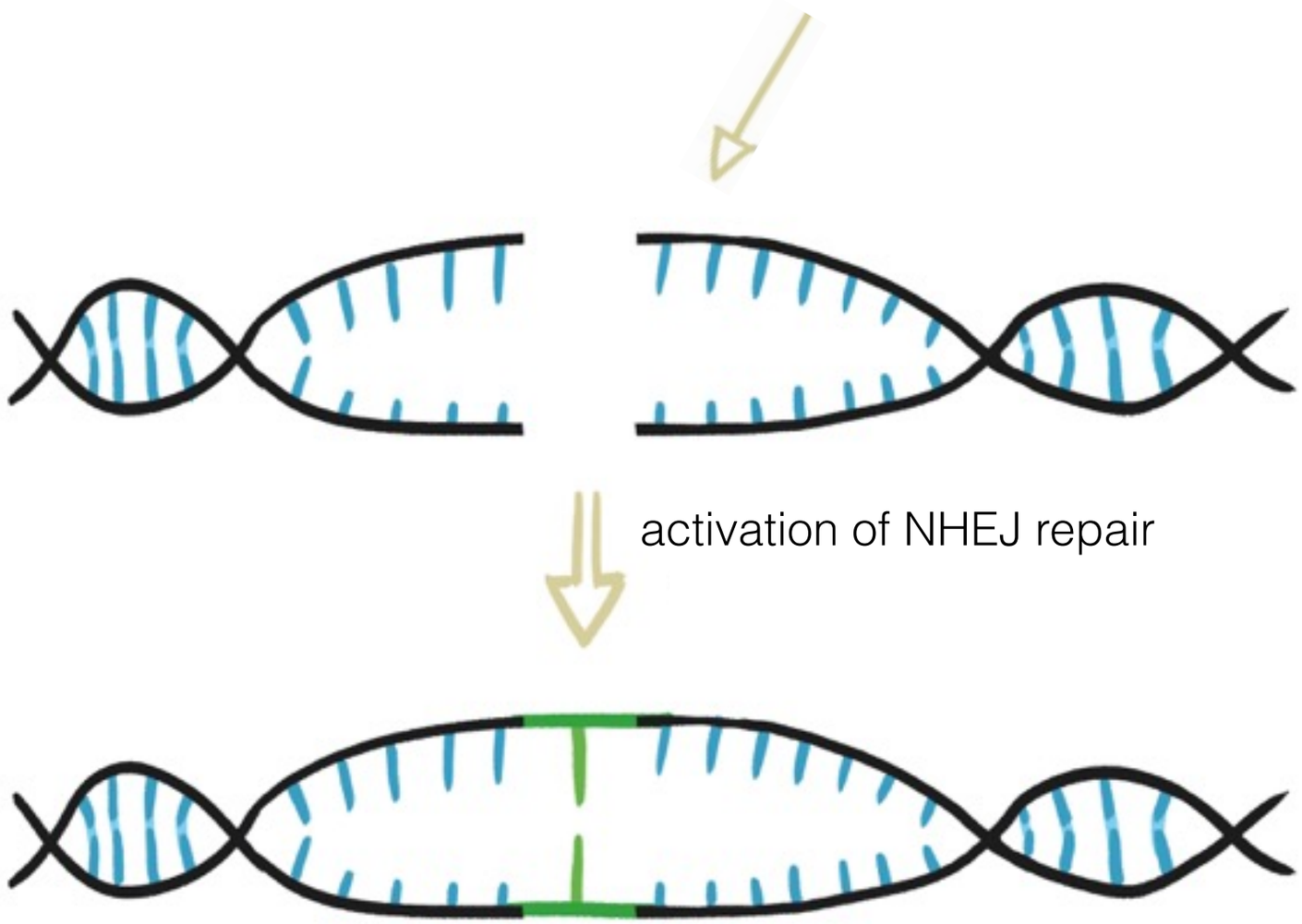
Bundesministerium für Umwelt, Naturschutz und nukleare Sicherheit

Genome Editing using CRISPR/Cas9




GUIDE RNA

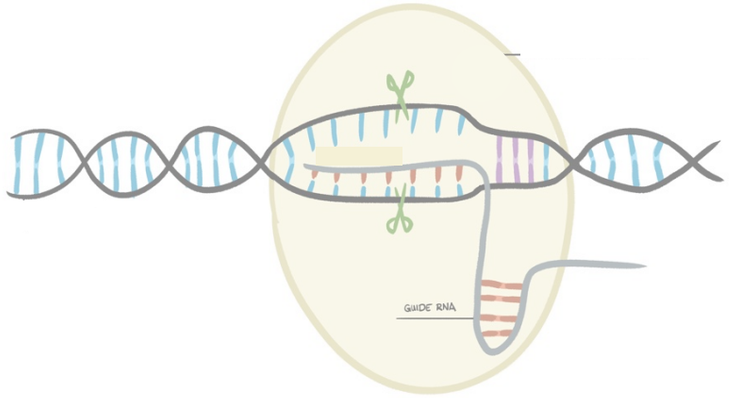


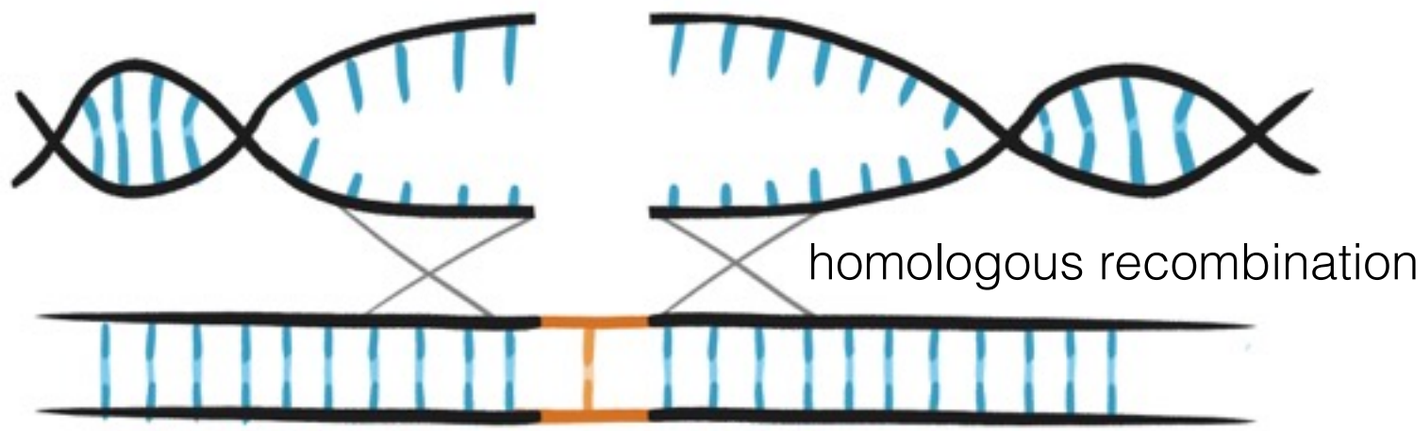


activation of NHEJ repair

random base substitutions

NHEJ: Non-homologous end joining



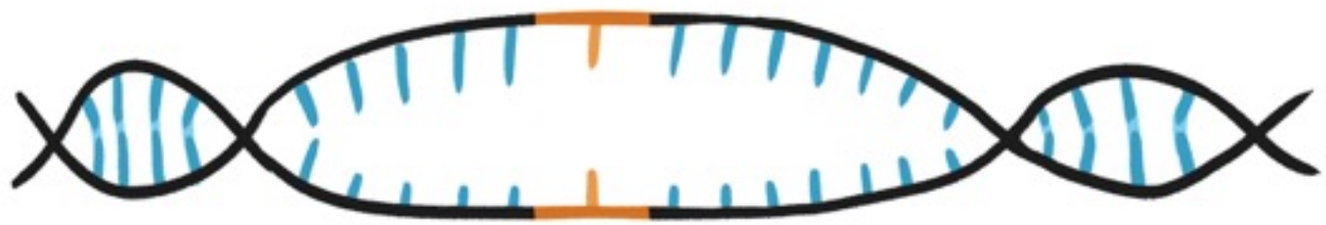


homologous recombination

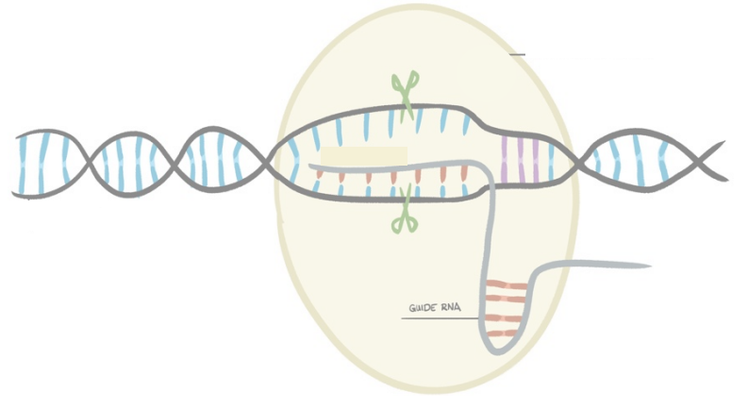
DNA template

activation of HDR

intended change of
single nucleotides



HDR: Homology Directed Repair

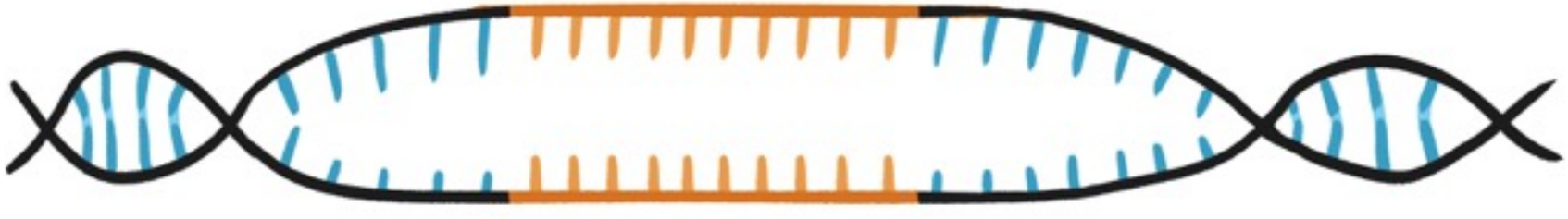


homologous recombination

DNA template

activation of HDR

integration of large genomic regions



Efficiency of CRISPR/Cas

The efficiency of CRISPR/Cas depends on the

- genome size of the target organism
- design of the guide RNA
- experimental setup: length of exposition, concentration of the nuclease
- accessibility of the target sequence (epigenetics)
- Singleplexing vs Multiplexing
- NHEJ versus HDR

Balance between efficiency and unwanted side effects

CRISPR/Cas is a promising tool for basic research, pest control, medical research and agricultural breeding.

For the protection of humans, nature, animals and plants one should consider risks.

Precautionary Principle

What side effects of CRISPR/Cas are already known?

Off-target effects

- Off-target effects are caused by nucleases cutting at unintended sites of the genome
- Cas9 and other nucleases are prone to cause off-target effects
- NHEJ repair might introduce unintended changes at these sites
- genetic variations in a species can cause unforeseen off-target sites in individuals (SNPs/reference genome)

References

High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. Fu, et al. Nature Biotechnology. (2013)

Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases. Cho, et al. Genome Res. (2014)

CRISPR off-target analysis in genetically engineered rats and mice. Anderson, et al. Nature Methods. (2018).

On-target effects

- after introduction of a double-strand break at the target sequence

→ integration of unwanted DNA fragments (e.g. degradation fragments of Cas DNA)

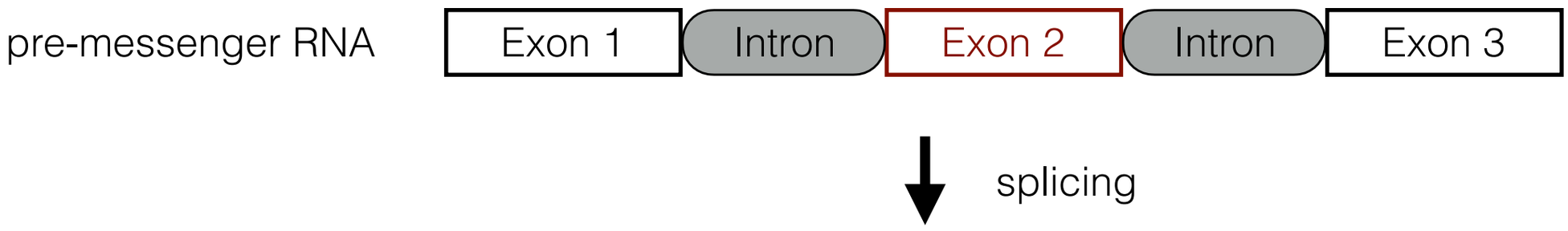
- can also be found at unintended off-target sites of the genome

References

Cas9-Guide RNA Directed Genome Editing in Soybean. Li, et al. Plant Physiology (2015).

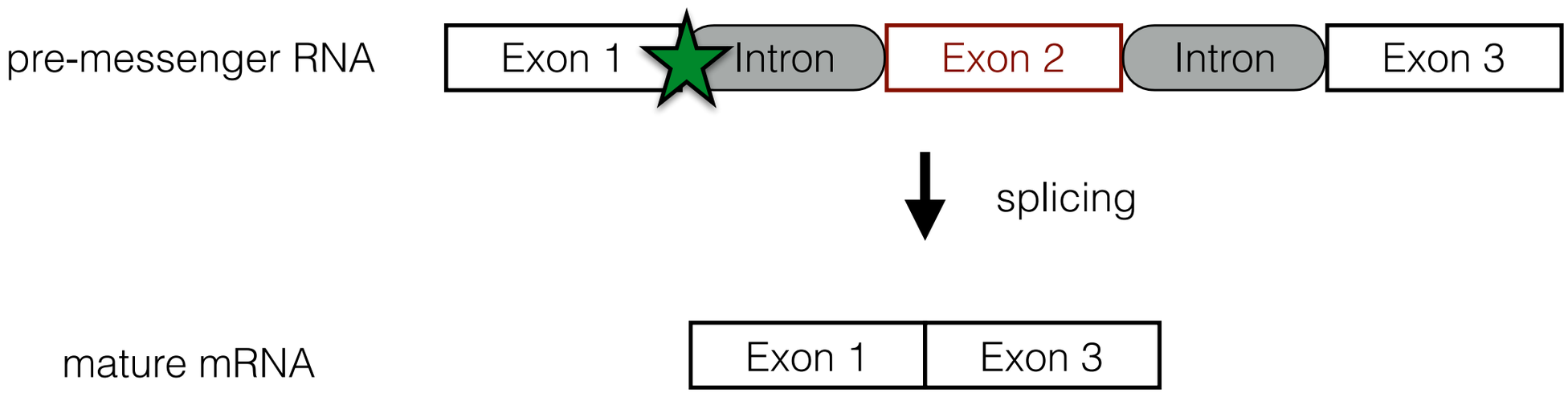
Efficient DNA-free genome editing of bread wheat using CRISPR/Cas9 ribonucleoprotein complexes. Liang, et al. Nat Commun (2017).

Exon Skipping



Splicing removes the non-coding parts (introns) from the gene-coding regions (exons).

CRISPR/Cas can cause Exon Skipping



References

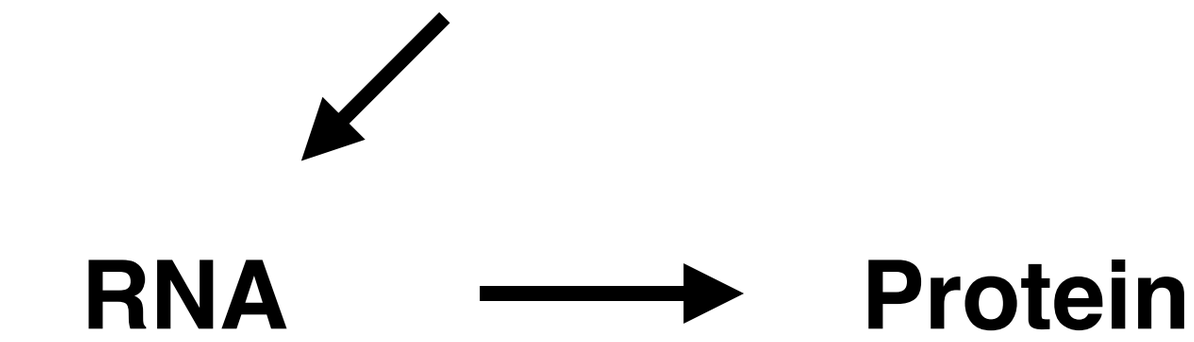
Random Splicing of Several Exons Caused by a Single Base Change in the Target Exon of CRISPR/Cas9 Mediated Gene Knockout. Kapahnke, et al, Cells (2016).

Frameshift indels introduced by genome editing can lead to in-frame exon skipping. Lalonde, et al. PLoS One (2017).

CRISPR/Cas9-mediated genome editing induces exon skipping by alternative splicing or exon deletion. Mou, et al. Genome Biol (2017).

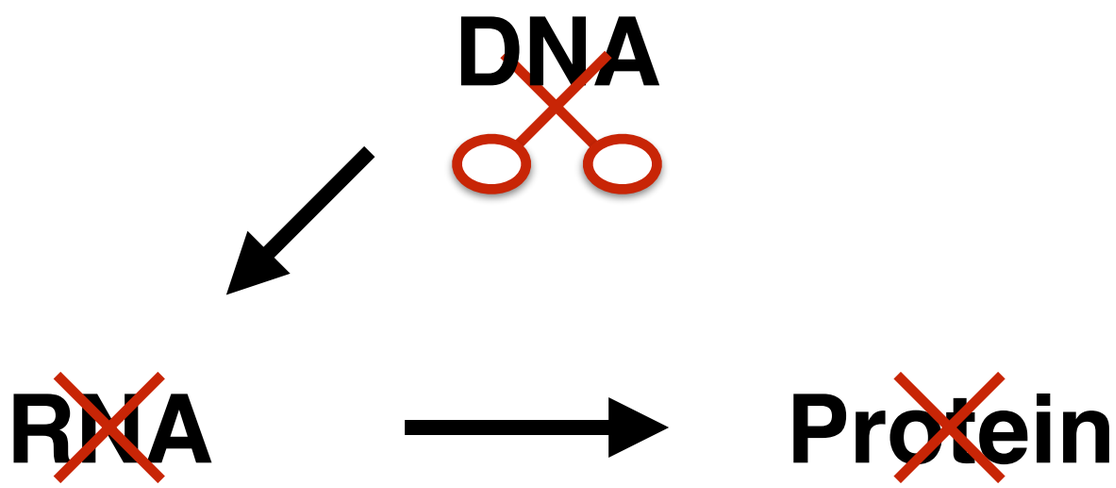
What effects can be caused by the intended changes of the genome?

Central Dogma of Molecular Biology



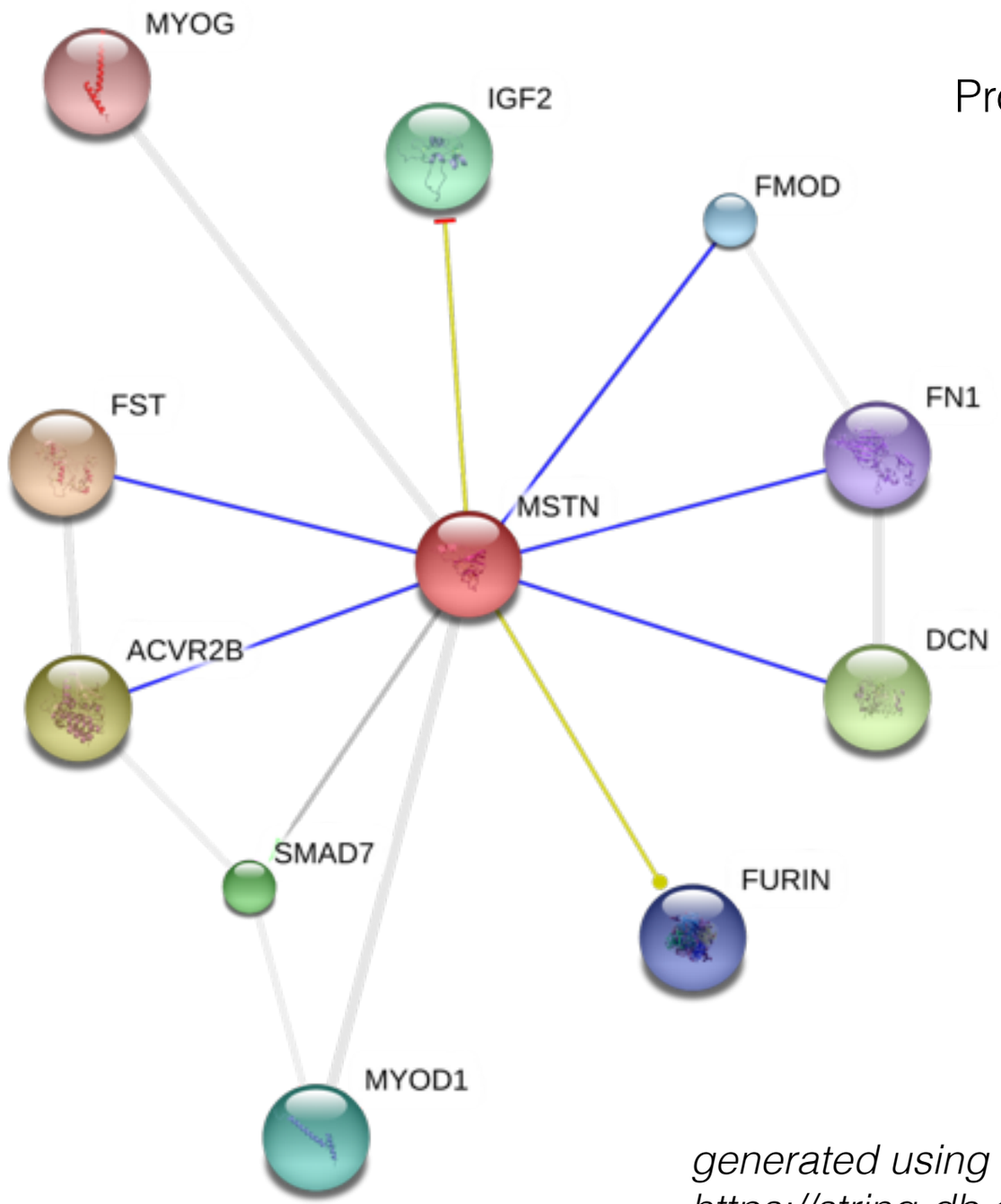
Flow of information between biomolecules

Central Dogma of Molecular Biology



Flow of information between biomolecules

Complexitiy of a biological system



Protein Interaction: Myostatin
from *Bos taurus*

generated using STRING v9
https://string-db.org/cgi/input.pl

Risk assessment: -omics technologies

Genomics: Whole Genome Sequencing

Transcriptomics: RNA-seq, Microarrays

Proteomics: Mass-Spectrometry

Metabolomics: Chromatography, Mass-Spectrometry

Microbiome: 16S rRNA sequencing, Metagenomics, Metatranscriptomics

Applications based on dead Cas9

Base Editing

Enzymatic conversion of one base type into another

Surrounding nucleotides can be changed unintentionally

Epigenome Editing

Enzymatic changes of epigenetic modifications

Genome wide changes of epigenetic modifications

References

Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Komor, et al, Nature (2016).

DNA epigenome editing using CRISPR-Cas SunTag-directed DNMT3A. Huang, et al, Genome Biol (2017).

Genome-wide tracking of dCas9-methyltransferase footprints. Galonska, et al, Nature Comm (2018).

Summary

- CRISPR/Cas is a new set of molecular techniques for various applications
- multiple factors have an influence on the efficiency of CRISPR/Cas
- side-effects of CRISPR/Cas:
    - off-target effects
    - unwanted on-target effects
    - exon skipping
- intended changes might interfere with other signaling pathways
- dead Cas9 approaches are still prone to failure

Thank you!

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