Targeted Nucleases

Molecular tools for genome editing and more

Giulia Pavani - 2 Maggio 2018

Summary

- Introduction to targeted nucleases
- RNA-guided nucleases: CRISPR/Cas9 System
- Mechanisms of Double-Stranded DNA Break Repair and their consequences on genome editing approaches
- Cas9 applications
- New tools:
  - Catalytically inactive nucleases for gene regulation/visualization
  - Base editors

What is a targeted nuclease?

Targeted Nuclease

- Enzyme that cleaves phosphodiester bonds between monomers of nucleic acids
- Nuclease that recognize a specific DNA (or RNA) sequence

Why are they so important?

- A nuclease that can recognize a specific DNA sequence it's an incredible tool to modify a genome

- We can induce a double strand break (DSB) at any genomic location (Knock Out, Knock In, deletions, inversions and much more…)

Protein-based nucleases

Hybrid Meganuclease

ZFN

Zinc finger domains

TALEN

TALE subunits

active FokI catalytic subunit heterodimer

From Wikipedia

Meganucleases

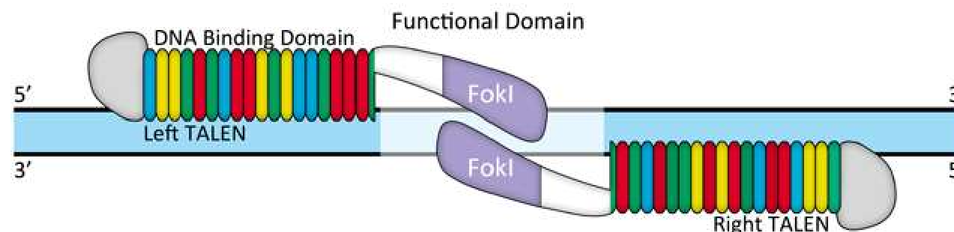
- Endonucleases that can recognize and cut large DNA sequences (from 12 to 40 base pairs)
- best known meganucleases proteins in the LAGLIDADG family
  - I-SceI (discovered in the mitochondria Saccharomyces cerevisiae)
  - I-CreI (from the chloroplasts of the green algae Chlamydomonas)
- Two methods for creating custom meganucleases:
  - Mutagenesis
  - Combinatorial assembly (subunits from different enzymes can be associated or fused)

Zinc-Fingers Nucleases

- Zinc finger motifs occur in several transcription factors
- Each ZFN containing 3 to 5 zinc finger motifs that recognize 3 base pair sequences and half of the FokI endonuclease complex.
- When a pair of ZFNs bind closely enough for their FokI domains to dimerize, they make a DSB.
- Engineering new pairs is complex

TALEN

- TALENs have 15–30 repeats of a 35 amino acid transcription activator-like effector (TALE).
- A TALE recognizes one base pair determined by which repeat variable di-residues (RVD) a TALE contains.
- TALE + FOK I (nuclease) = TALEN
- Function as a dimer (similar to ZFNs) but with an easier and more modular assembly



From Genecopoeia

CRISPR/Cas9: milestones

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

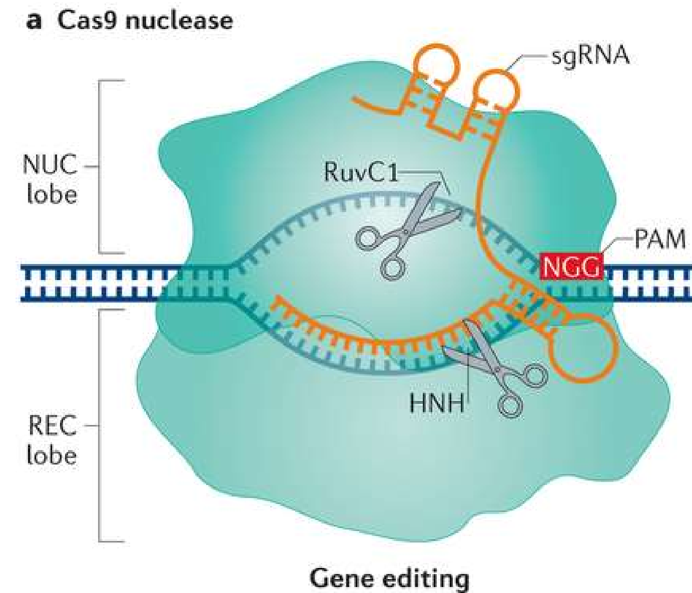
Martin Jinek,[1,2]* Krzysztof Chylinski,[3,4]* Ines Fonfara,[4] Michael Hauer,[2]† Jennifer A. Doudna,[1,2,5,6]‡ Emmanuelle Charpentier[4]‡

Science, 2012

Multiplex Genome Engineering Using CRISPR/Cas Systems

Le Cong,[1,2]* F. Ann Ran,[1,4]* David Cox,[1,3] Shuailiang Lin,[1,5] Robert Barretto,[6] Naomi Habib,[1] Patrick D. Hsu,[1,4] Xuebing Wu,[7] Wenyan Jiang,[8] Luciano A. Marraffini,[8] Feng Zhang[1]†

Science, 2013

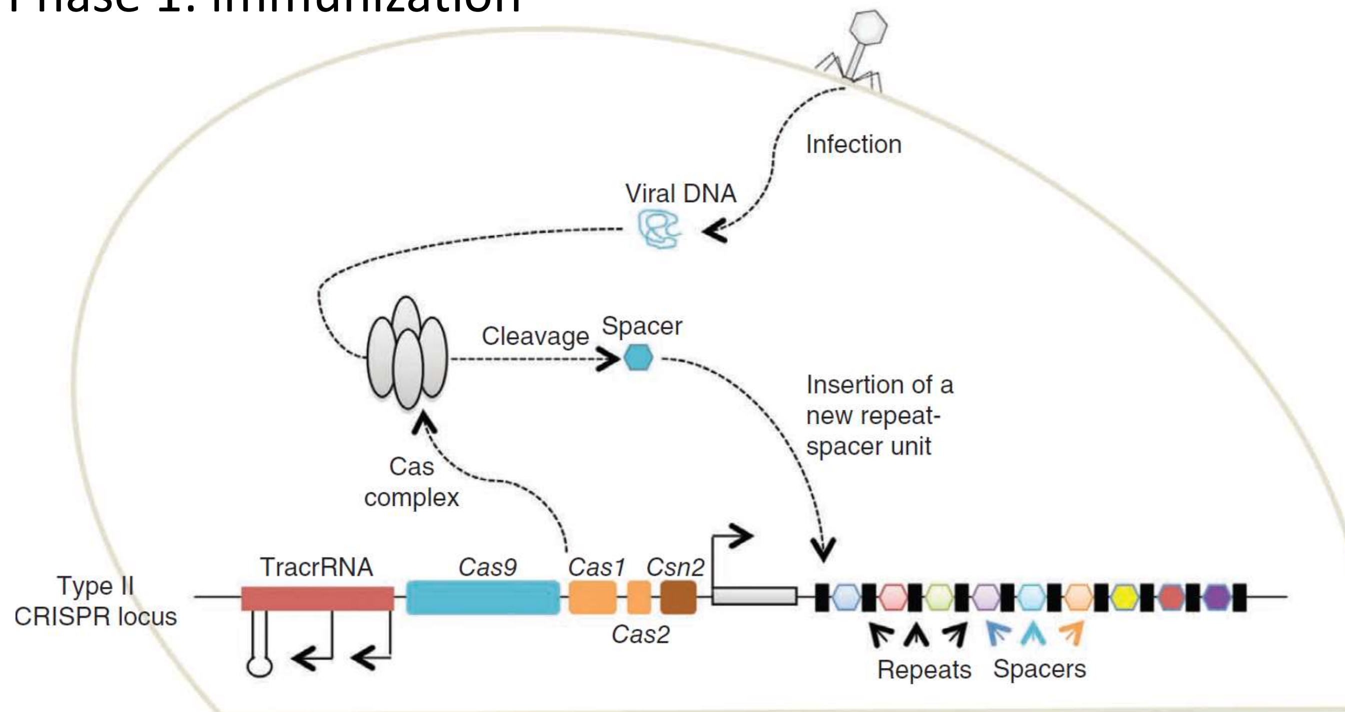


Komor, Cell, 2016

Nature cover, March 2016

CRISPR/Cas

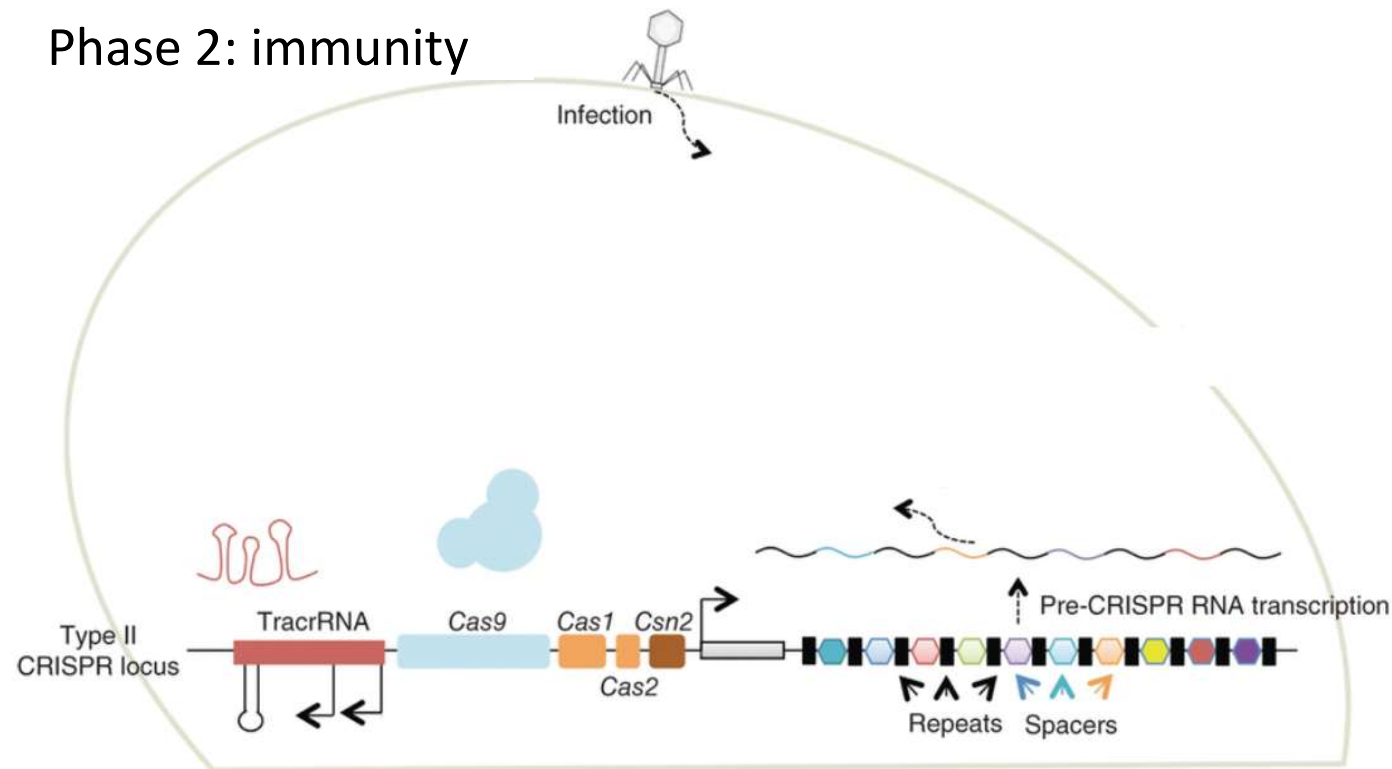
Phase 1: immunization



Mali, Nat. Biotechnol., 2013

- CRISPR: clustered regulatory interspaced short palindromic repeats
- Cas: CRISPR associated protein

CRISPR/Cas

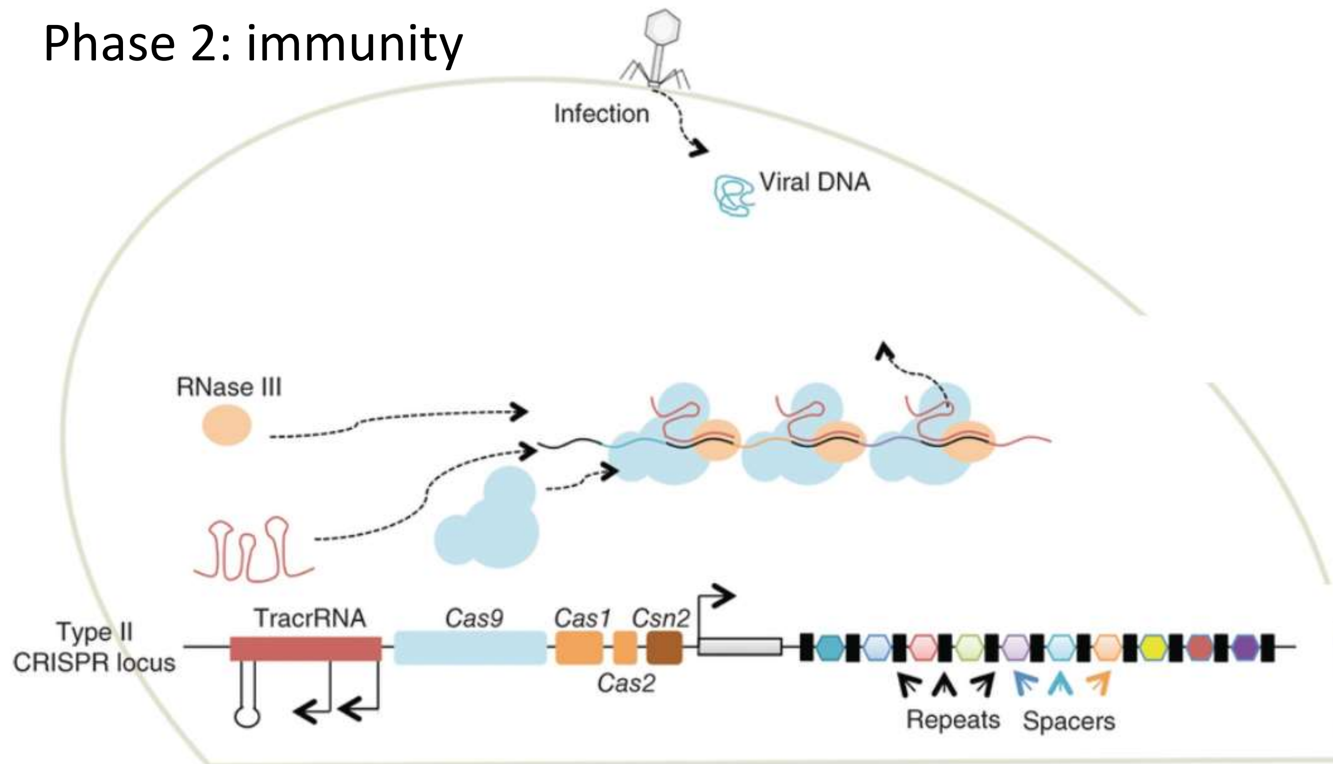


Mali, Nat. Biotechnol., 2013

- crRNA: CRISPR RNA
- tracrRNA: trans-activating crRNA

CRISPR/Cas

Phase 2: immunity



Mali, Nat. Biotechnol., 2013

- crRNA: CRISPR RNA
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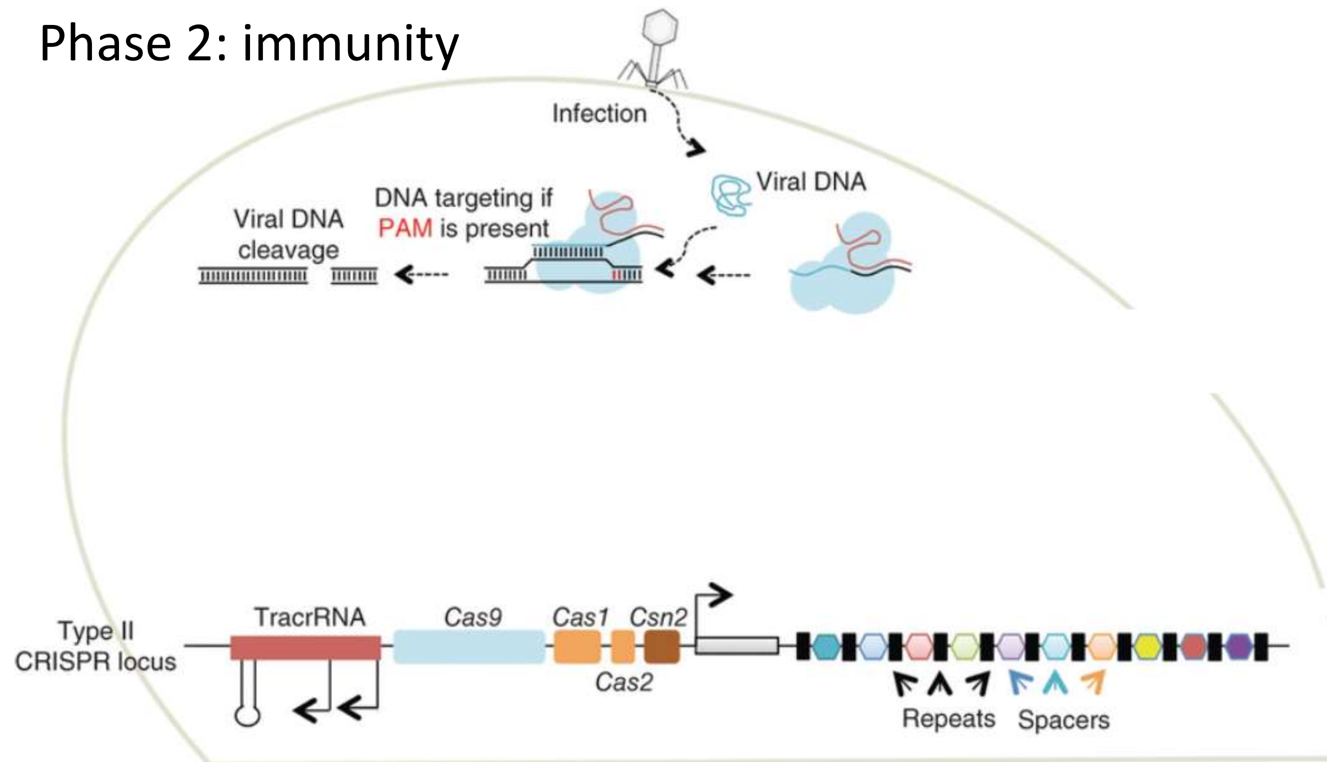
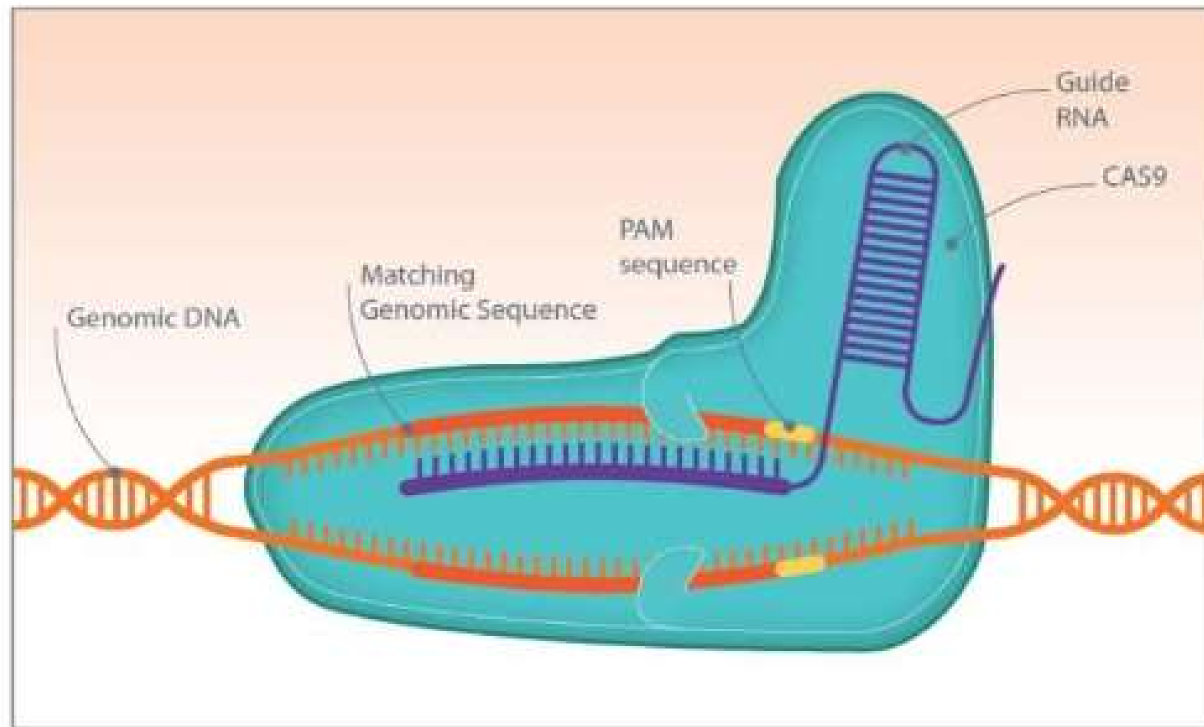
CRISPR/Cas



Mali, Nat. Biotechnol., 2013

- crRNA: CRISPR RNA
- tracrRNA: trans-activating crRNA
- PAM: protospacer adjacent motif

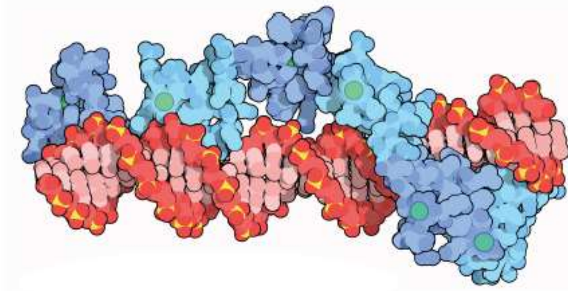
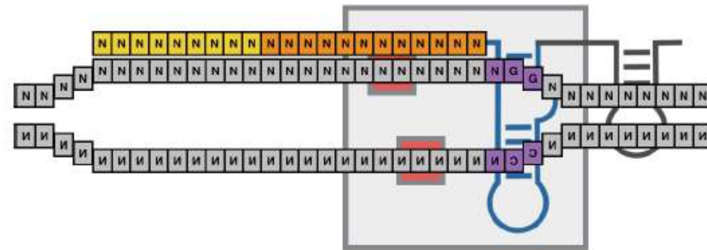
CAS9 + guide RNA



gRNA target sequence | PAM

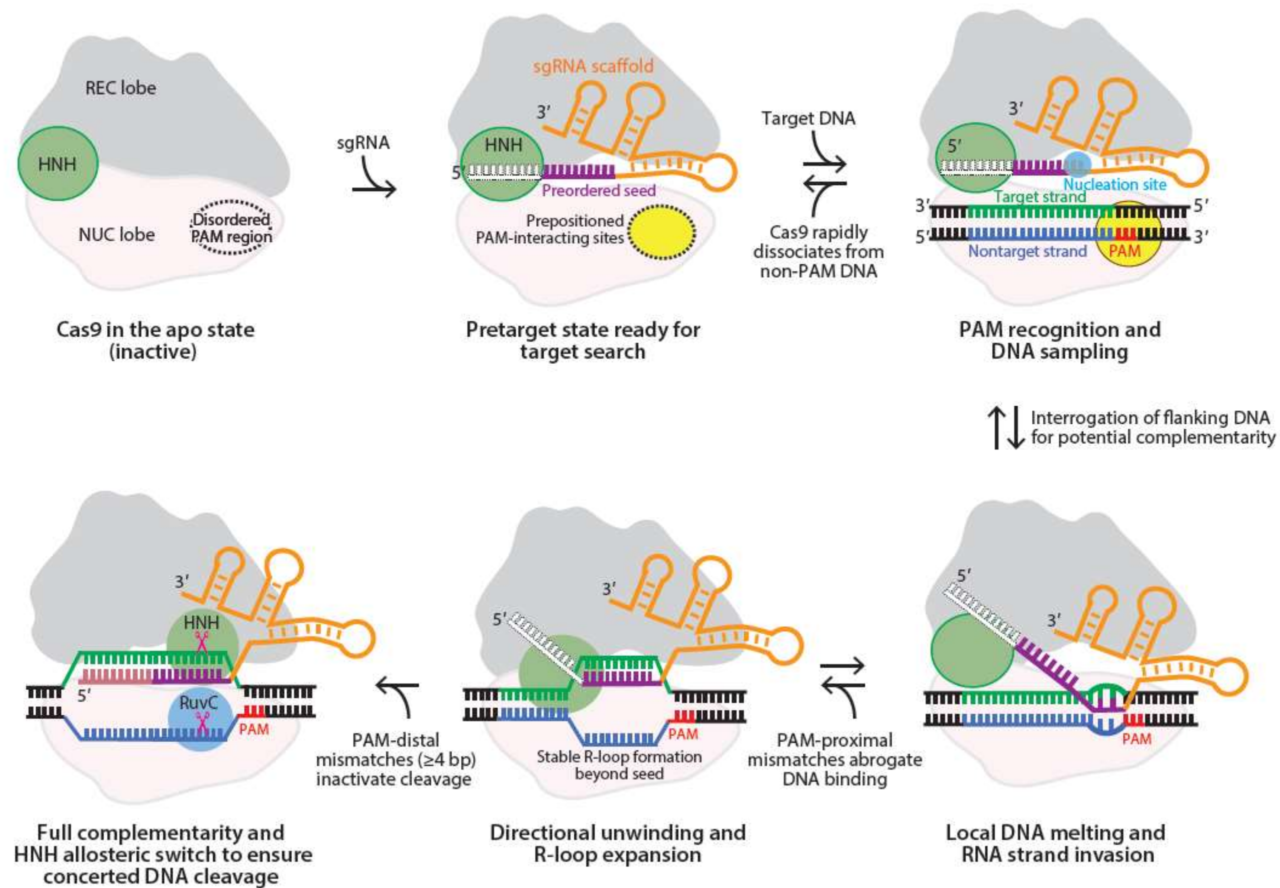
AGCTGGGATCAACTATAGCG CGG

Comparing nucleases

Protein-based	CRISPR
	 *RNA*-guided endonuclease(*RGEN*)
1. Protein and DNA interface is very complex	1. Watson-Crick base pairing is straight-forward and predictable
2. Construction is relatively expensive and complicated	2. Construction is fast, easy, and cheap

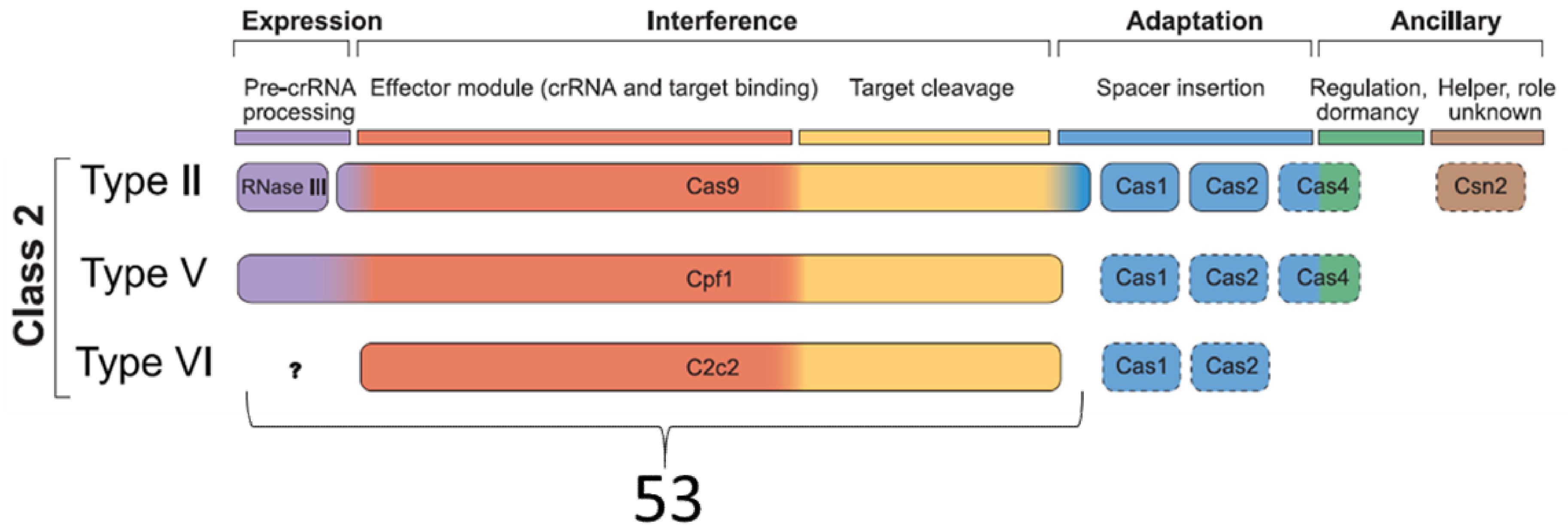
e.g. ZFN and Talen

CRISPR/Cas9 mechanism



Sternberg, Nature, 2014

CRISPR/Cas classes



Type VI target RNA

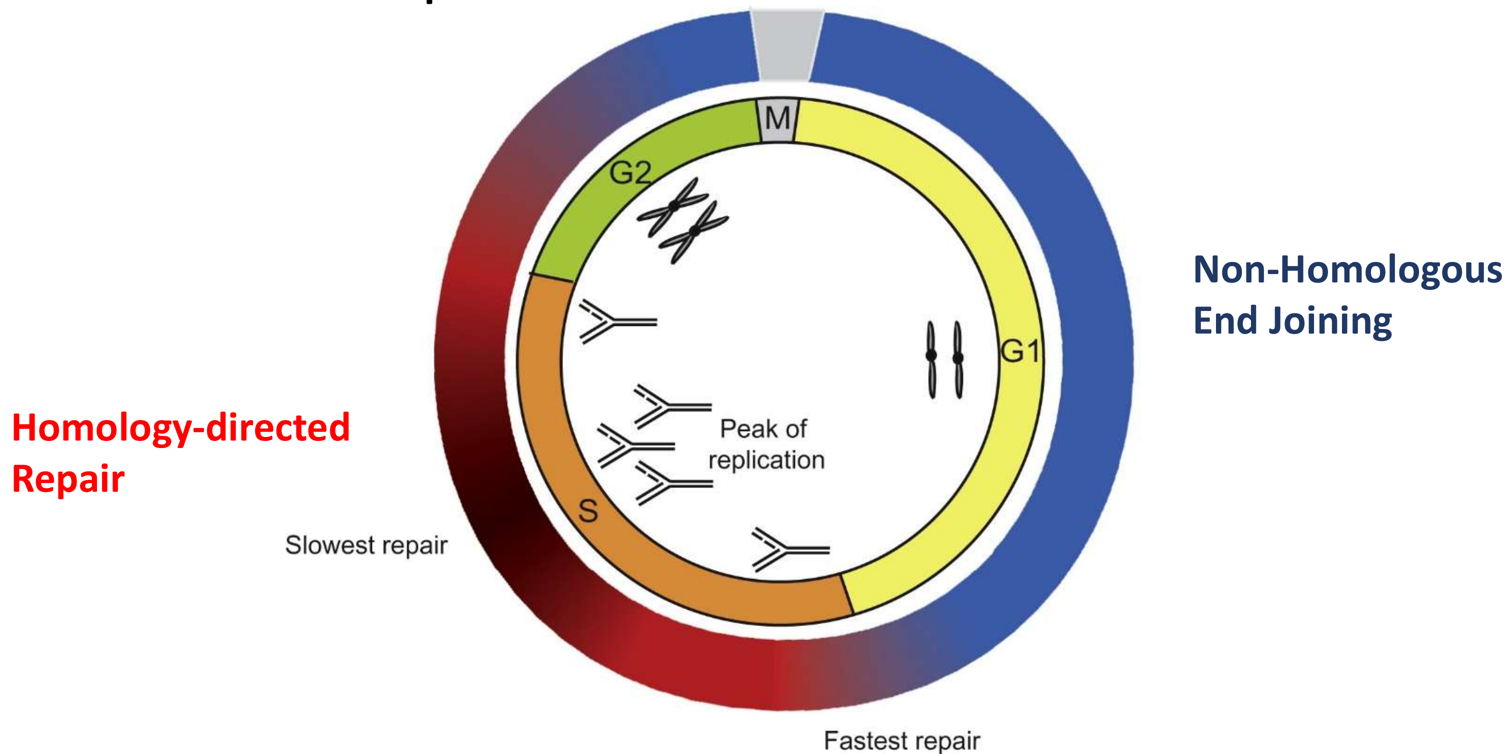
Cox DBT, Science 2017
Abudayyeh OO, Science 2017
Gootenberg JS, Science 2017

Summary

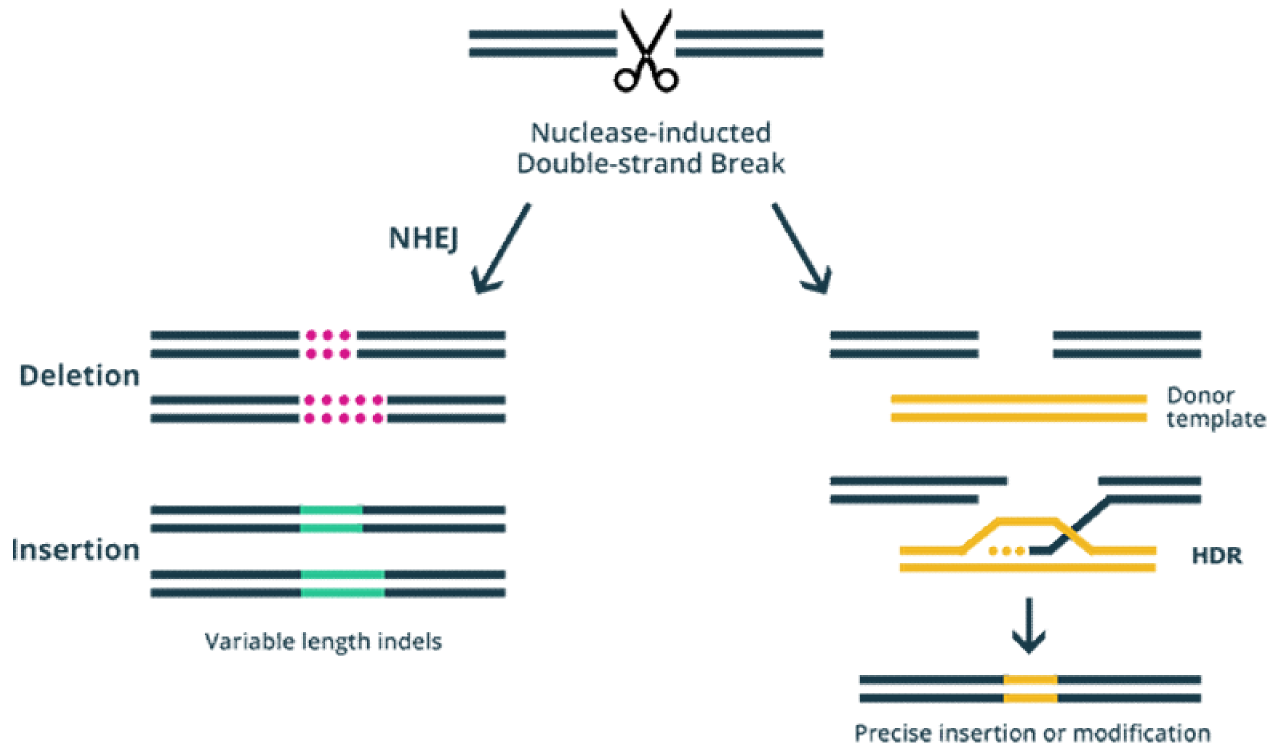
- Cas9 is a RNA-guided nuclease discovered in prokaryotes
- Cas9 can induce double-strand break in selected locations (as long as there is a PAM sequence next to the guide)
- It’s a fast/easy/relatively cheap system to edit genomes

Nucleases cut DNA, but the cells repair

- Cell cycle stage and DNA sequence dictate how the cut will be repaired



Molecular Cell 2012 47, 320-329DOI: (10.1016/j.molcel.2012.05.052)



NHEJ is the prominent repair mechanism of the cell. It is usually associated with the introduction of **indel** mutations at the cut site, which may cause genomic modifications.

HDR is active during late phase S/early G2, which mediates a strand-exchange process to repair DNA damage accurately, based on the availability of homologous DNA sequences.

Questions

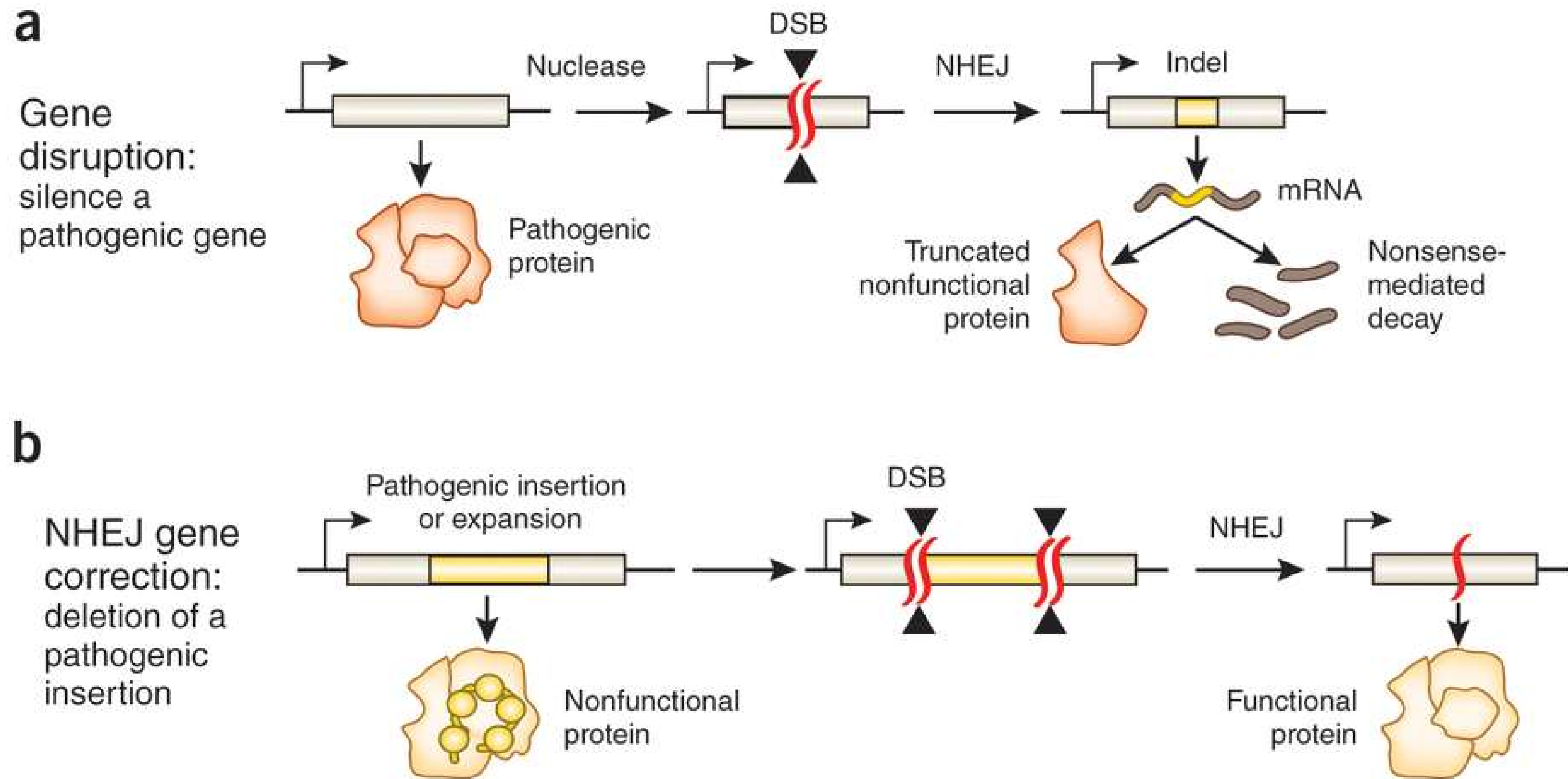
- Which DNA repair system would you use to generate a Knock-out of protein X?

    NHEJ: active most of the time, very efficient

- Which DNA repair system would you use to generate a Knock-In ?

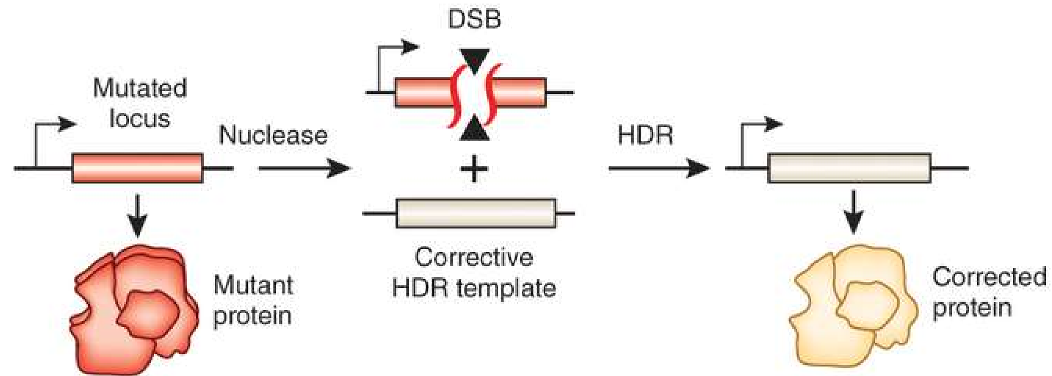
    HDR: precise, low efficiency

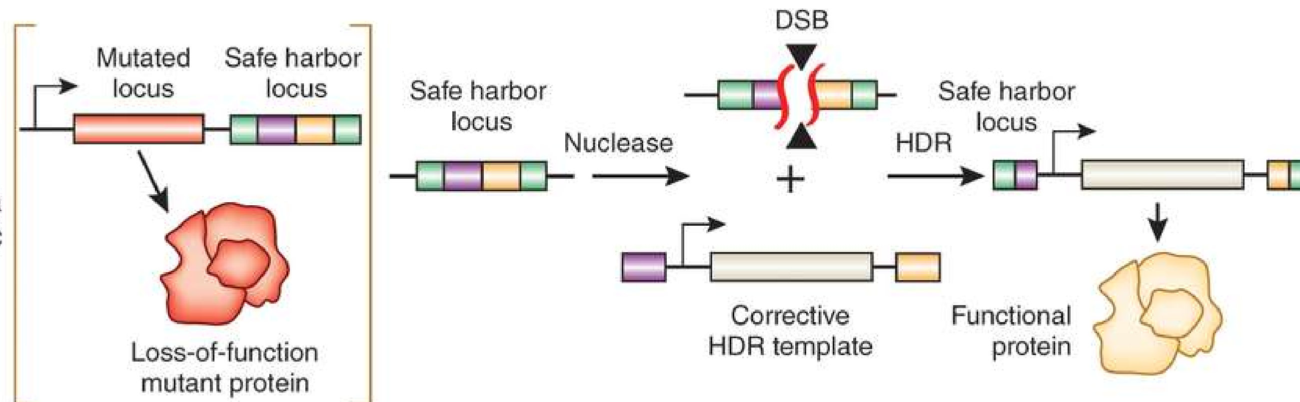
CRISPR/Cas9: NHEJ



Cox et al., Nat. Med., 2015

CRISPR/Cas9: HDR

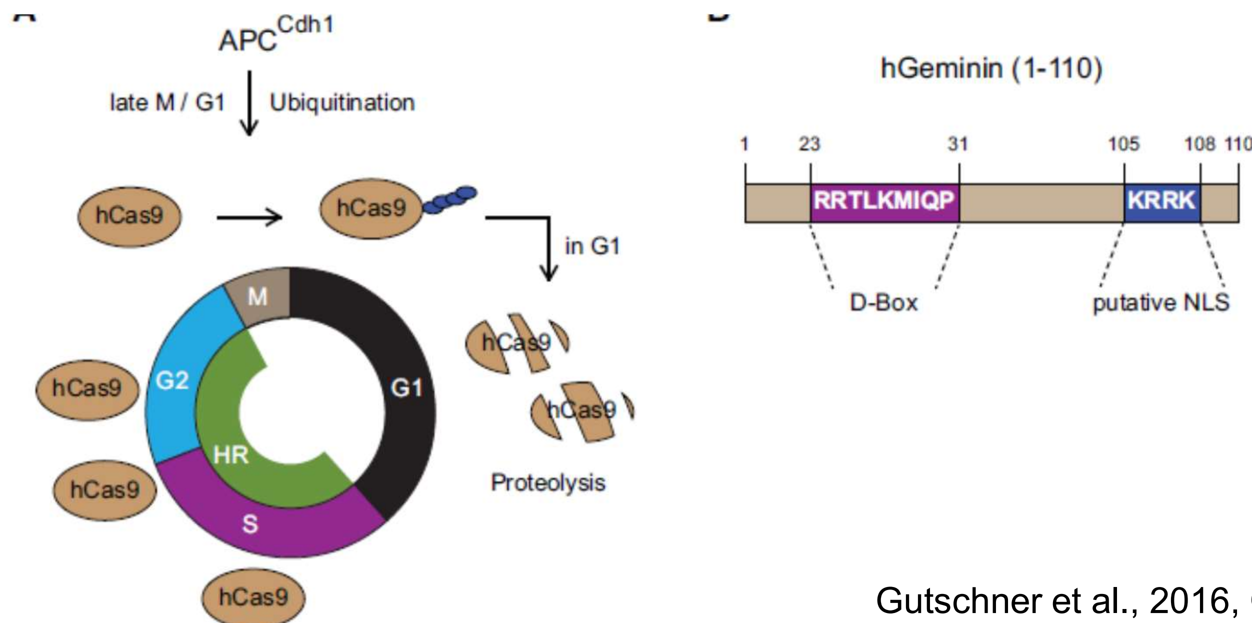




Cox et al., Nat. Med., 2015

Cas9-Geminin

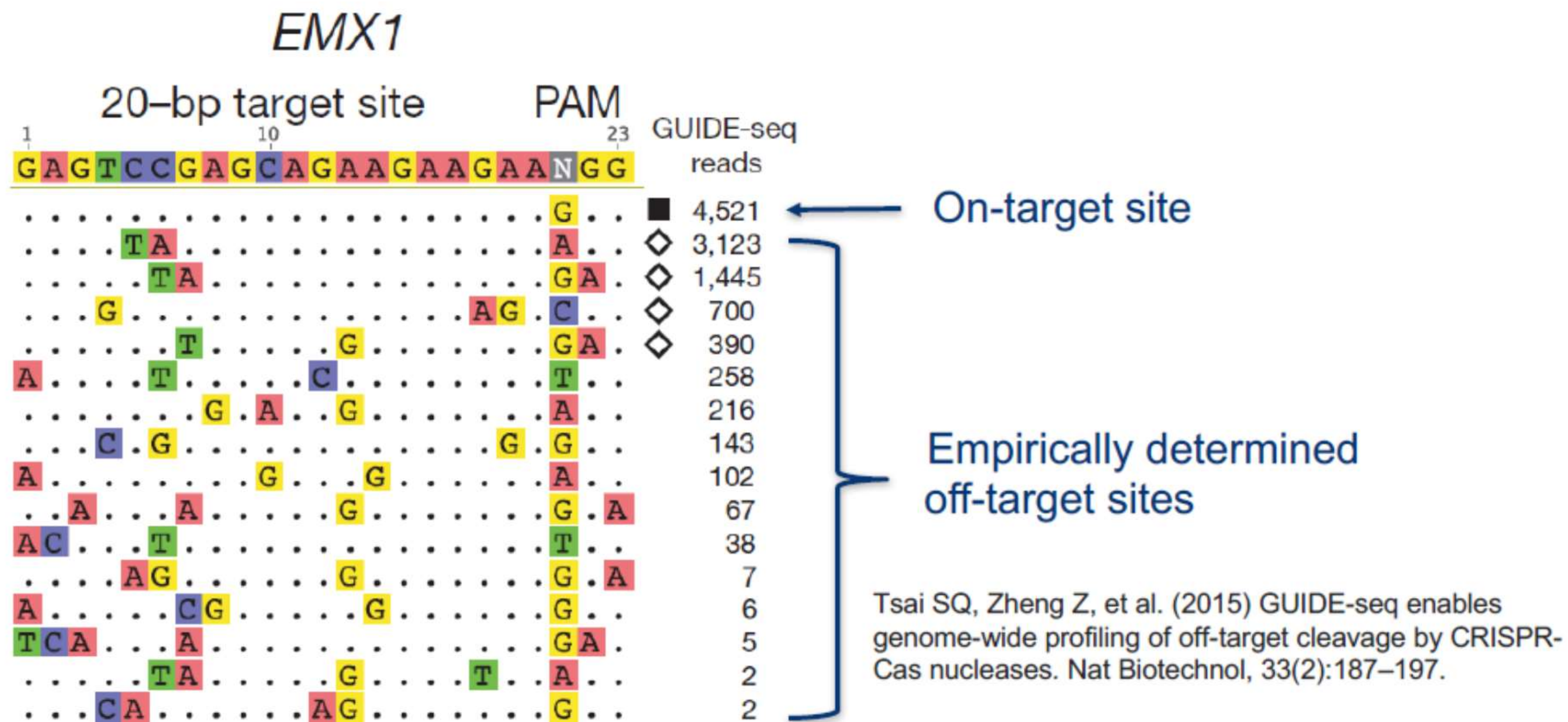
- Fusion of Cas9 to the N-term of human Geminin converted CAs9 into a substrate for the E3 ubiquitin ligase complex APC/Cdh1.
- time specific expression with low levels in G1 but high expression in S/G2/M -> Increase in HDR vs NHEJ



Gutschner et al., 2016, Cell Reports

Off-targets

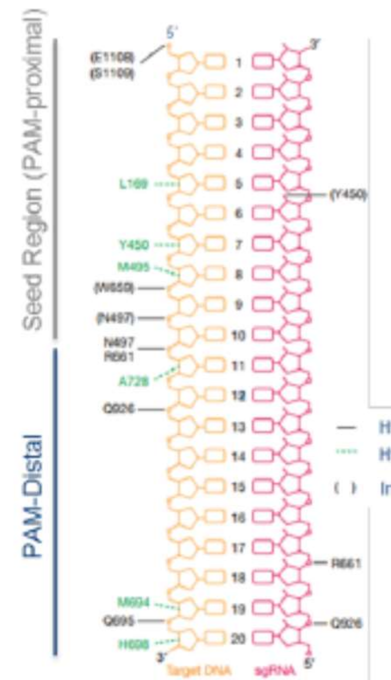
- Cas9 can cut similar sequences in the genome
- DSB in unwanted locations can be detrimental for the cells



How to minimize off-targets

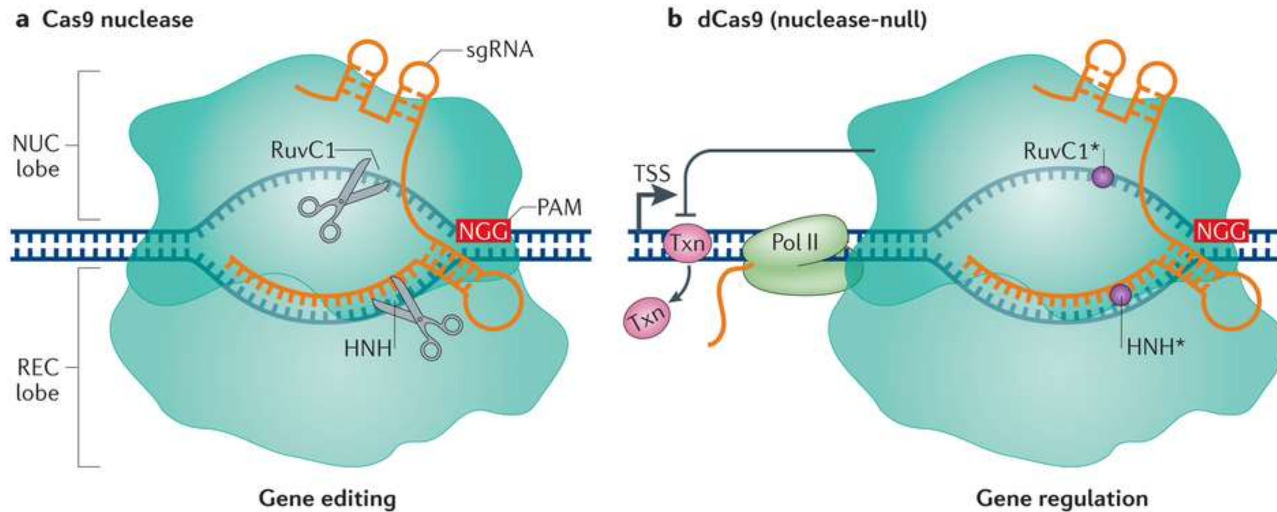
- Limit Cas9 presence in the cell (delivery method)
- Use engineered Cas9

- eSpCas9
  - Slaymaker IM, Gao L, et al. (2016) Rationally engineered Cas9 nucleases with improved specificity. Science, 351(6268):84–88.
- SpCas9-HF1
  - Kleinstiver BP, Pattanayak V, et al. (2016) High-fidelity CRISPR–Cas9 nucleases with no detectable genome-wide off-target effects. Nature, 529(7587):490–495.



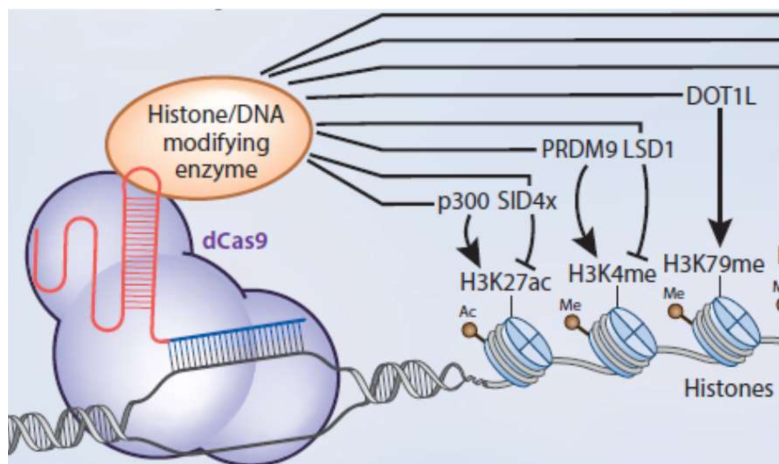
What can we do with a “dead” Cas9?

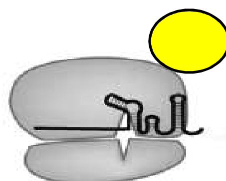
Transcriptional repression via steric hindrance

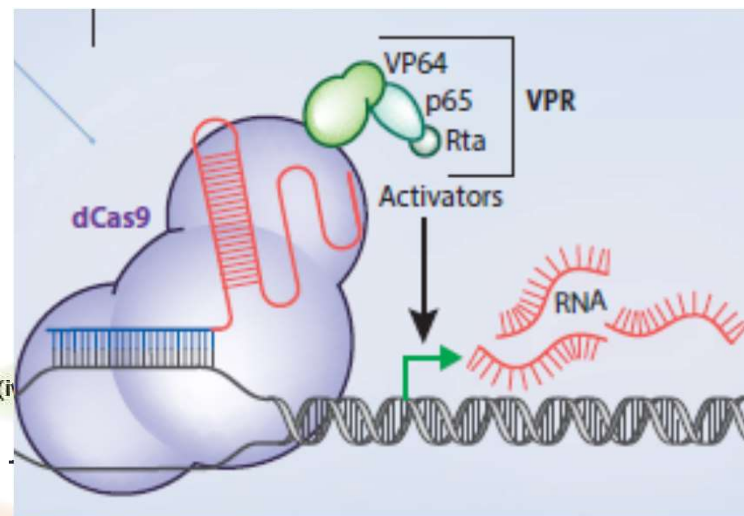


Antonia A, 2016

dCas9 applications







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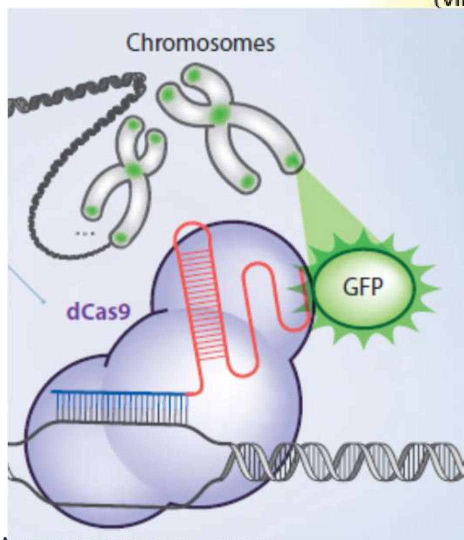
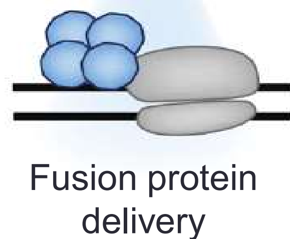
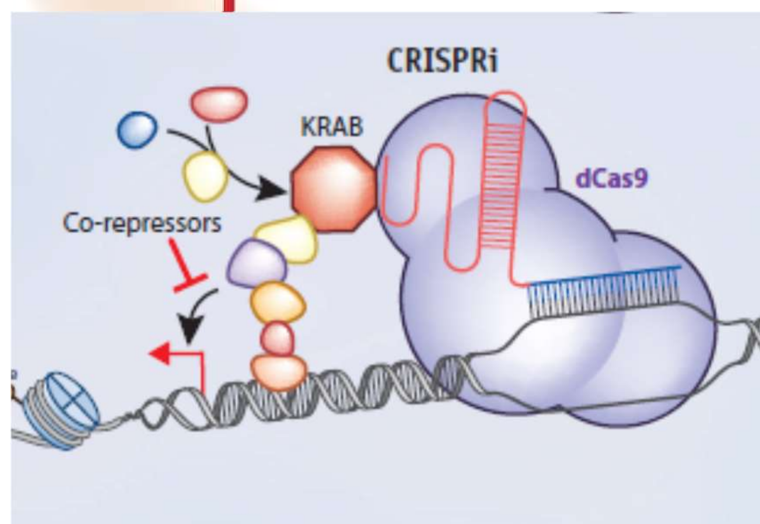




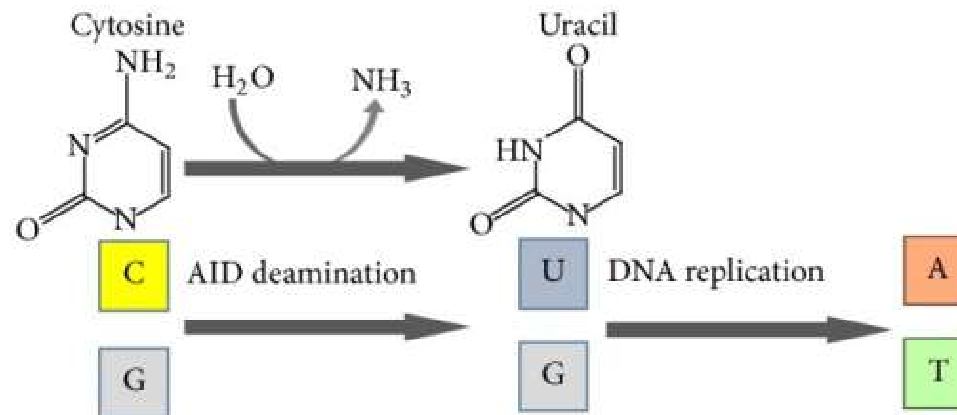
Fusion protein delivery



Barrangou, Nat Biotechnol, 2015

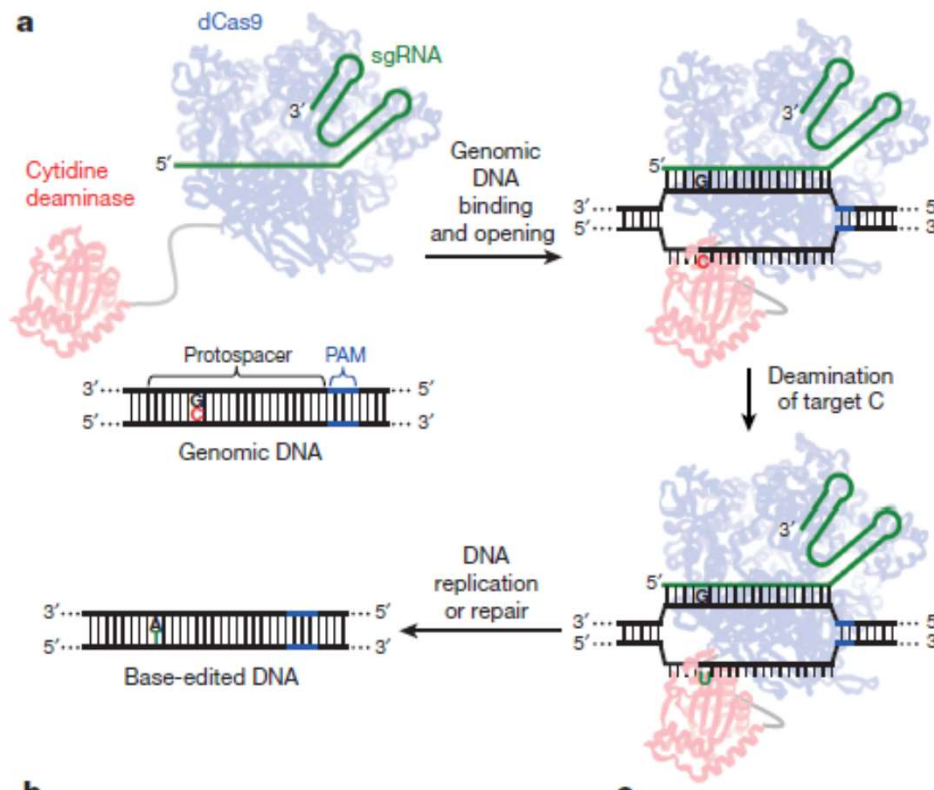
Base Editors

- Genome editing most of the time looks like genome mutilation.
- Cells don't like DSB
- Cutting the DNA can be messy (genomic rearrangement, off target…)



Base Editors

- dCAS9 + APOBEC1 = first Base editor



Cytidine deamination by a tethered APOBEC1 enzyme (red) converts the single-stranded target C→U. The resulting G:U heteroduplex can be permanently converted to an A:T bp following DNA replication or DNA repair

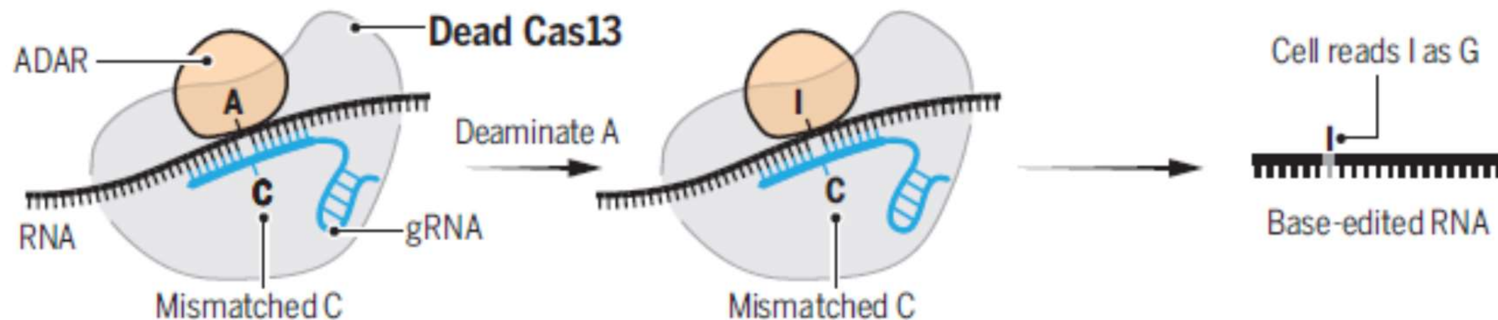
Base Editors

- New fusion proteins available for A/T to G/C edit!

Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage

Nicole M. Gaudelli[1,2,3], Alexis C. Komor[1,2,3]†, Holly A. Rees[1,2,3], Michael S. Packer[1,2,3]†, Ahmed H. Badran[1,2,3],
David I. Bryson[1,2,3]† & David R. Liu[1,2,3]

- RNA base editors were developed



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Conclusions

- CRISPR/Cas9 is rapidly changing the way we study genomes and what we can do with them
- It's a versatile tool that can be adapted for different genomic applications
- It shows promising results for therapeutic applications
- It needs to be improved in terms of efficacy and safety for therapeutic application in humans