

CG920 Genomics

Lesson 5

RNA Interference and Genome Editing

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Functional Genomics and Proteomics of Plants,
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M U N I
S C I



Outline

- Knocking-down the genes using RNA interference
 - Mechanism of RNAi
- Genome Editing
 - Principle of genome editing using Site Directed Nucleases, (SDNs)
 - Zinc-Finger Nucleases (ZFNs)
 - Transcription Activator-Like Effectors (TALENs)
 - Clustered Regularly Interspaced Short Palindromic Repeats/Cas9 (CRISPR/Cas9)

Outline

- Knocking-down the genes using RNA interference
 - Mechanism of RNAi

RNA interference

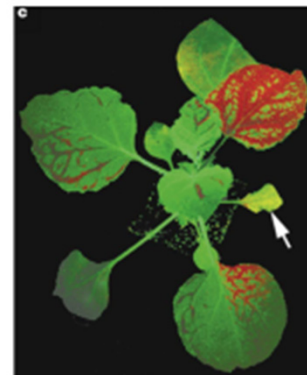
- **Molecular mechanism of post-transcriptional gene silencing (PTGS)**
 - RNAi discovered in plants, later in *Coenorhabditis elegans*
 - In plants identified as „sense effect“ in systemic negative regulation of gene activity

Silencing the Expression via Introducing Additional Gene Copy for Flavonoid Biosynthesis



van der Krol et al., Plant Cell (1998)

Systemic effect in the regulation of GFP expression



- *Nicotiana benthamiana* expressing GFP
- Retransformation of one of the leaves by construct for GFP expression
- Absence of GFP can be seen as a red chlorophyll fluorescence

Volken and Szulcman, Nature (1997)

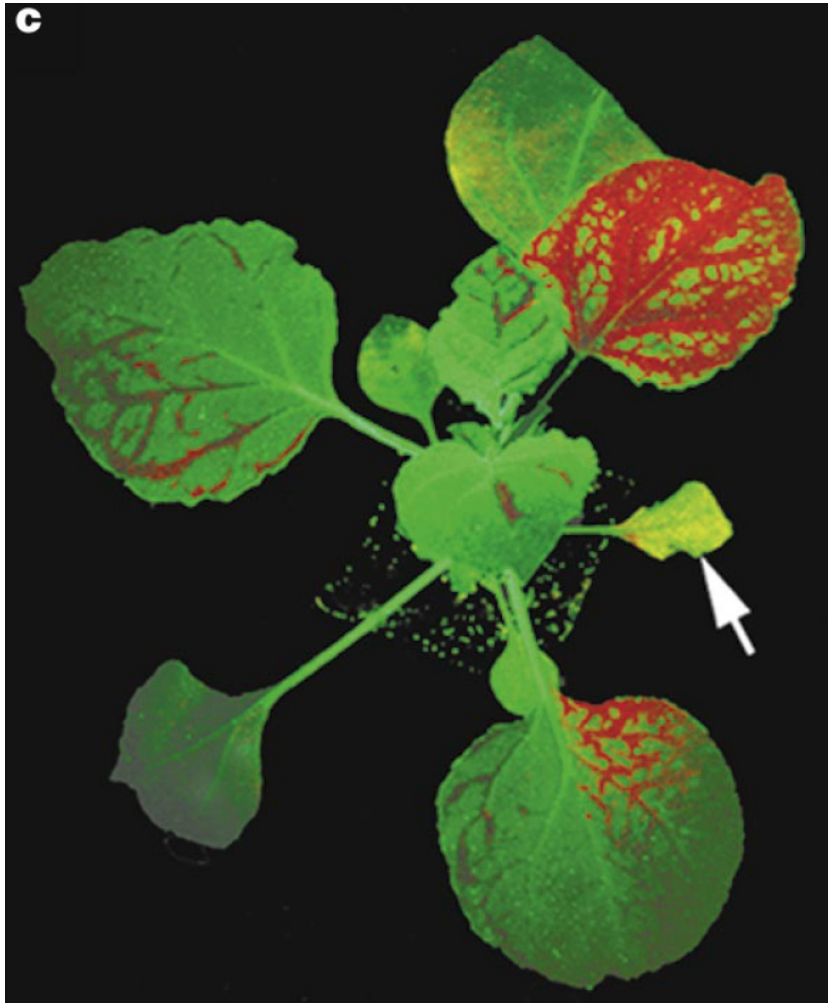
Silencing the Expression via Introducing Additional Gene Copy for Flavonoid Biosynthesis

p35S::DFR



van der Krol et al., *Plant Cell* (1990)

Systemic effect in the regulation of GFP expression

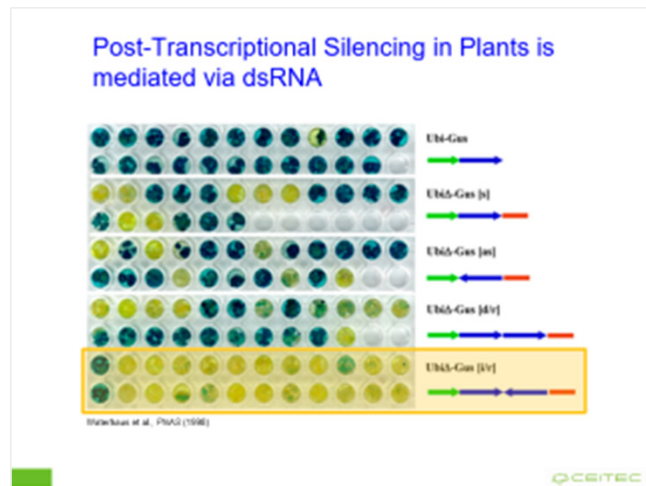


Voinnet and Baulcombe, *Nature* (1997)

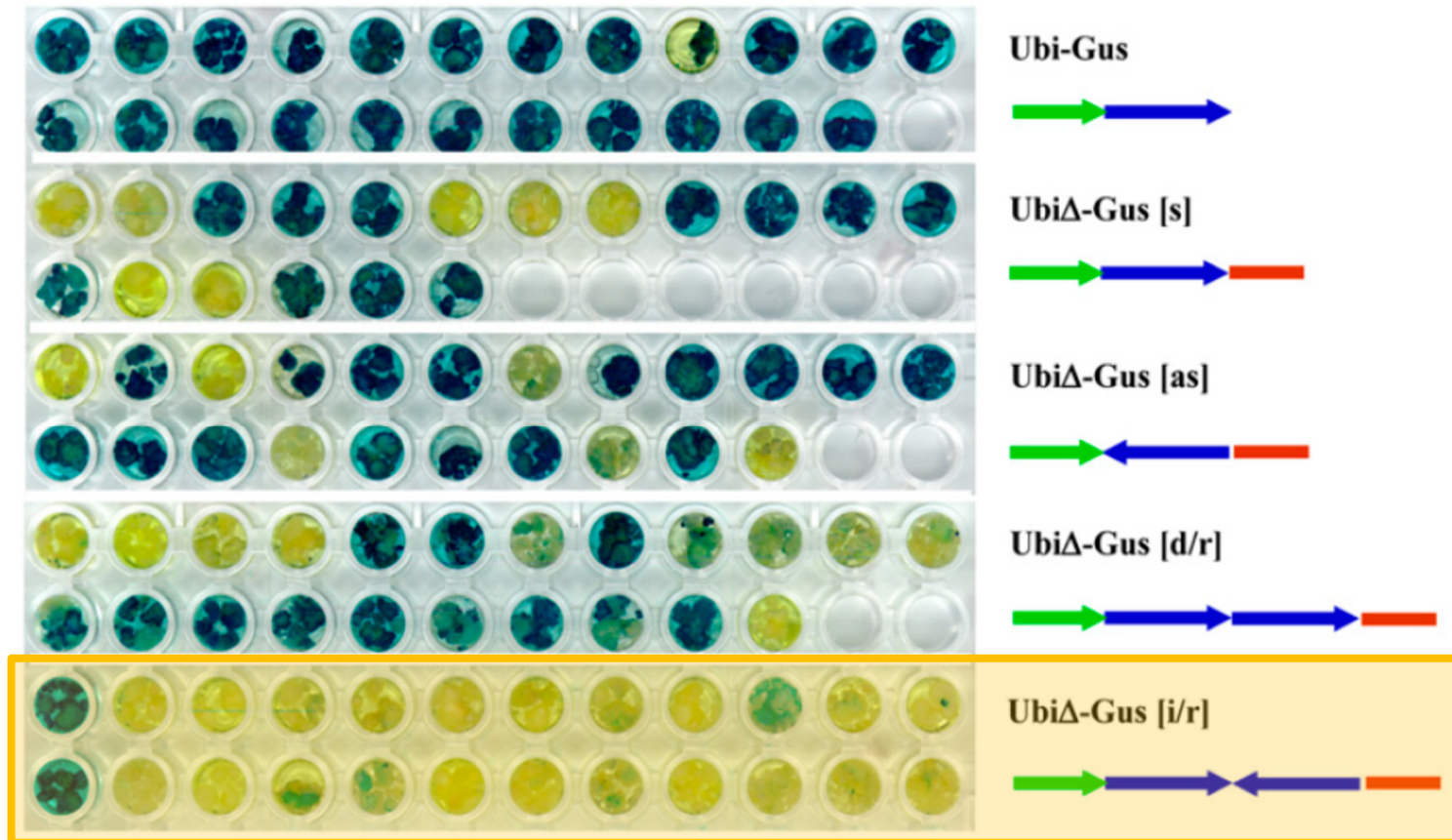
- *Nicotiana benthamiana* expressing GFP
- Retransformation of one of the leaves by construct for GFP expression
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RNA interference

- Molecular mechanism of post-transcriptional gene silencing (PTGS)
 - RNAi discovered in plants, later in *Coenorhabditis elegans*
 - In plants identified as „sense effect“ in systemic negative regulation of gene activity
 - Gene silencing induced via both sense and anti-sense RNA
 - dsRNA induced gene silencing approx. 100x more efficiently



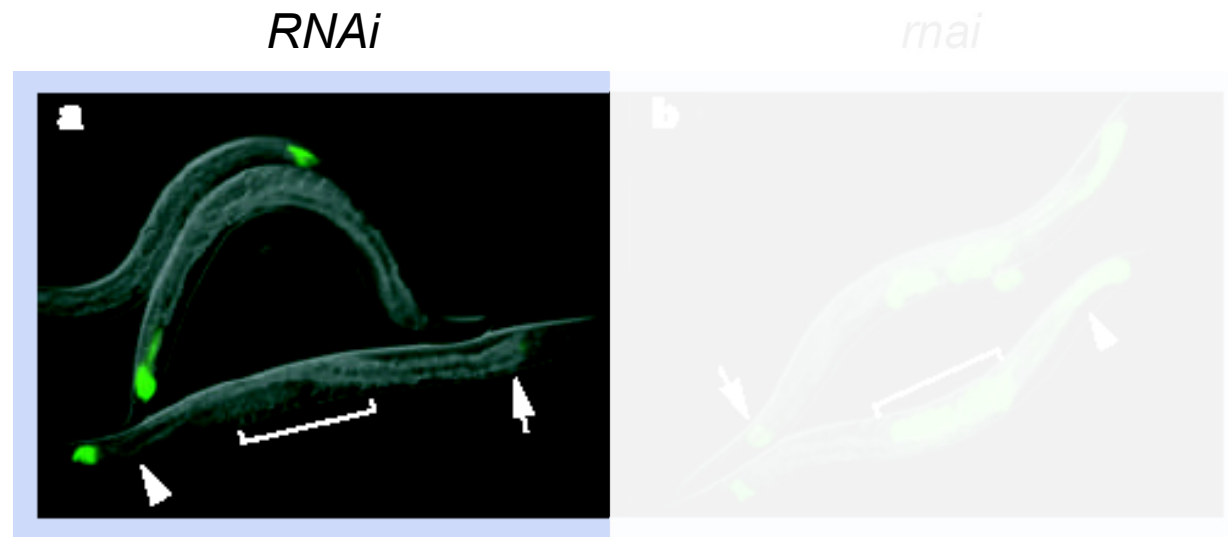
Post-Transcriptional Silencing in Plants is mediated via dsRNA



Waterhaus et al., *PNAS* (1998)

RNA interference

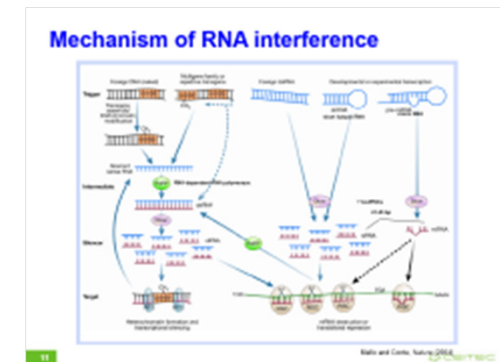
- **Molecular basis of posttranscriptional gene silencing (PTGS)**
 - dsRNA induction is dependent on own genes
 - gene identification using forward genetics



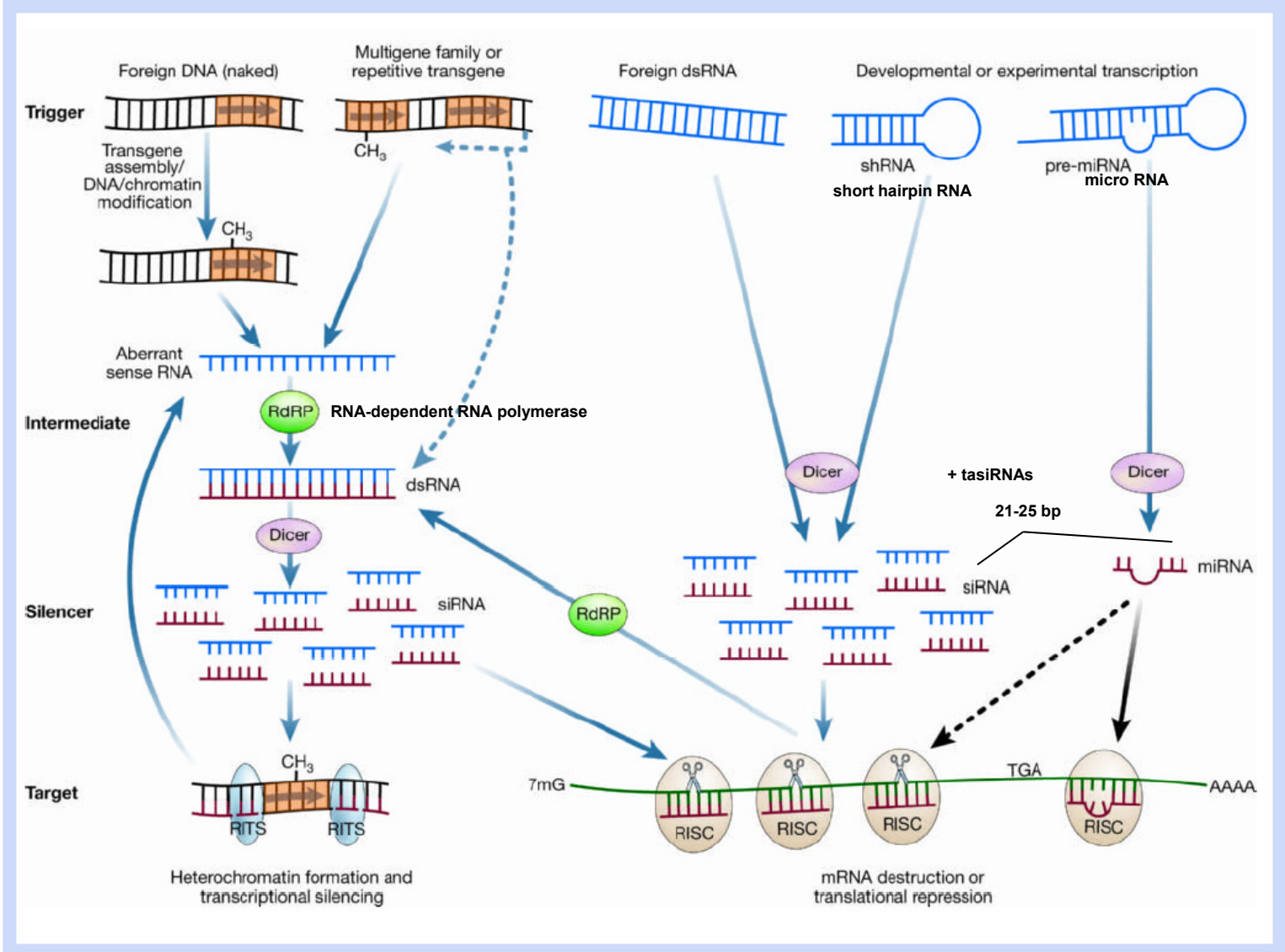
Mello and Conte, *Nature* (2004)

RNA interference

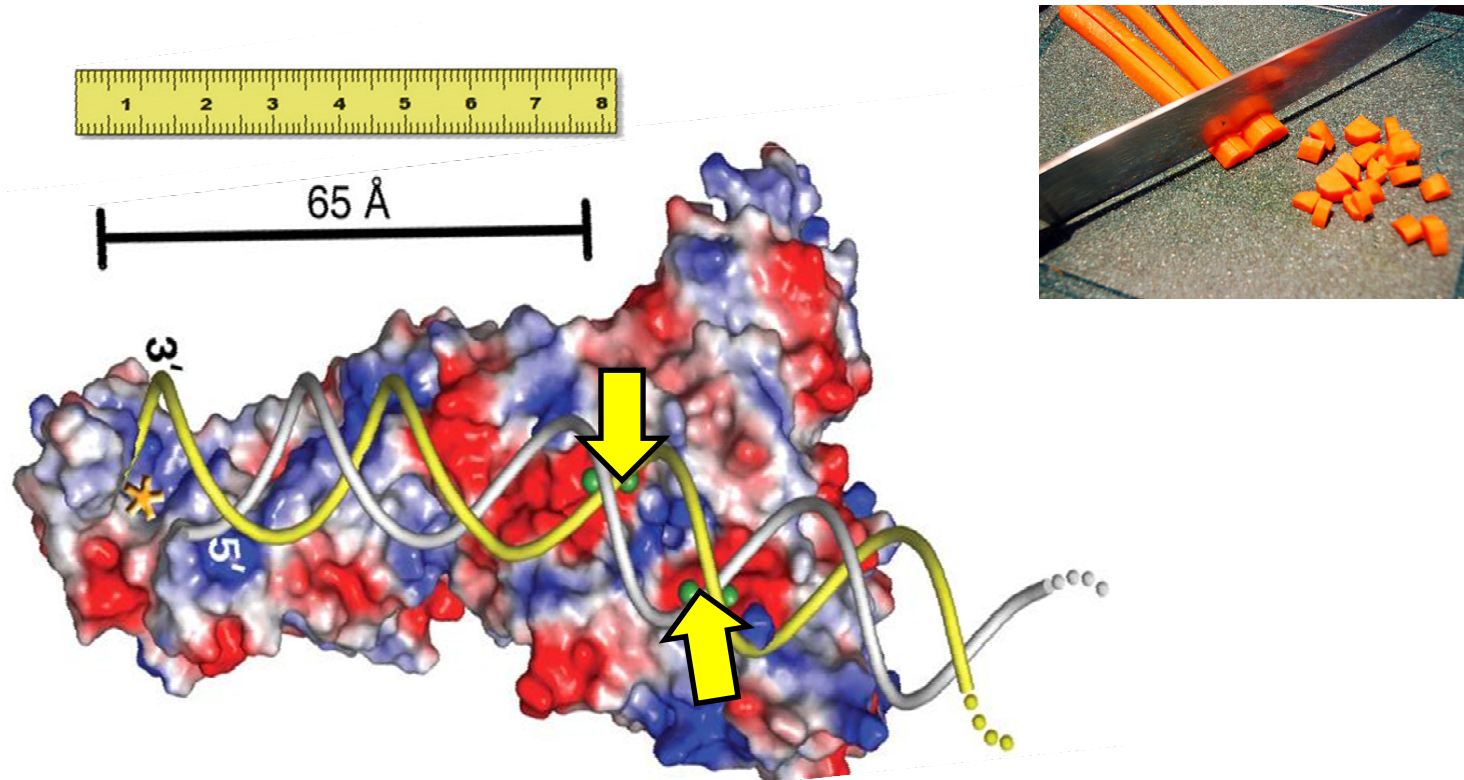
- **Molecular basis of posttranscriptional gene silencing (PTGS)**
 - RNAi found in *Coenorhabditis elegans* and in plants
 - It is a **natural mechanism** of regulation of gene expression in all eukaryotes
 - The principle is **creating dsRNA**, which can be triggered in several ways:
 - By presence of **foreign „aberrant“ DNA**
 - **Specific transgenes** containing **inverted repeats** of the cDNA parts
 - Transcription of own genes for **shRNA** (short hairpin RNA) or **miRNA** (micro RNA, endogenous hairpin RNA)
 - dsRNA is processed by enzyme complex (DICER), which leads to the formation of **siRNA** (short interference RNA), which is then bound to enzyme complex **RITS** (RNA-induced transcriptional silencing complex) or **RISC** (RNA-induced silencing complex)
 - **RISC** mediates either **degradation of mRNA** (in case of full similarity of siRNA and the target mRNA) or leads only to **termination of translation** (in case of incomplete homology, e.g. as in the case of miRNA)
 - **RITS** mediates **reorganization of genomic DNA** (heterochromatin formation and inhibition of transcription)



Mechanism of RNA interference

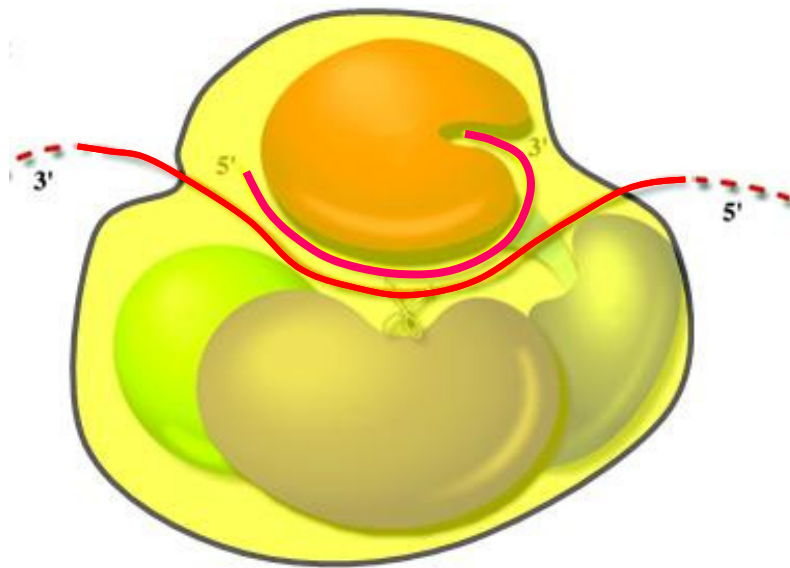


Dicer and Dicer-like proteins

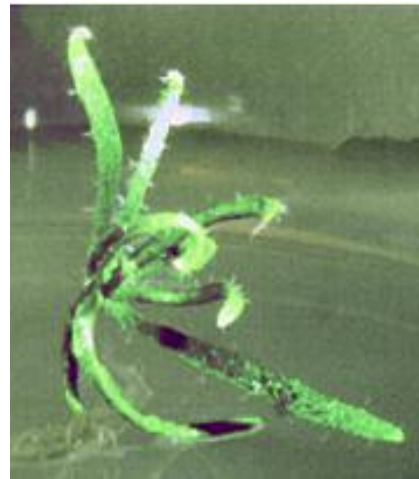


From MacRae, I.J., Zhou, K., Li, F., Repic, A., Brooks, A.N., Cande, W., Adams, P.D., and Doudna, J.A. (2006) Structural basis for double-stranded RNA processing by Dicer. *Science* 311: [195-198](#). Reprinted with permission from AAAS. Photo credit: [Heidi](#)

Argonaute proteins



ago1



Argonauta argo

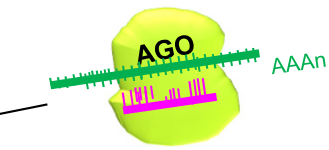


Reprinted by permission from Macmillan Publishers Ltd: EMBO J. Bohmert, K., Camus, I., Bellini, C., Bouchez, D., Caboche, M., and Benning, C. (1998) *AGO1* defines a novel locus of *Arabidopsis* controlling leaf development. EMBO J. 17: [170–180](#). Copyright 1998; Reprinted from Song, J.-J., Smith, S.K., Hannon, G.J., and Joshua-Tor, L. (2004) Crystal structure of Argonaute and its implications for RISC slicer activity. Science 305: [1434 – 1437](#). with permission of AAAS.

transcriptional gene silencing

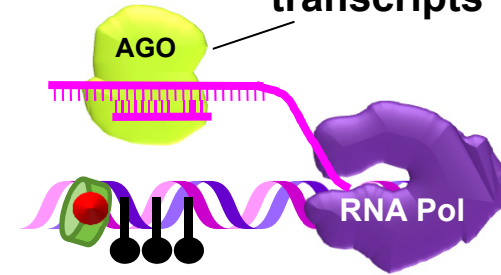
siRNA

binding to DNA



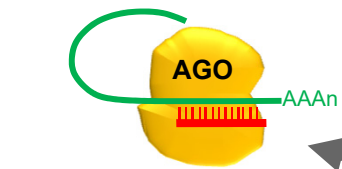
post-transcriptional gene silencing

binding to specific transcripts

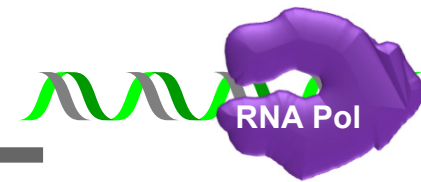
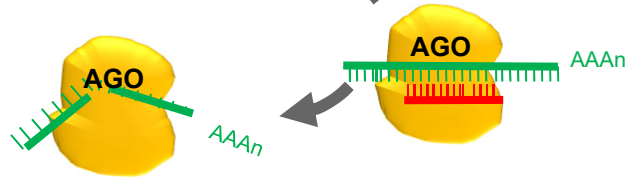
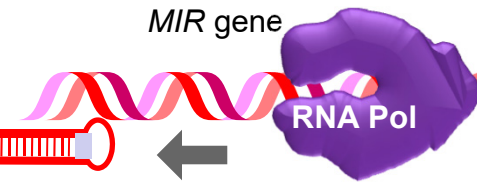


miRNA

translational repression



MIR gene



transcriptional slicing

The Nobel Prize in Physiology or Medicine 2006



Andrew Z. Fire

USA

Stanford University
School of Medicine
Stanford, CA, USA

b. 1959



Craig C. Mello

USA

University of
Massachusetts Medical
School
Worcester, MA, USA

b. 1960

The Nobel Prize in Physiology or Medicine 2006



Andrew Z. Fire

USA



Craig C. Mello

USA



David Baulcombe

UK

CORRESPONDENCE NATURE|Vol 443|26 October 2006

RNAi Nobel ignores vital groundwork on plants

SIR — The Nobel prize, by recognizing the individuals behind breakthroughs, inspires all scientists to do great science. The discovery of RNA interference (RNAi) changed the face of gene regulation, a feat deservedly recognized with this year's Nobel Prize in Physiology or Medicine¹.

As undergraduates, we witnessed with great excitement the discovery of gene silencing. At that time, almost all research in that area was being conducted by plant

values at the centre of the prize and is sending a discouraging message, especially to young researchers.

Marc Bots*, **Spencer Maughan†**,
Jeroen Nieuwland†

*Flanders Interuniversity Institute for Biotechnology, Technologiepark 927, BE-9052 Gent, Belgium

†Institute of Biotechnology, University of Cambridge, Cambridge CB2 1QT, UK

1. *Nature* **443**, 488 (2006).
2. Baulcombe, D. C. *Plant Mol. Biol.* **32**, 79–88 (1996).
3. Van der Krol, A. R. et al. *Plant Cell* **2**, 291–299 (1990).
4. Voimmet, O. & Baulcombe, D. C. *Nature* **389**, 553 (1997).
5. Metzlaif, M., O'Dell, M., Cluster, P. D. & Flavell, R. B. *Cell* **88**, 845–854 (1997).

will not do so in the future. We believe that Iranian scientists can and will respond appropriately to the country's needs.

Kamran B. Lankarani
Ministry of Health and Medical Education of I. R. Iran, Tehran, I. R. Iran

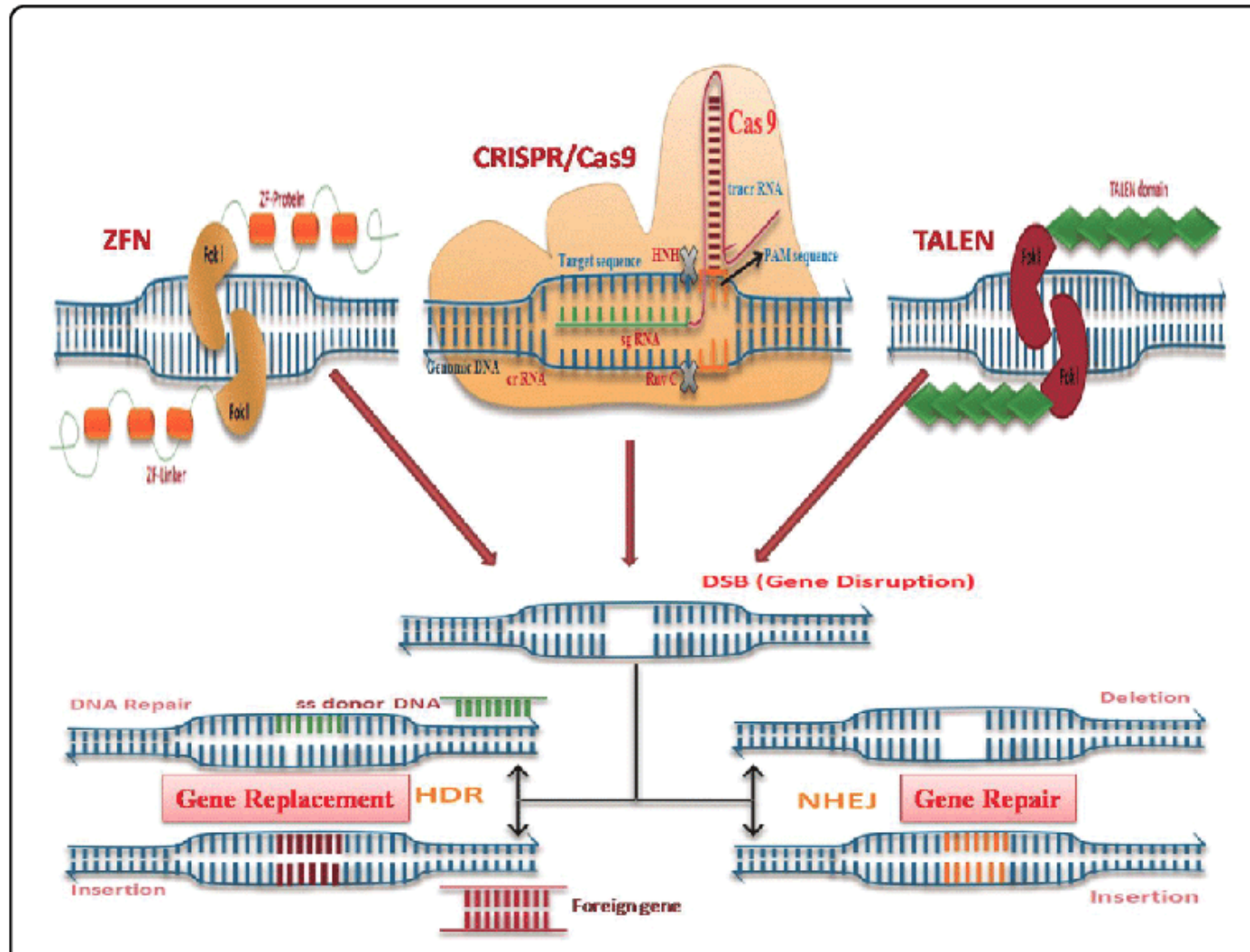
Iran: productivity is not simple to evaluate

SIR — Eran Meshorer, in Correspondence ("Iran is sixth, not second, in Middle East publication list" *Nature* **443**, 271; 2006), states:

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Genome Editing via SDNs

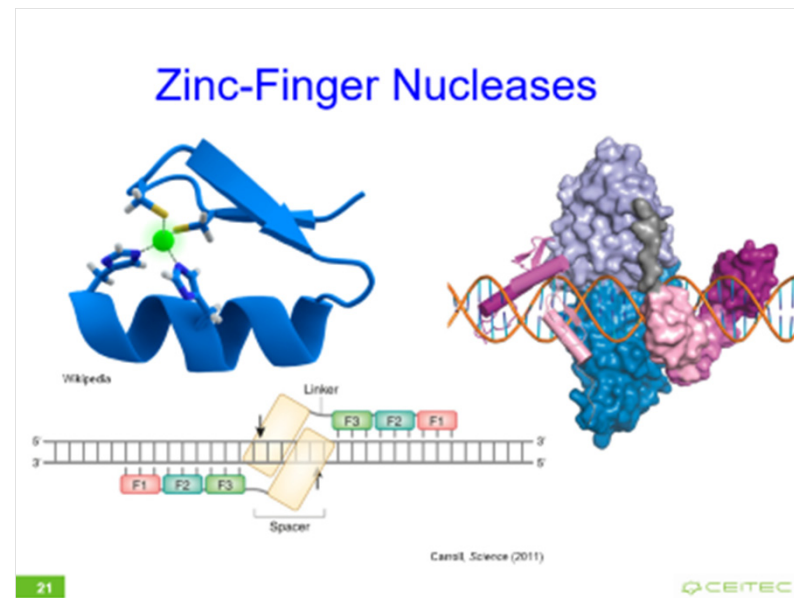


Outline

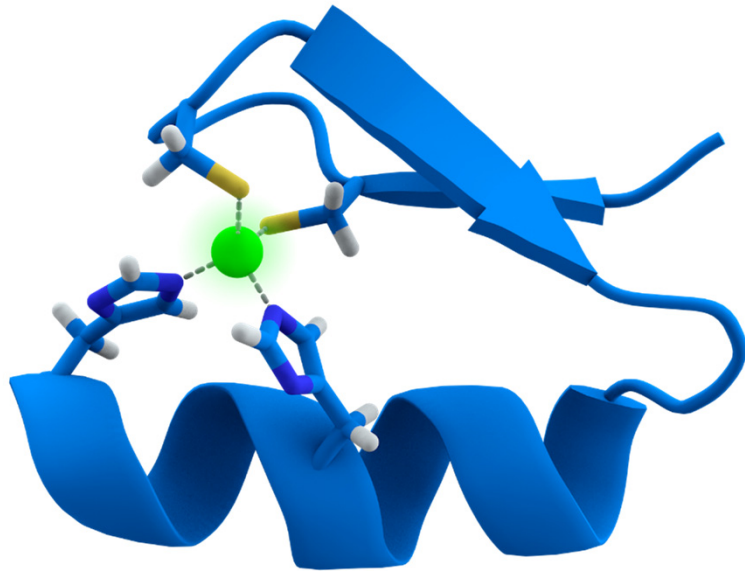
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Zinc-Finger Nucleases - ZFNs

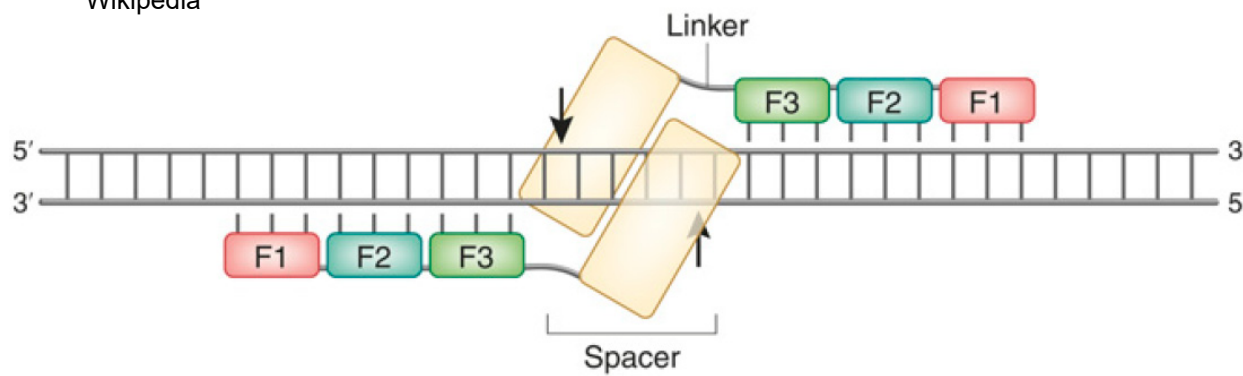
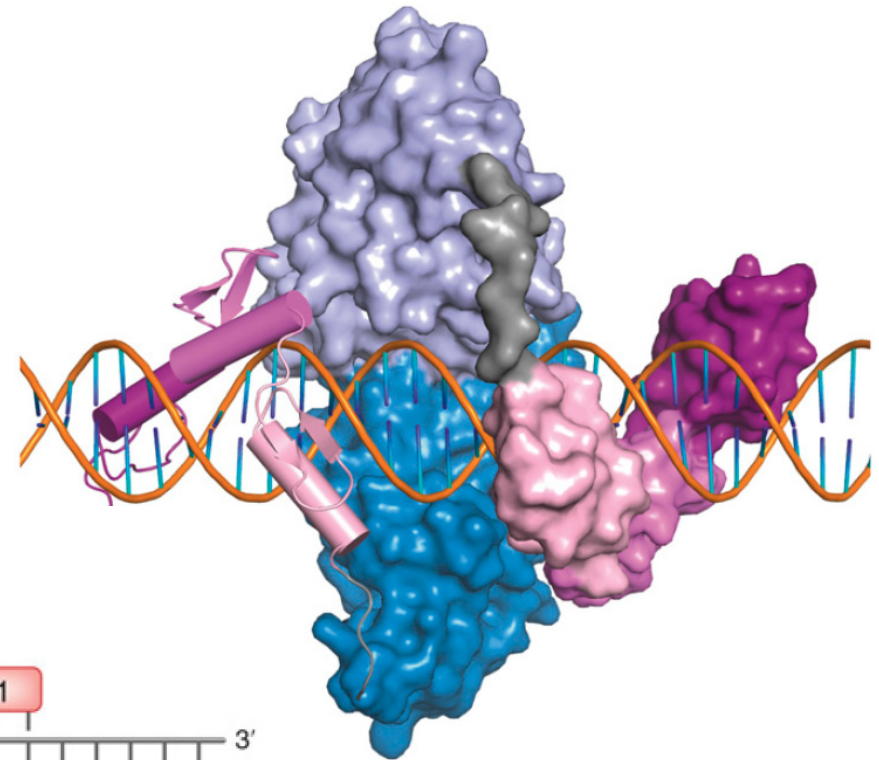
- **Sequence-specific endonucleases recognizing the target sequence via set of “zinc fingers”**
 - Each zinc „finger“ is recognizing nucleotide triplet
 - Nuclease domain acts as heterodimer – possibility to enhance the specificity by designing the set of „fingers“ recognizing 9 bp on both sides of the target sequence
- **Shortcomings**
 - Difficult to “program”
 - Delimited specificity



Zinc-Finger Nucleases



Wikipedia



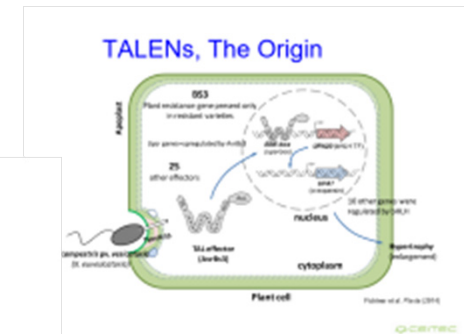
Carroll, *Science* (2011)

Outline

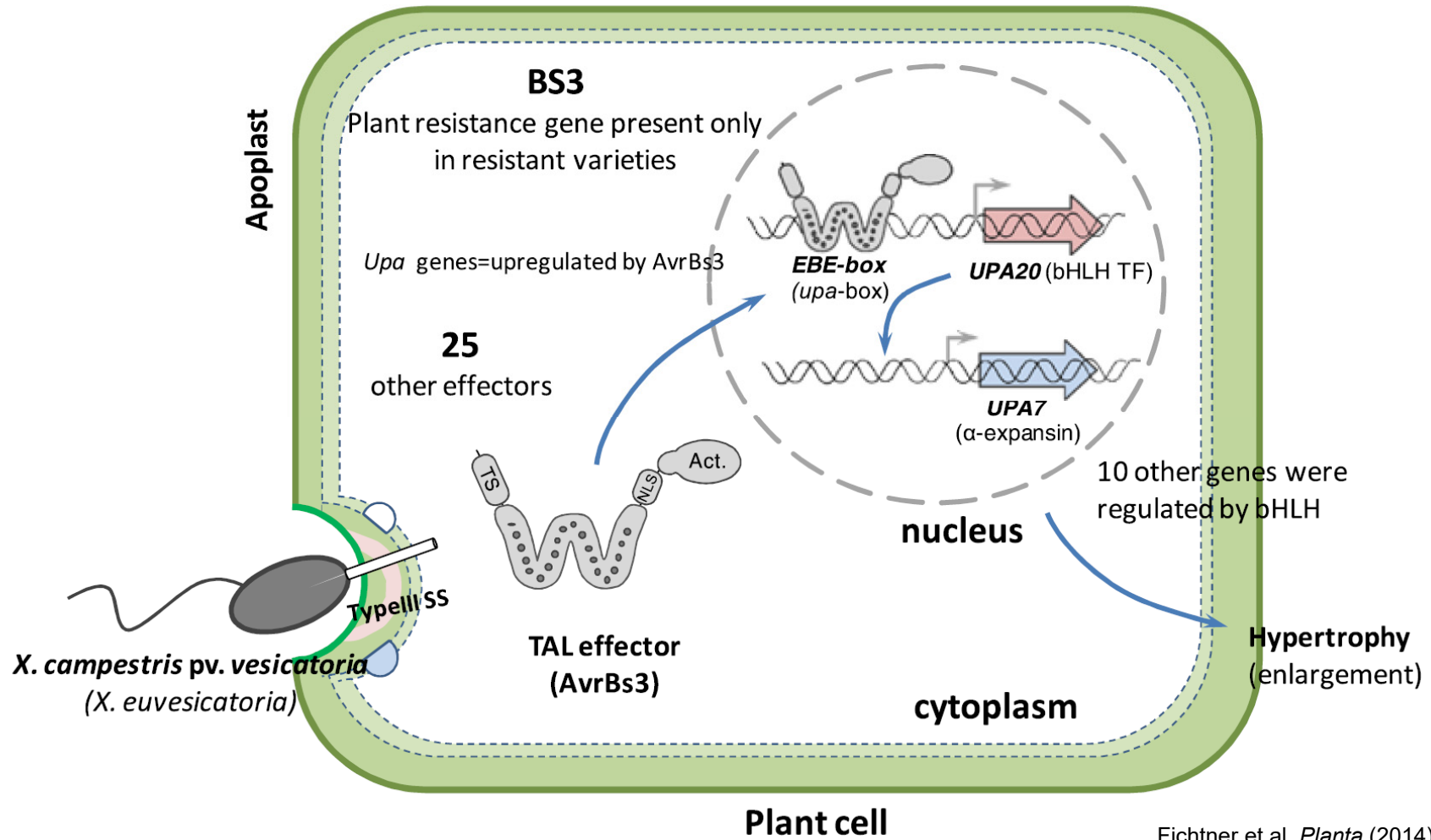
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Transcription Activator-Like Effectors - TALENs

- **Proteins derived from sequence-specific transcription activators**
 - Identified (so far only) in plant pathogenic bacteria *Xanthomonas sp.* as bacterial effectors, able to control the transcription of target genes in plants
 - Sekvenční specificity determined by aminoacid sequence of DNA-binding repeats
 - Possible to use for various modification types
- **Shortcomings**
 - Difficult to “program”
 - Delimited specificity

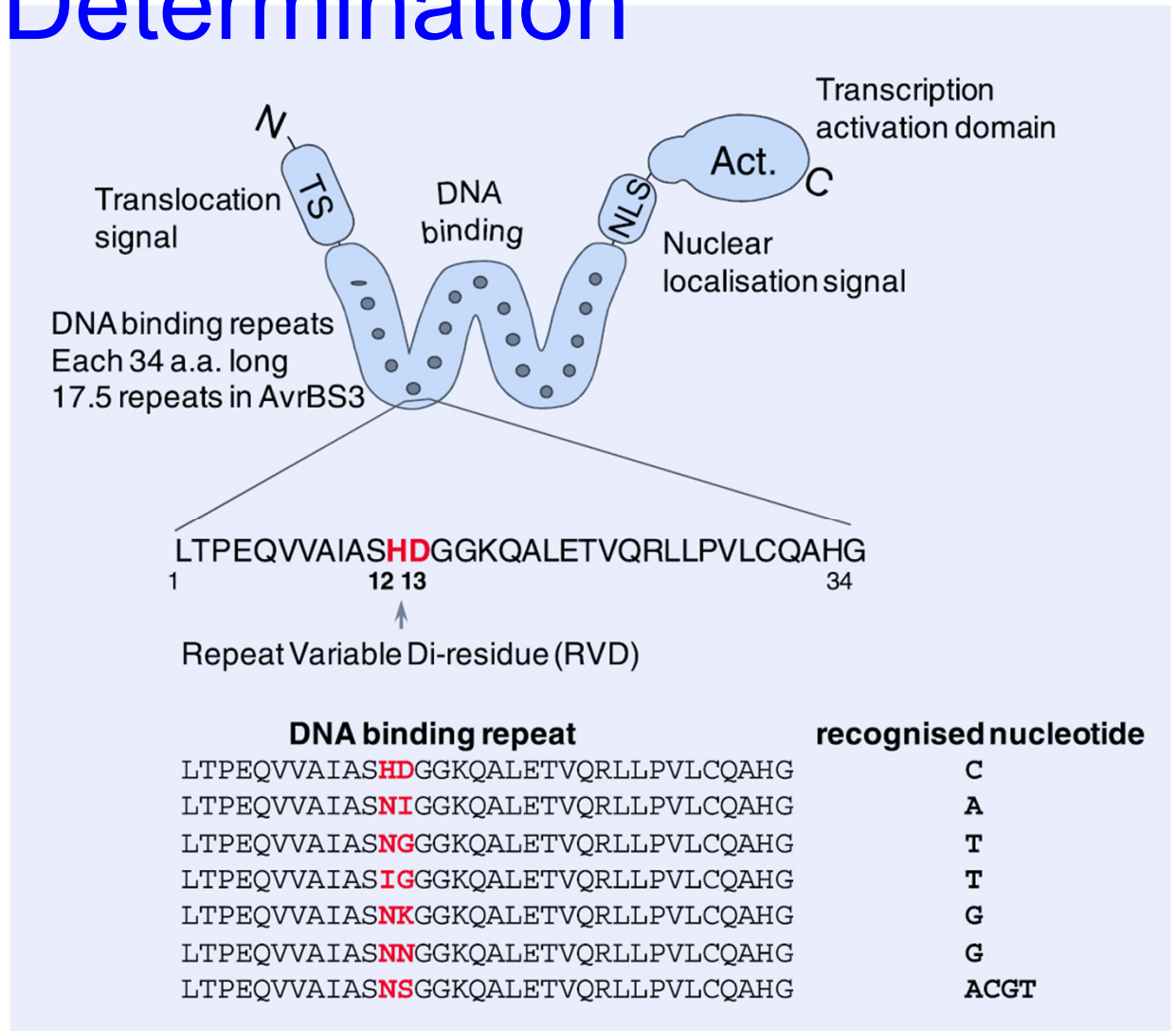


TALENs, The Origin



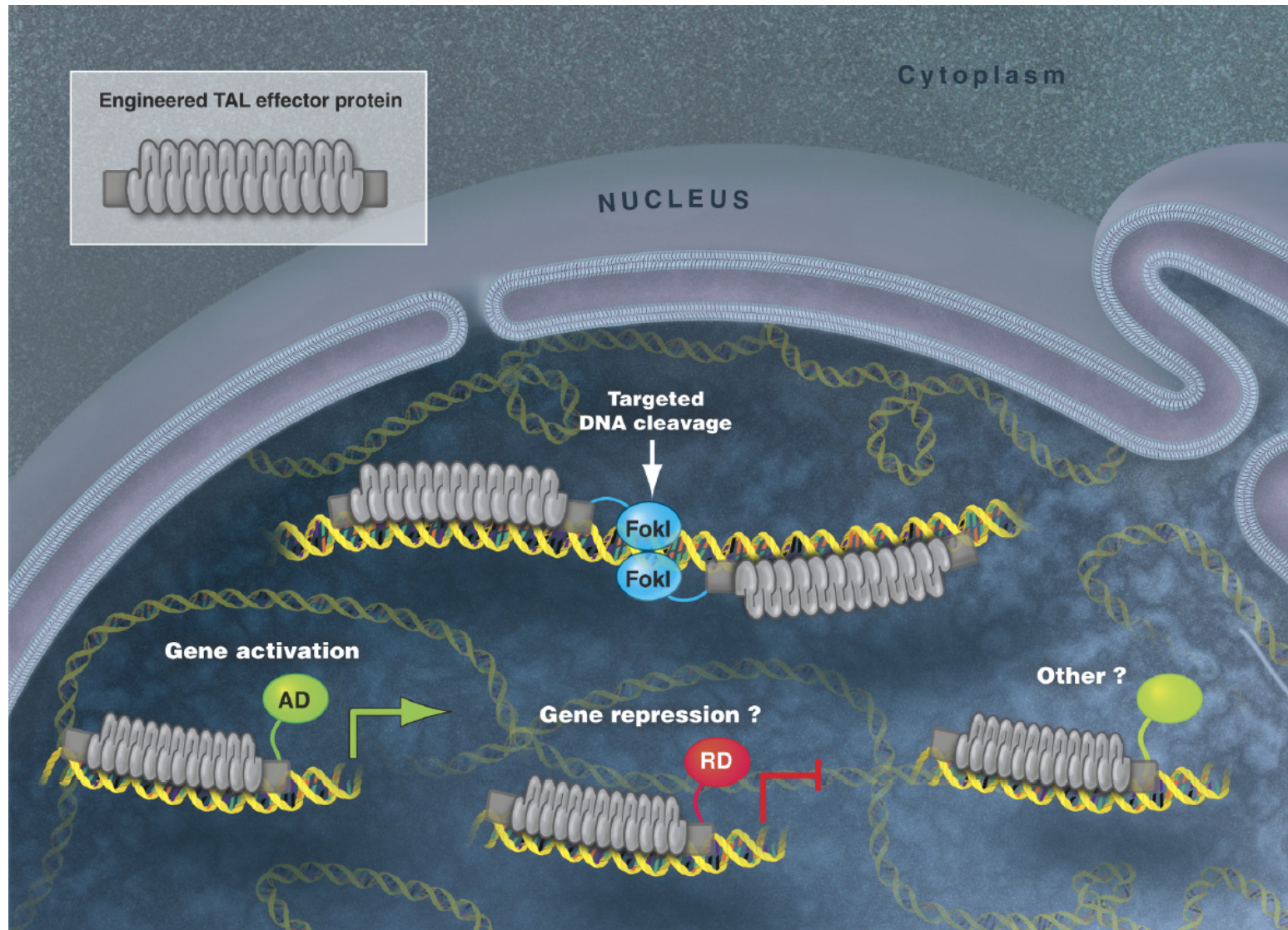
Fichtner et al. *Planta* (2014)

TALENs, Specificity Determination



Fichtner et al. *Planta* (2014)

TALENs, Applications

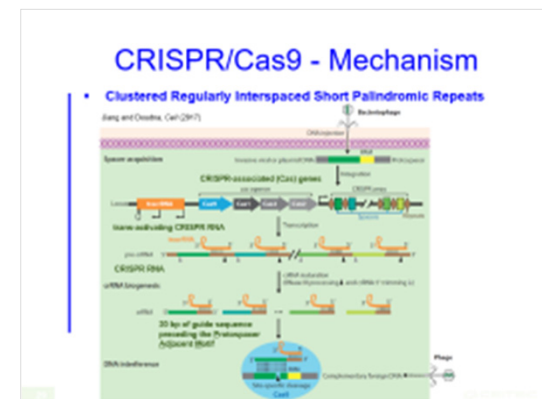


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 - Clustered Regularly Interspaced Short Palindromic Repeats/Cas9 (CRISPR/Cas9)

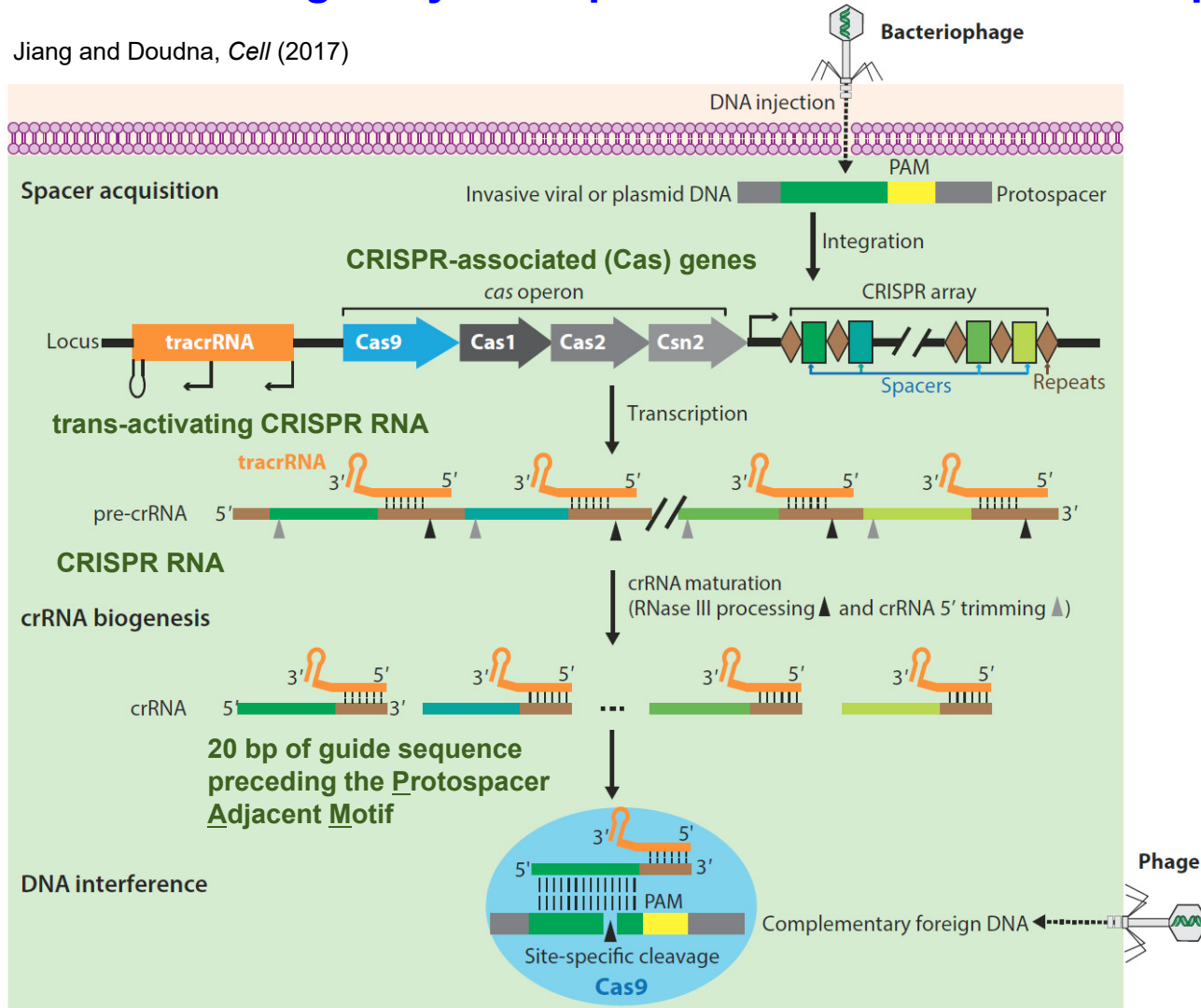
Clustered Regularly Interspaced Short Palindromic Repeats/Cas9 - CRISPR/Cas9

- **Discovered as a mechanism of bacterial immune system**
 - The principle is targeted insertion of foreign DNA (typically phage DNA) into specific bacterial genomic loci
 - Transcription of trans-activating CRISPR RNA (tracrRNA) and the region with inserted foreign DNA followed by RNA processing allows formation of crRNA–tracrRNA complex
 - crRNA–tracrRNA binds Cas9 nuclease, targeting it to complementary (foreign/phage) DNA, that is then digested
 - crRNA–tracrRNA is in the targeted genome editing replaced by a single guide RNA (sgRNA or gRNA)
 - **Advantages**
 - Easy to „program“
 - High specificity
 - Number of further applications possible



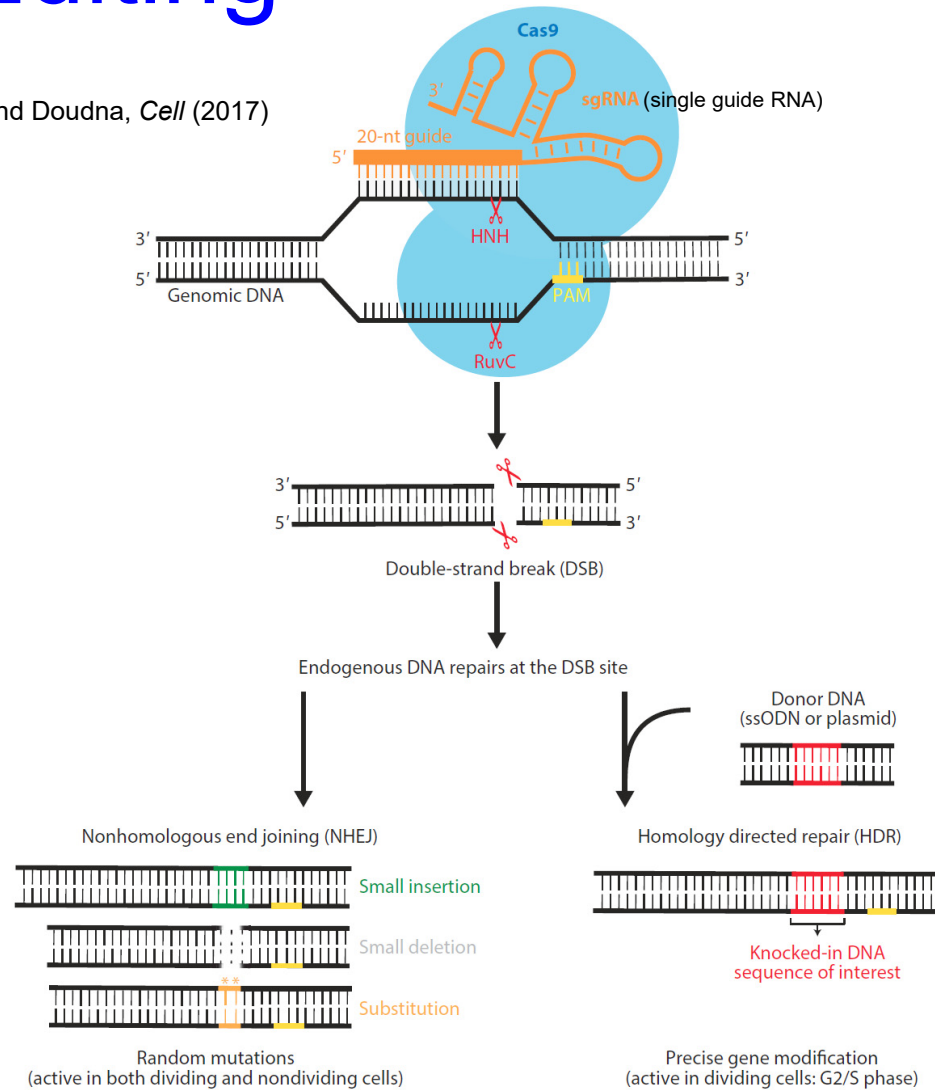
CRISPR/Cas9 - Mechanism

- Clustered Regularly Interspaced Short Palindromic Repeats



CRISPR/Cas9 – Genome Editing

Jiang and Doudna, *Cell* (2017)



CRISPR/Cas9 – Nobel Prize in 2020!



Francisco Mojica



Emmanuelle Charpentier



Jenifer Doudna



Martin Jinek

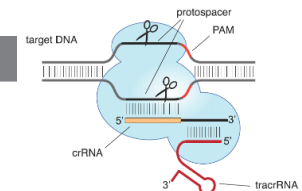
RESEARCH ARTICLE

A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity

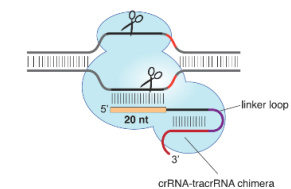
Martin Jinek,^{1,2*} Krzysztof Chylinski,^{3,4*} Ines Fonfara,⁴ Michael Hauer,^{2,†} Jennifer A. Doudna,^{1,2,5,6,‡} Emmanuelle Charpentier^{4,‡}

Jinek et al, *Science* (2012)

Cas9 programmed by crRNA:tracrRNA duplex



Cas9 programmed by single chimeric RNA



Key concepts

- RNAi
 - Natural mechanism controlling gene expression, partially explaining existence of large amount of non-coding DNA in various genomes
 - Possible use as a tool for specific gene expression control
- Genome editing
 - Sequence-specific high-precision genome modifications
 - Allows generation of both random mutations in a specific locus, as well as
 - introgression/replacement of defined sequence in the target locus, including gene therapy
 - CRISPR/Cas9 paved the way for easy, fast and accurate genome editing and further derived modifications

Discussion