CG920 Genomics

Lesson 5

RNA Interference and Genome Editing

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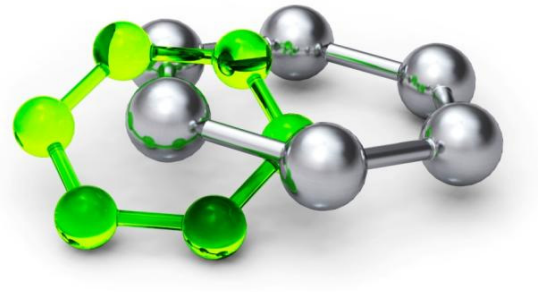
MUNI
SCI



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)

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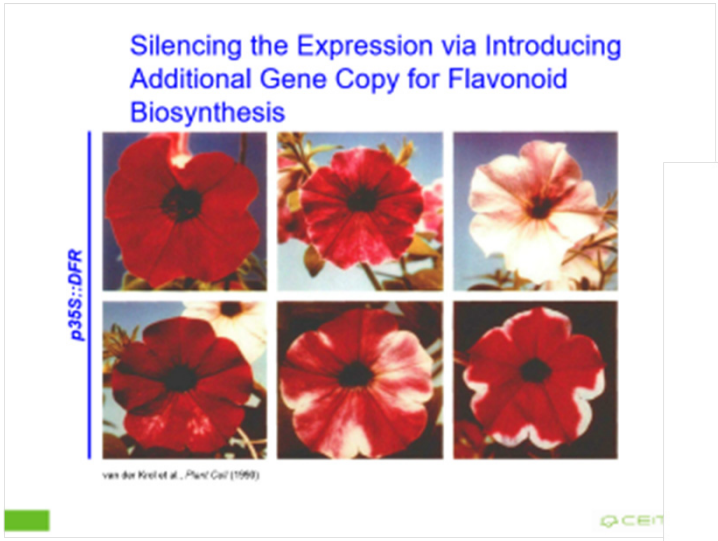
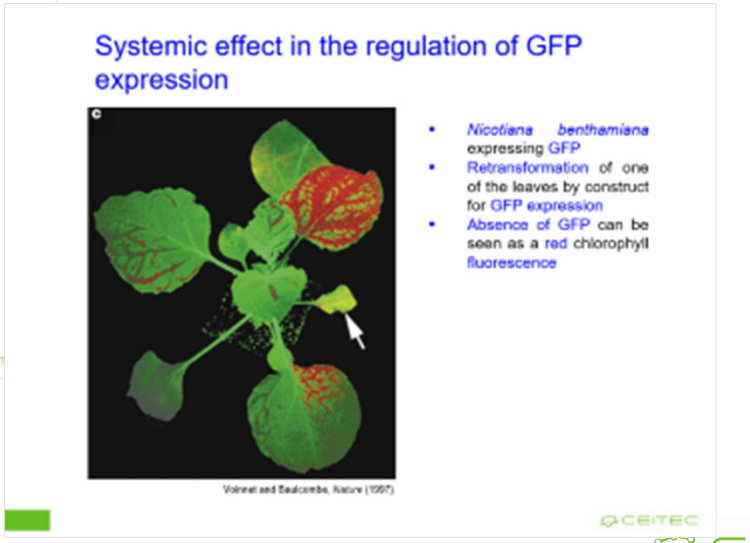
- Knocking-down the genes using RNA interference
  - Mechanism of RNAi

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



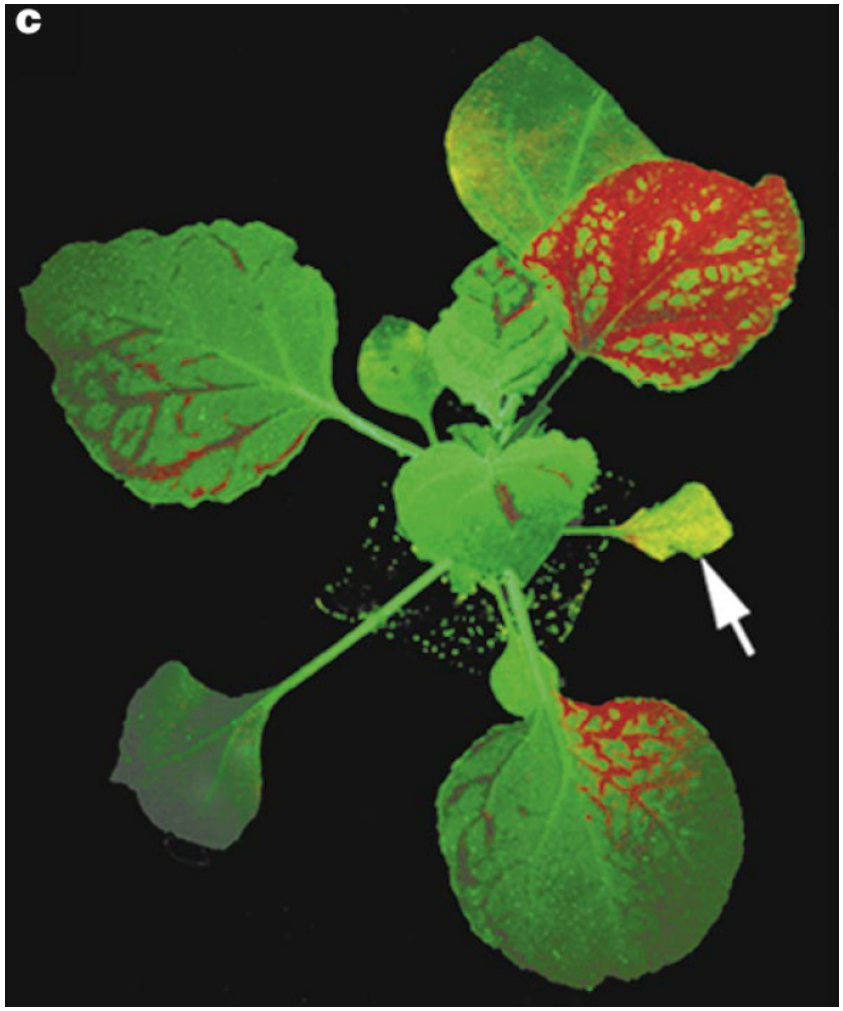


Silencing the Expression via Introducing Additional Gene Copy for Flavonoid Biosynthesis

p35S::DFR

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

Systemic effect in the regulation of GFP expression

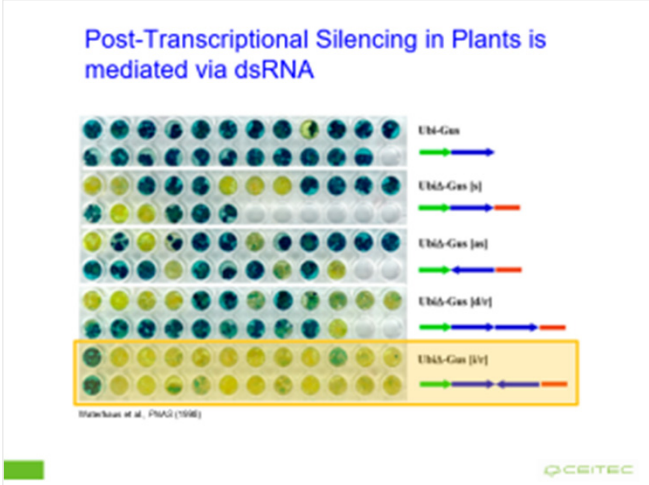


Voinnet and Baulcombe, *Nature* (1997)

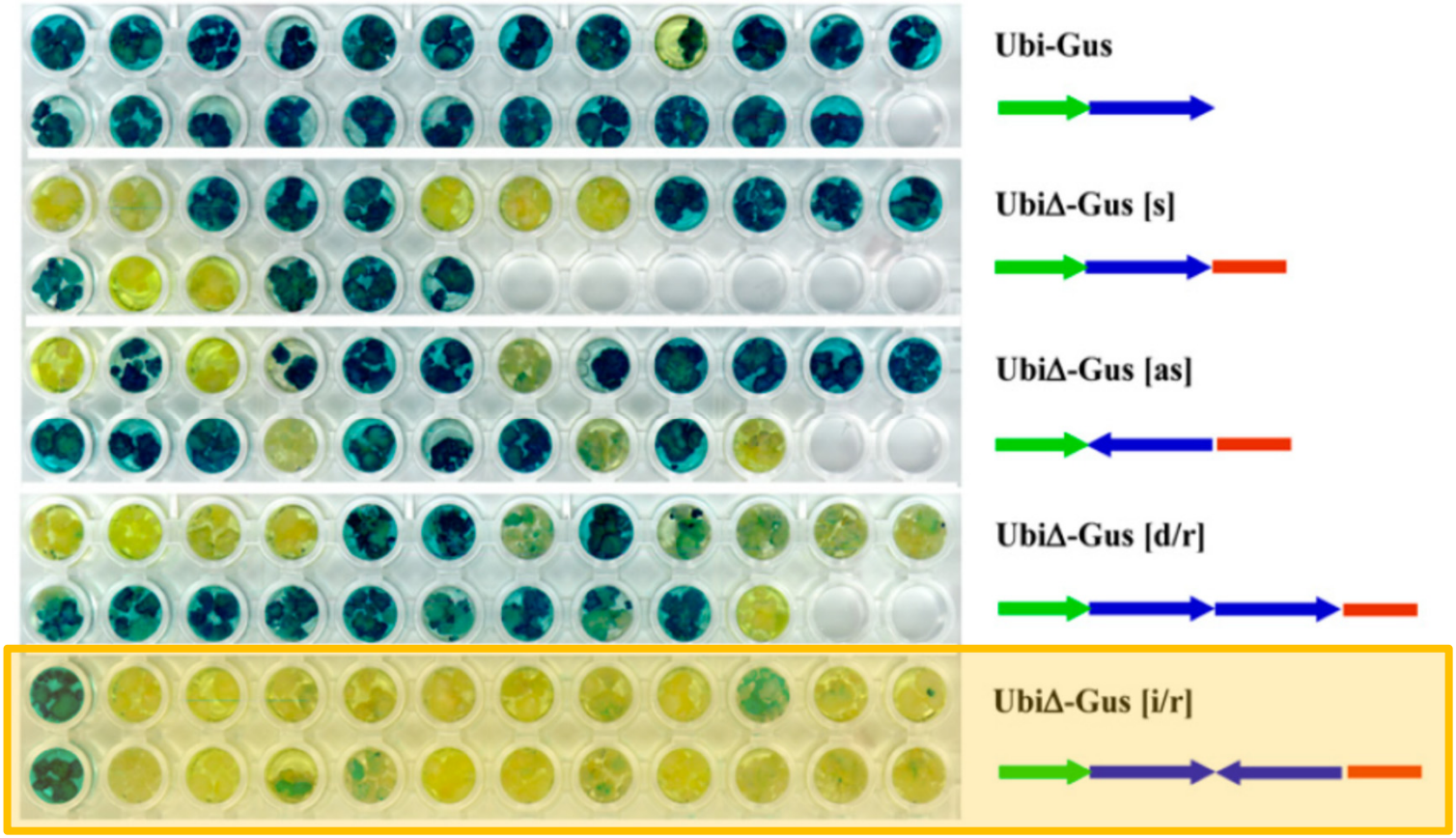
- *Nicotiana benthamiana* expressing GFP
- Retransformation of one of the leaves by construct for GFP expression
- Absence of GFP can be seen as a red chlorophyll fluorescence

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 aktivity
    - 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

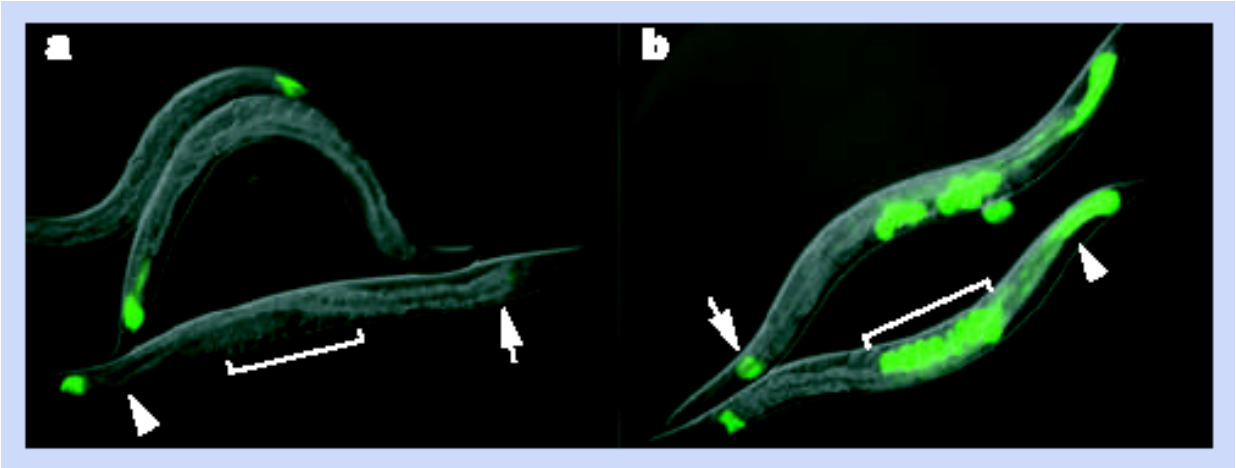


Waterhaus et al., *PNAS* (1998)

RNA interference

- **Molecular basis of posttranscriptional gene silencing (PTGS)**
  - dsRNA induction is dependent on its own genes – gene searching

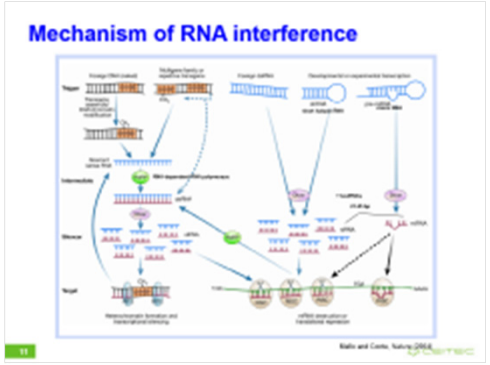
RNAi *rnai*



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 komplex)
  - **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)



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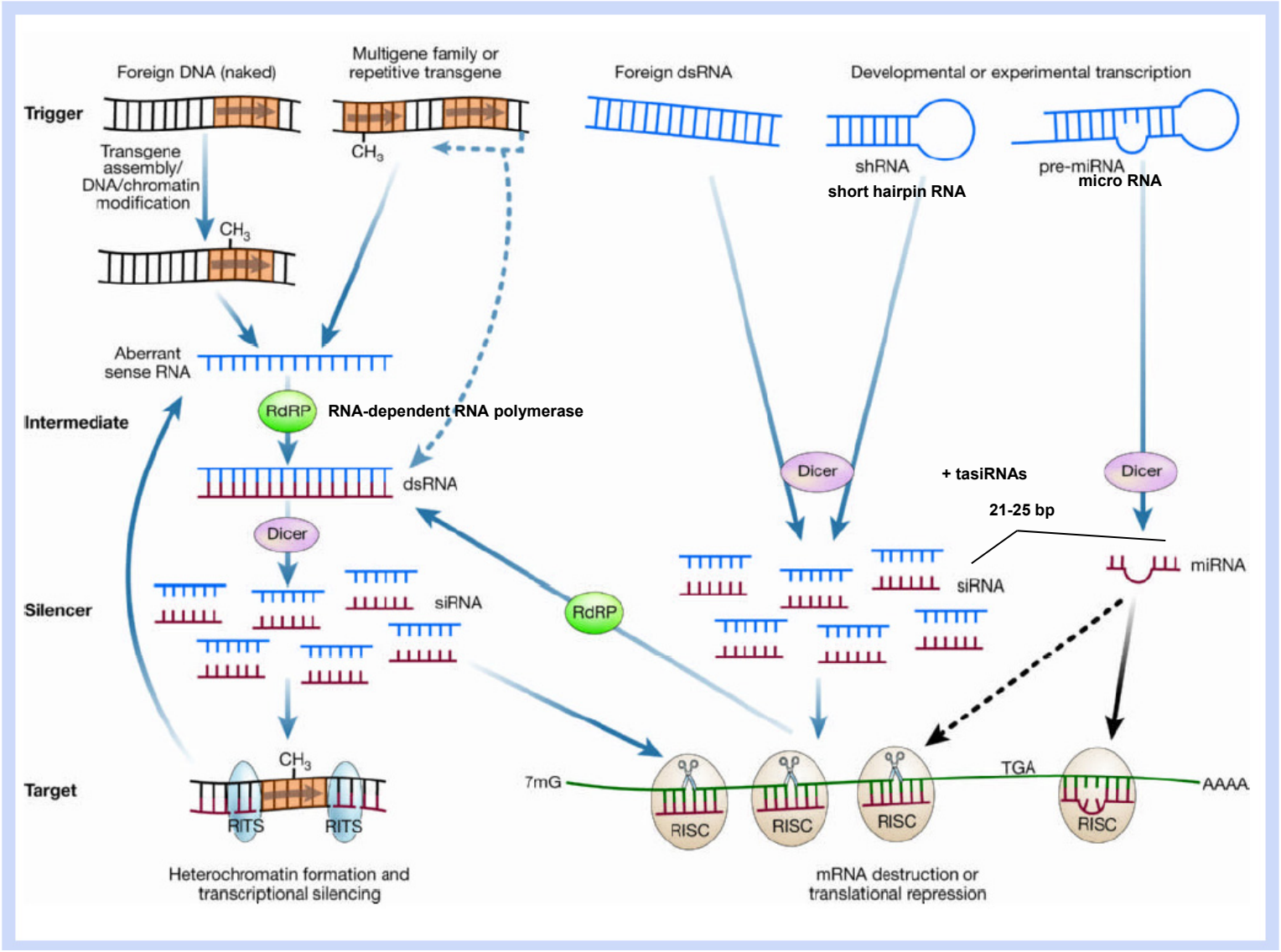
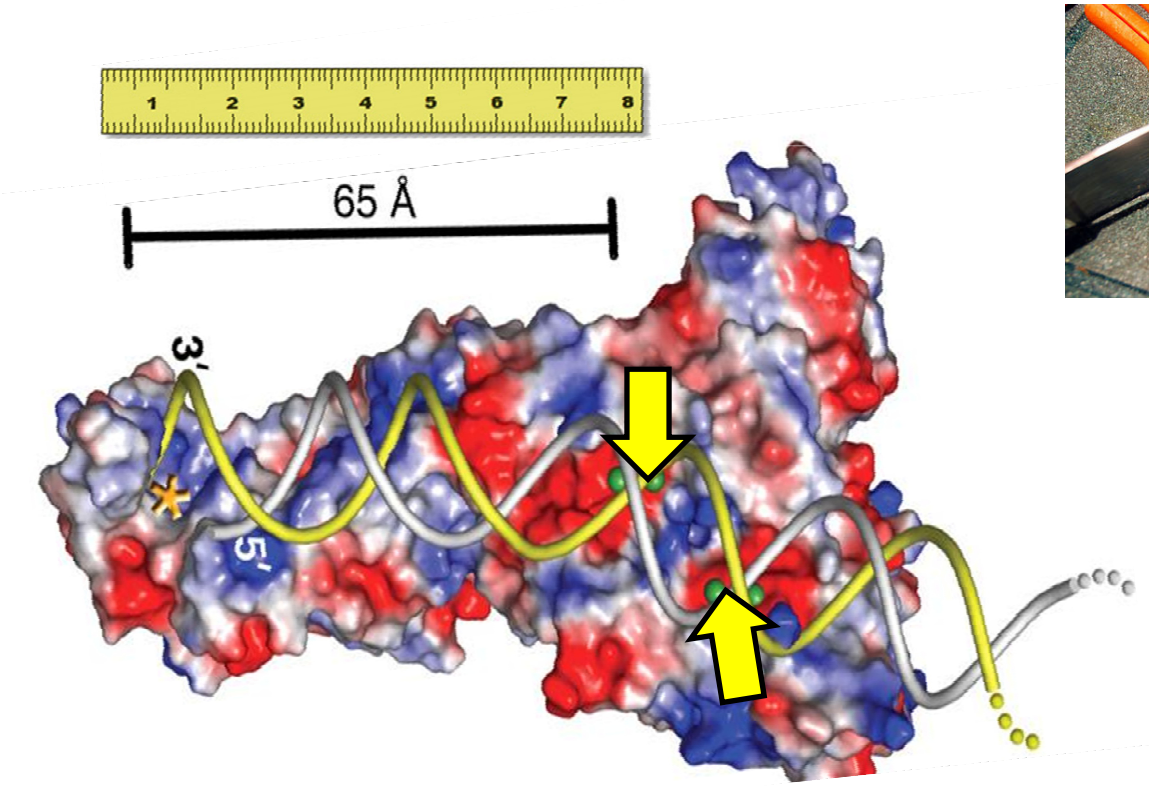
Mechanism of RNA interference



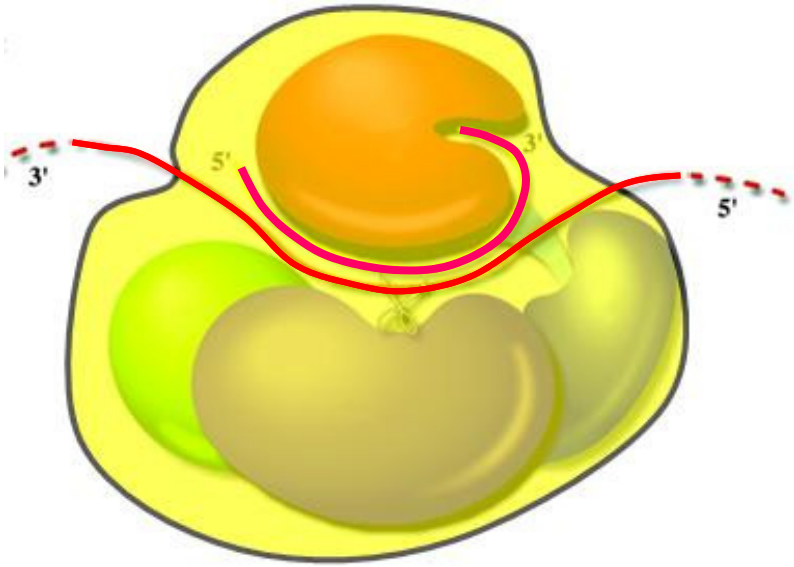
Mello and Conte, *Nature* (2004)

Dicer and Dicer-like proteins

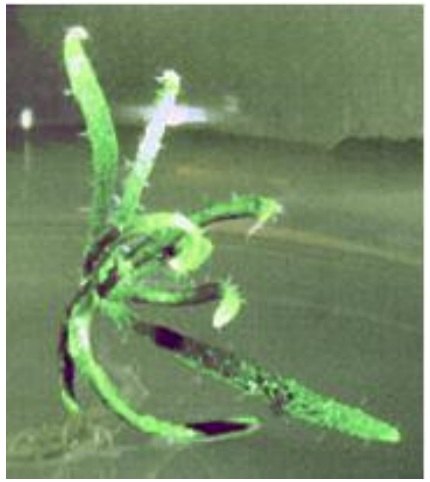


From MacRae, I.J., Zhou, K., Li, F., Repic, A., Brooks, A.N., Cande, W.., Adams, P.D., and Doudna, J.A. (2006) Structural basis for double-stranded RNA processing by Dicer. Science 311: 195 -198. Reprinted with permission from AAAS. Photo credit: Heidi

Argonaute proteins

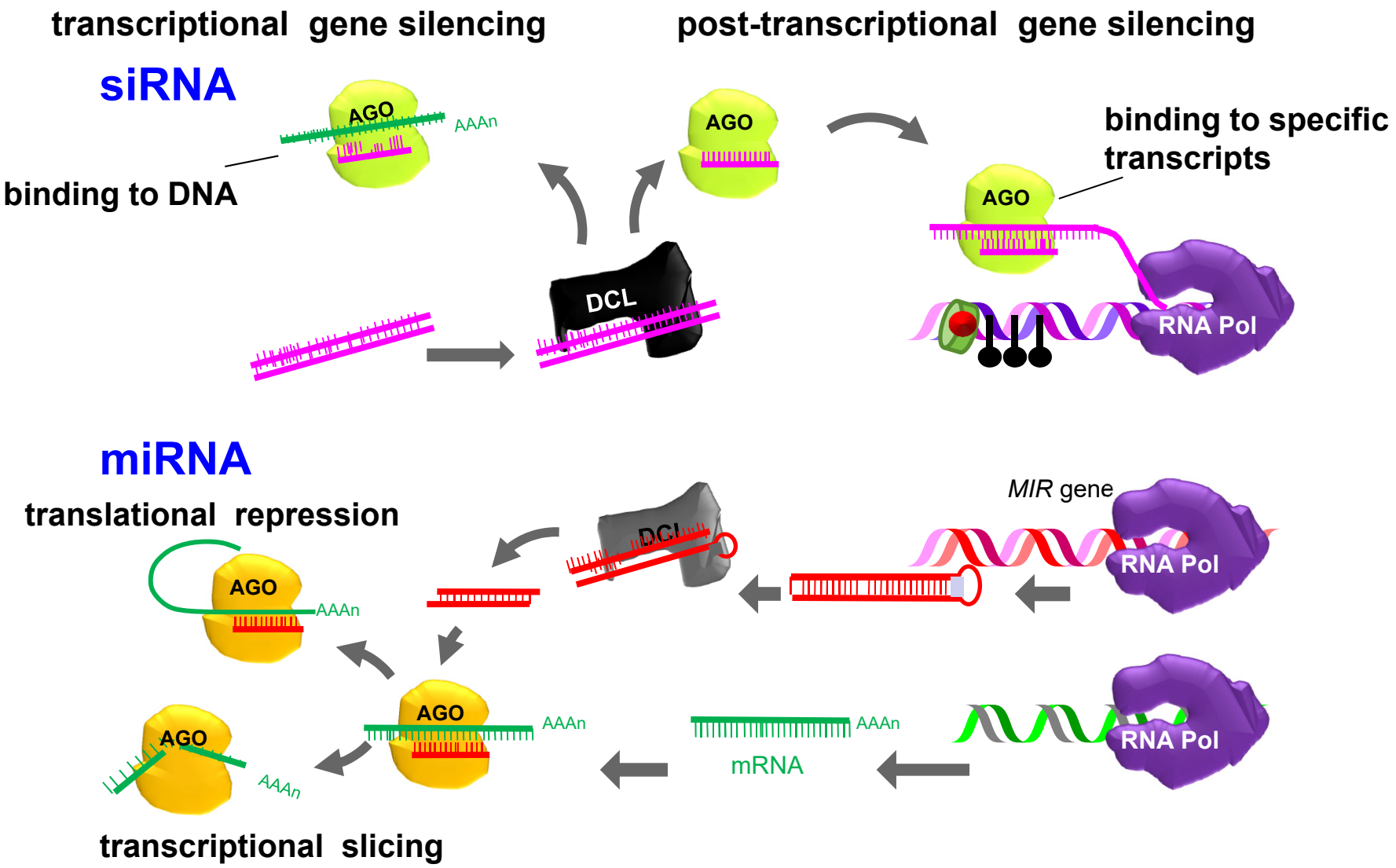


ago1



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
post-transcriptional gene silencing
siRNA
AGO
AAAn
binding to DNA
AGO
binding to specific transcripts
AGO
DCL
RNA Pol
miRNA
translational repression
MIR gene
DCL
RNA Pol
AGO
AAAn
AGO
AAAn
AAAn
mRNA
RNA Pol
AGO
AAAn
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 Medicine[1].

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.

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*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. Vainnet, O. & Baulcombe, D. C. *Nature* 389, 553 (1997).
5. Metzlaff, M., O'Dell, M., Cluster, P. D. & Flavell, R. B. *Cell* 88, 845–854 (1997).

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: 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

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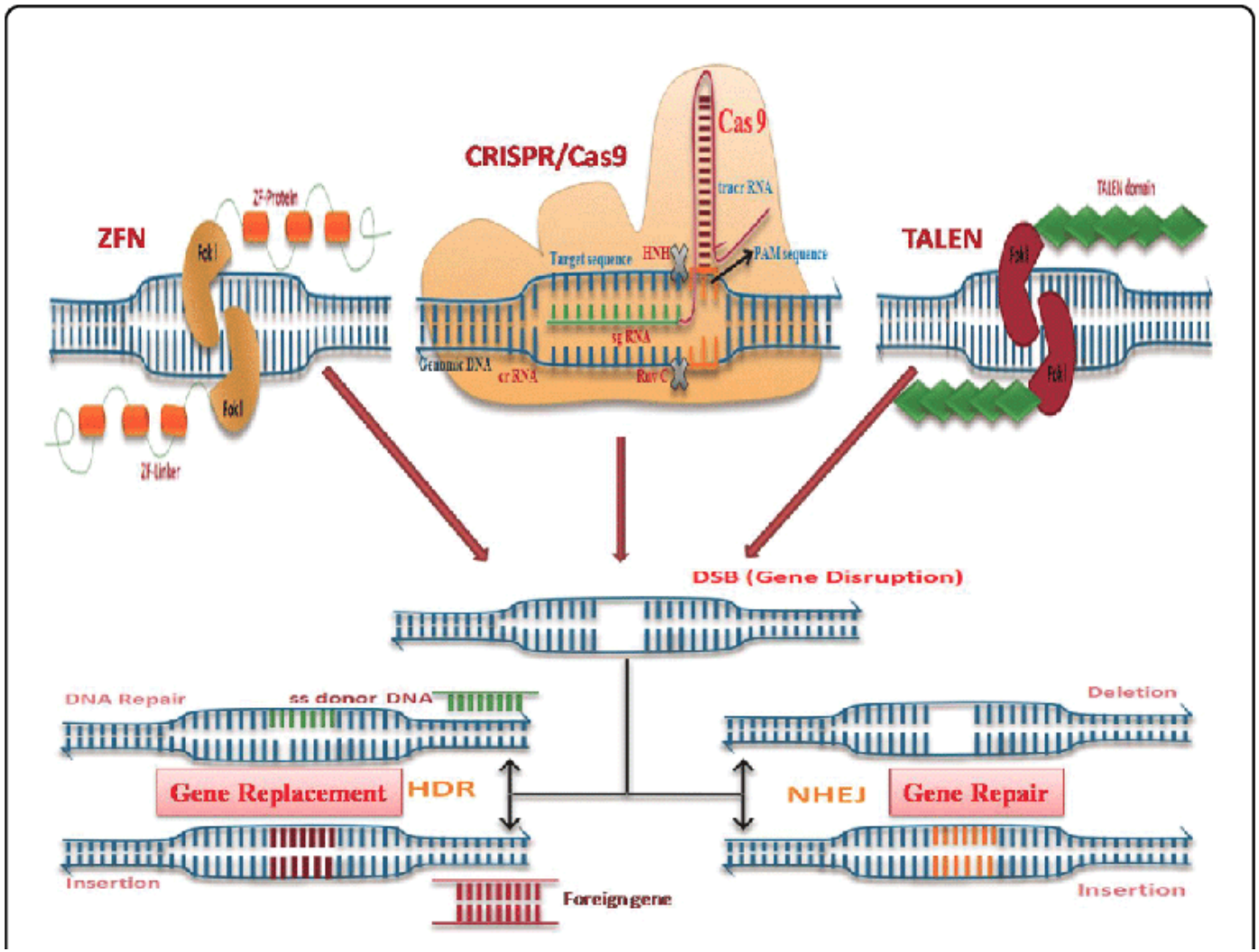
  - Principle of genome editing using Site Directed Nucleases, (SDNs)

Genome Editing via SDNs



Pandey et al, *Journal of Genetic Syndromes & Gene Therapy* (2011)

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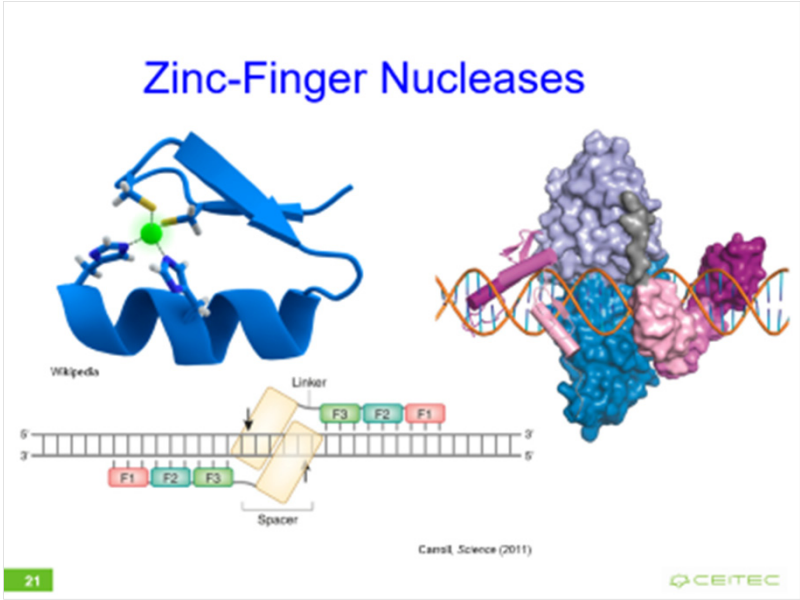
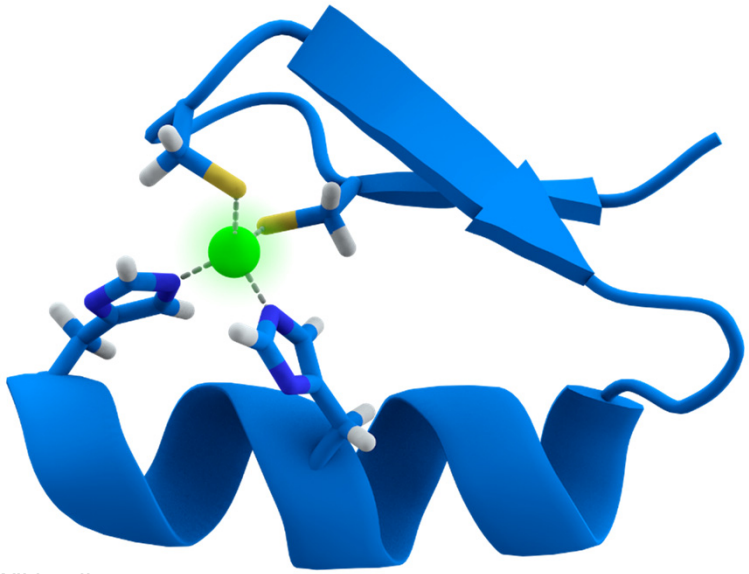
  - Zinc-Finger Nucleases (ZFNs)

Zinc-Finger Nucleases - ZFNs

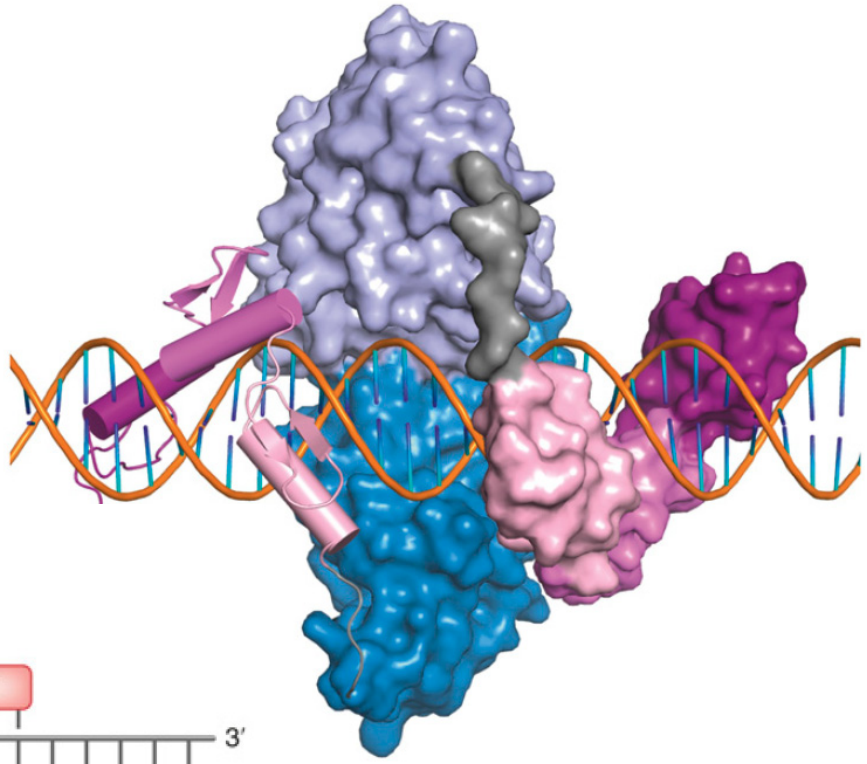
- **Sequence-specific endonucleases recognizing the target sequence via set of "zinc fingers"**
  - Each zinc „finger" is recognizing nucleotide triplet
  - Nuclease domain acts as heterodimer – possiblity to enhance the specificity by designing the set of „fingers" recognizing 9 bp on both sides of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

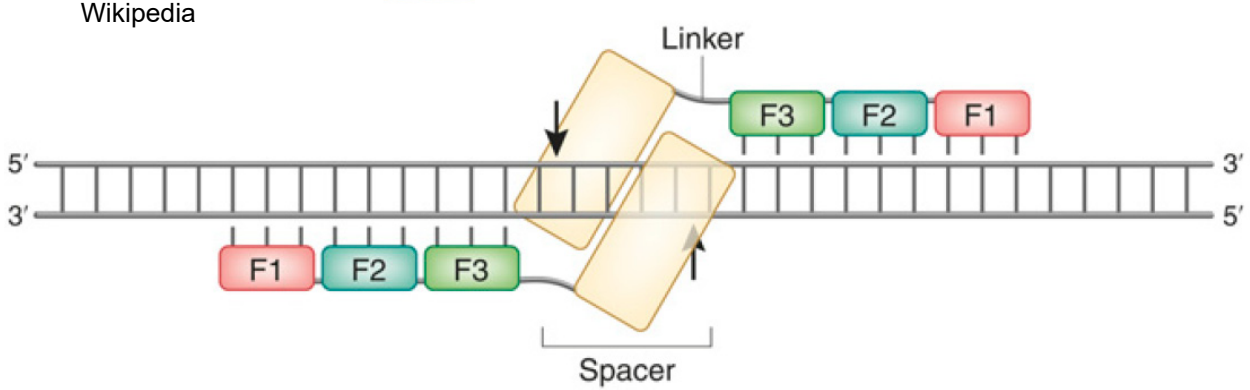


Zinc-Finger Nucleases



Wikipedia





Carroll, *Science* (2011)

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Transcription Activator-Like Effectors - TALENs

- **Proteins derived from sequence-specific transcription activators**
  - Identifiied (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

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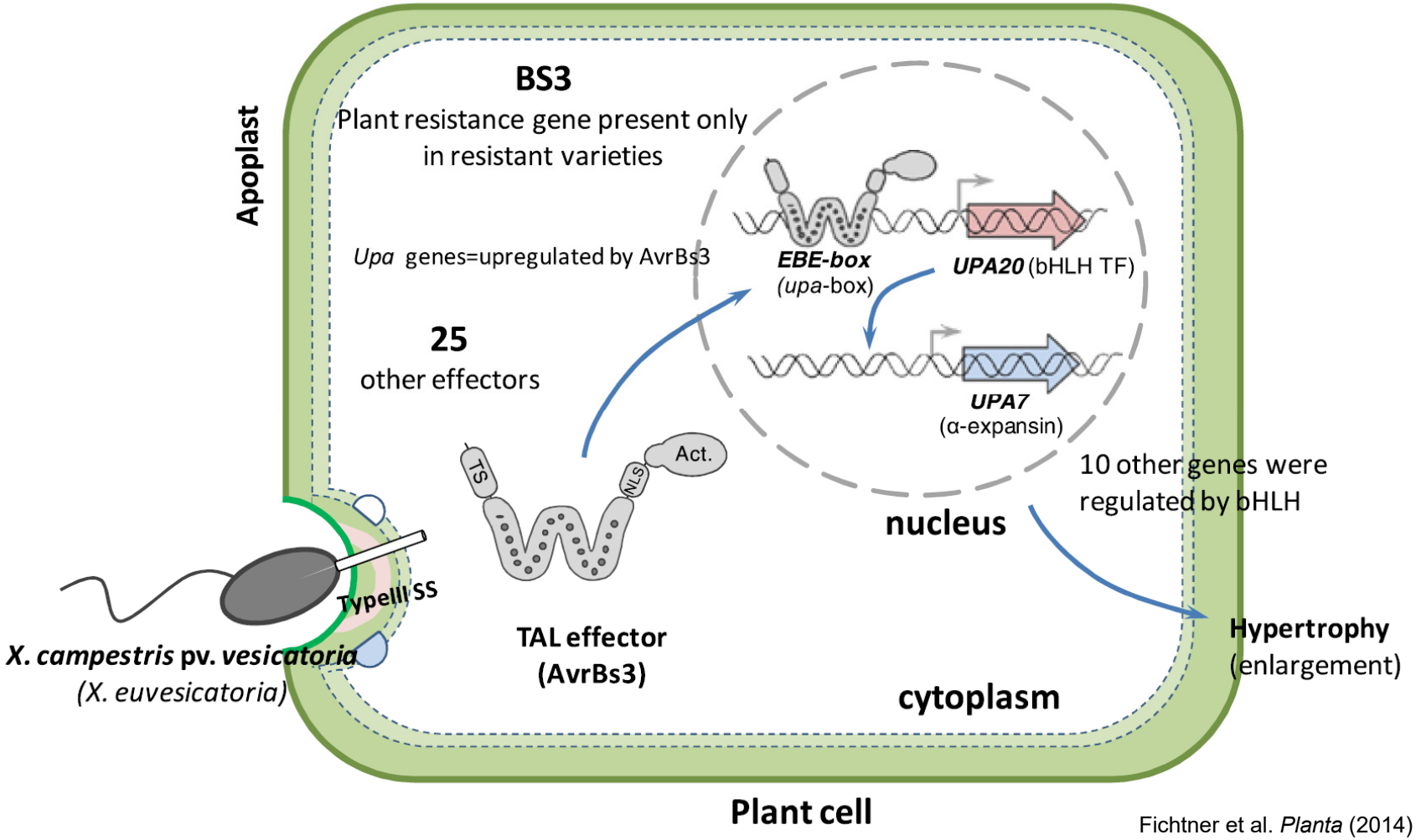
TALENs, The Origin



Fichtner et al. *Planta* (2014)

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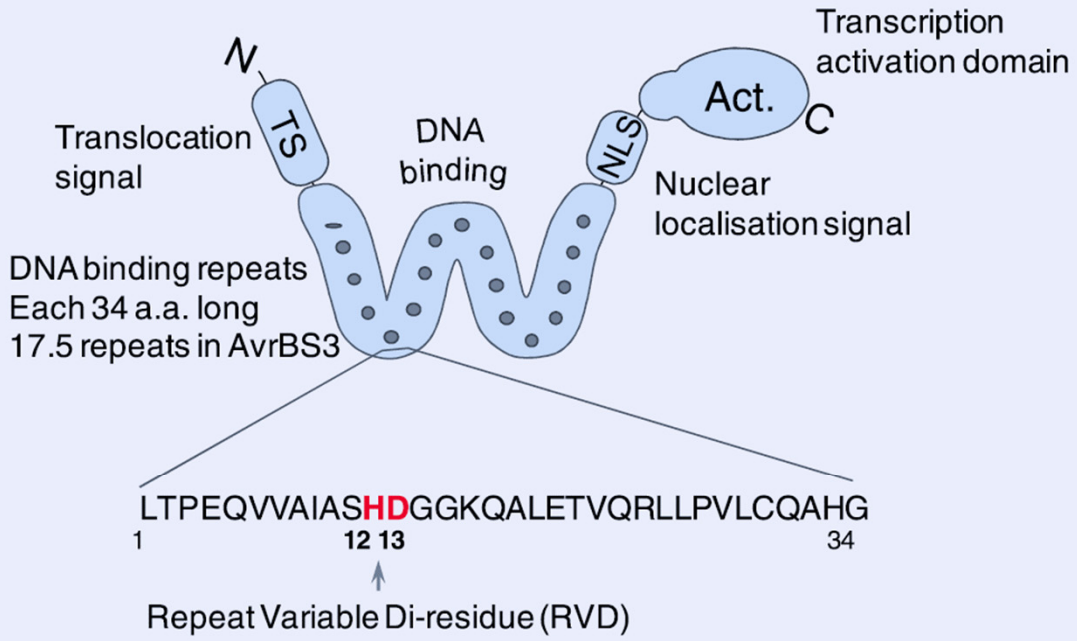
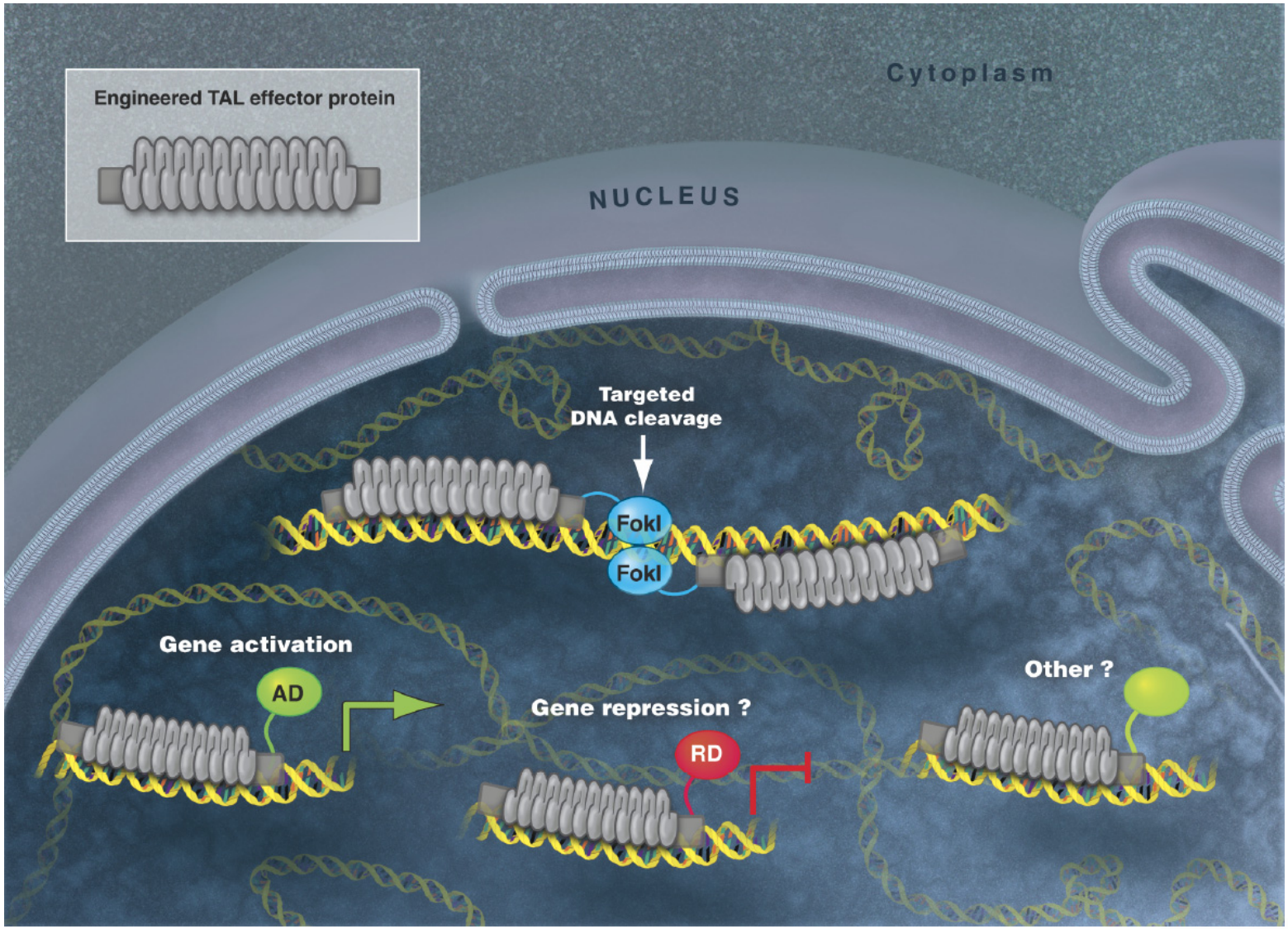
TALENs, Specificity Determination



DNA binding repeat	recognised nucleotide
LTPEQVVAIAS**HD**GGKQALETVQRLLPVLCQAHG	C
LTPEQVVAIAS**NI**GGKQALETVQRLLPVLCQAHG	A
LTPEQVVAIAS**NG**GGKQALETVQRLLPVLCQAHG	T
LTPEQVVAIAS**IG**GGKQALETVQRLLPVLCQAHG	T
LTPEQVVAIAS**NK**GGKQALETVQRLLPVLCQAHG	G
LTPEQVVAIAS**NN**GGKQALETVQRLLPVLCQAHG	G
LTPEQVVAIAS**NS**GGKQALETVQRLLPVLCQAHG	ACGT

Fichtner et al. *Planta* (2014)

TALENs, Applications



Bogdanove and Voytas, *Science* (2011)

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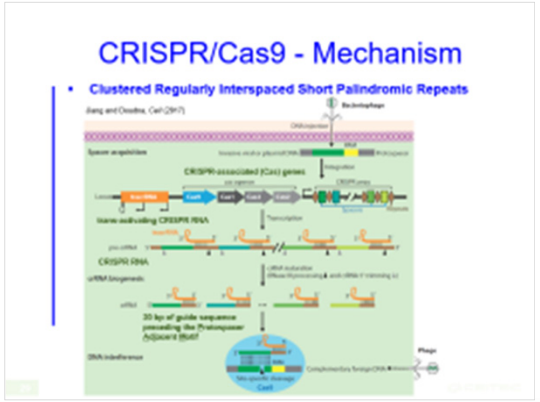
Clustered Regularly Interspaced Short Palindromic Repeats/Cas9 - CRISPR/Cas9

- **Discovered as a mechanism of bacterial immune system**
  - The principle is targeted insertion of foreign DNA (typically phage DNA) into specific bactrial genomu 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)
  - **Advanatges**
    - Easy to „program“
    - High specificity
    - Number of further applications possible



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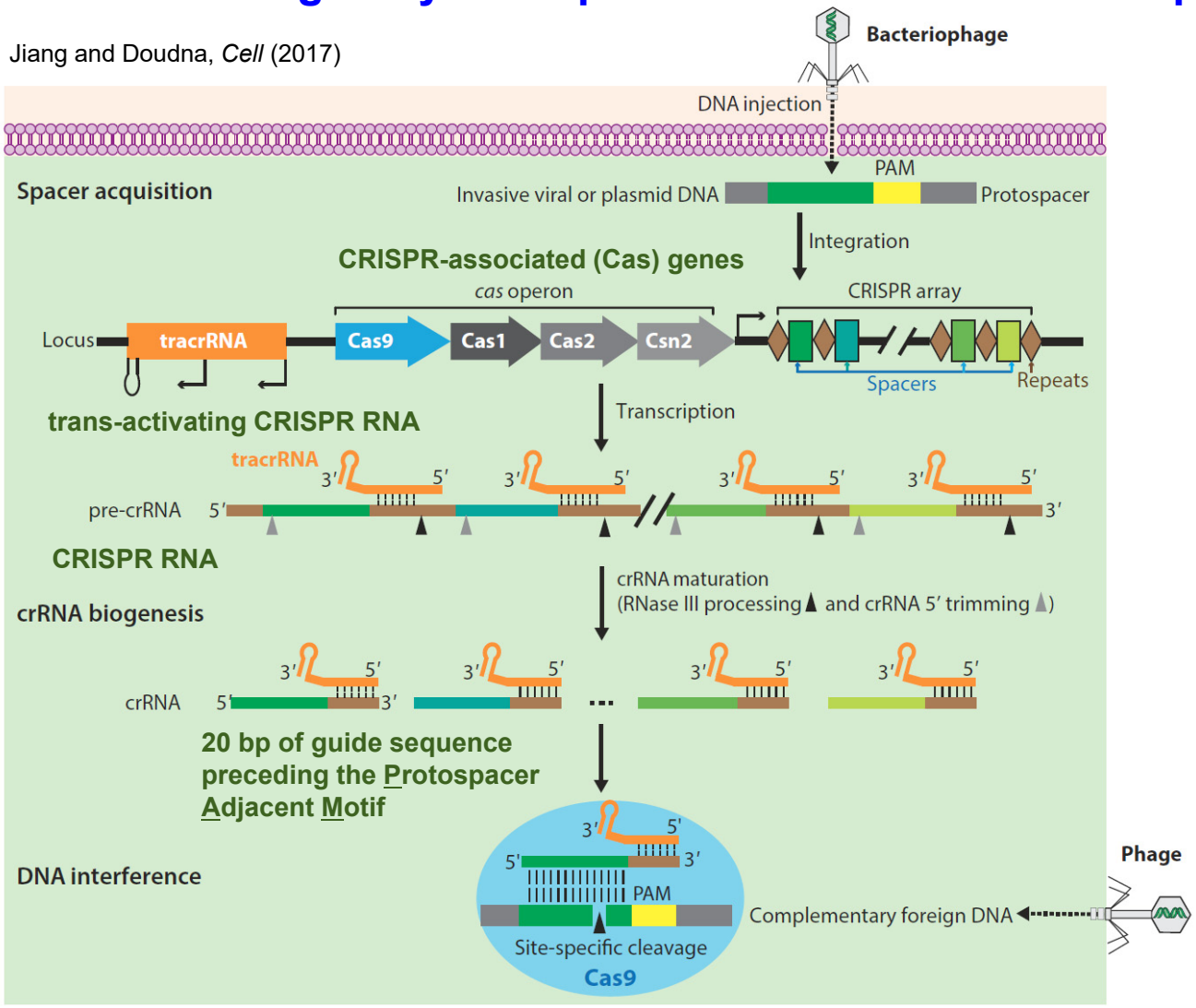
CRISPR/Cas9 - Mechanism

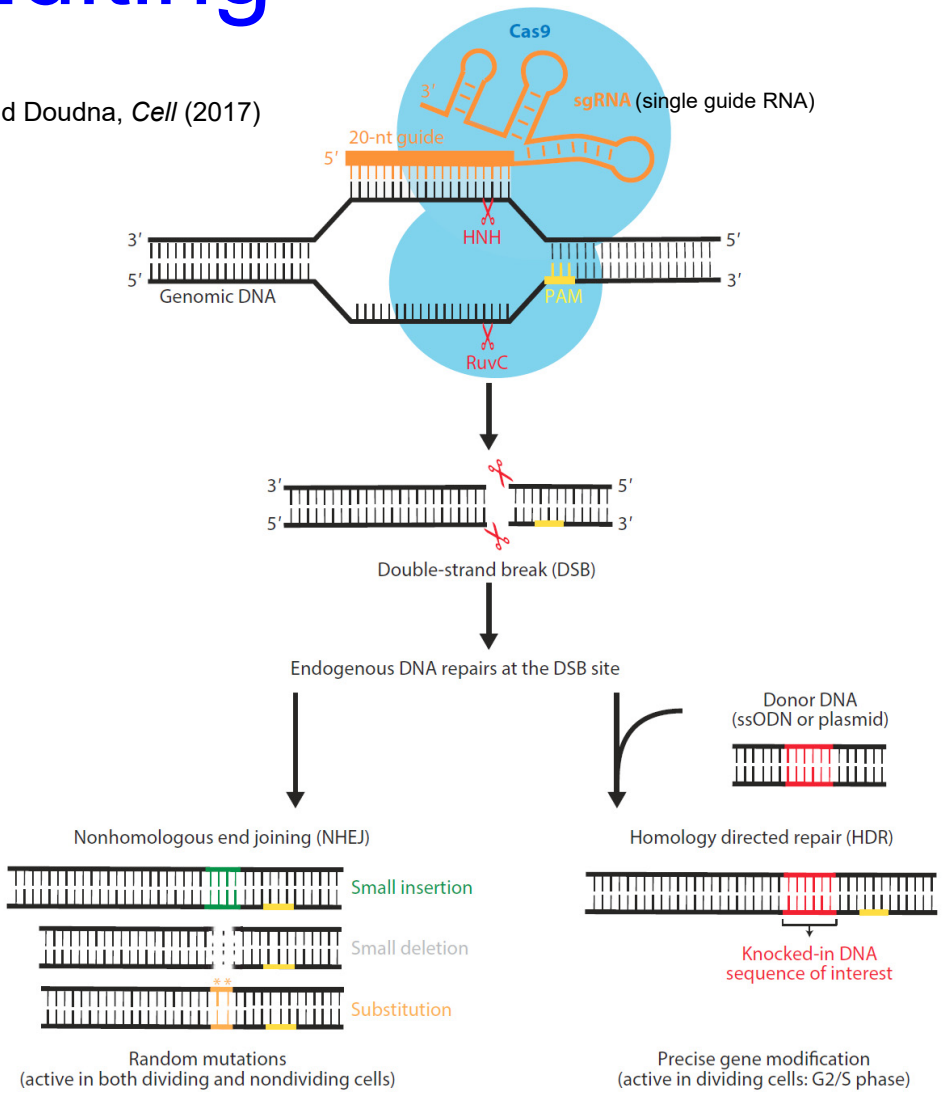
- **Clustered Regularly Interspaced Short Palindromic Repeats**

Jiang and Doudna, *Cell* (2017)



CRISPR/Cas9 – Genome Editing

Jiang and Doudna, *Cell* (2017)



CRISPR/Cas9 – Nobel Prize in 2020!

Francisco Mojica

Emmanuelle Charpentier

Jenifer Doudna

Martin Jinek

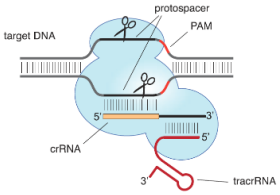
RESEARCH ARTICLE

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

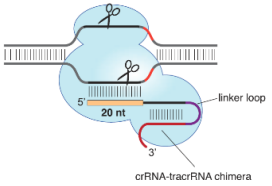
Martin Jinek,[1,2]* Krzysztof Chylinski,[3,4]* Ines Fonfara,[4] Michael Hauer,[2]† Jennifer A. Doudna,[1,2,5,6]‡ Emmanuelle Charpentier[4]‡

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