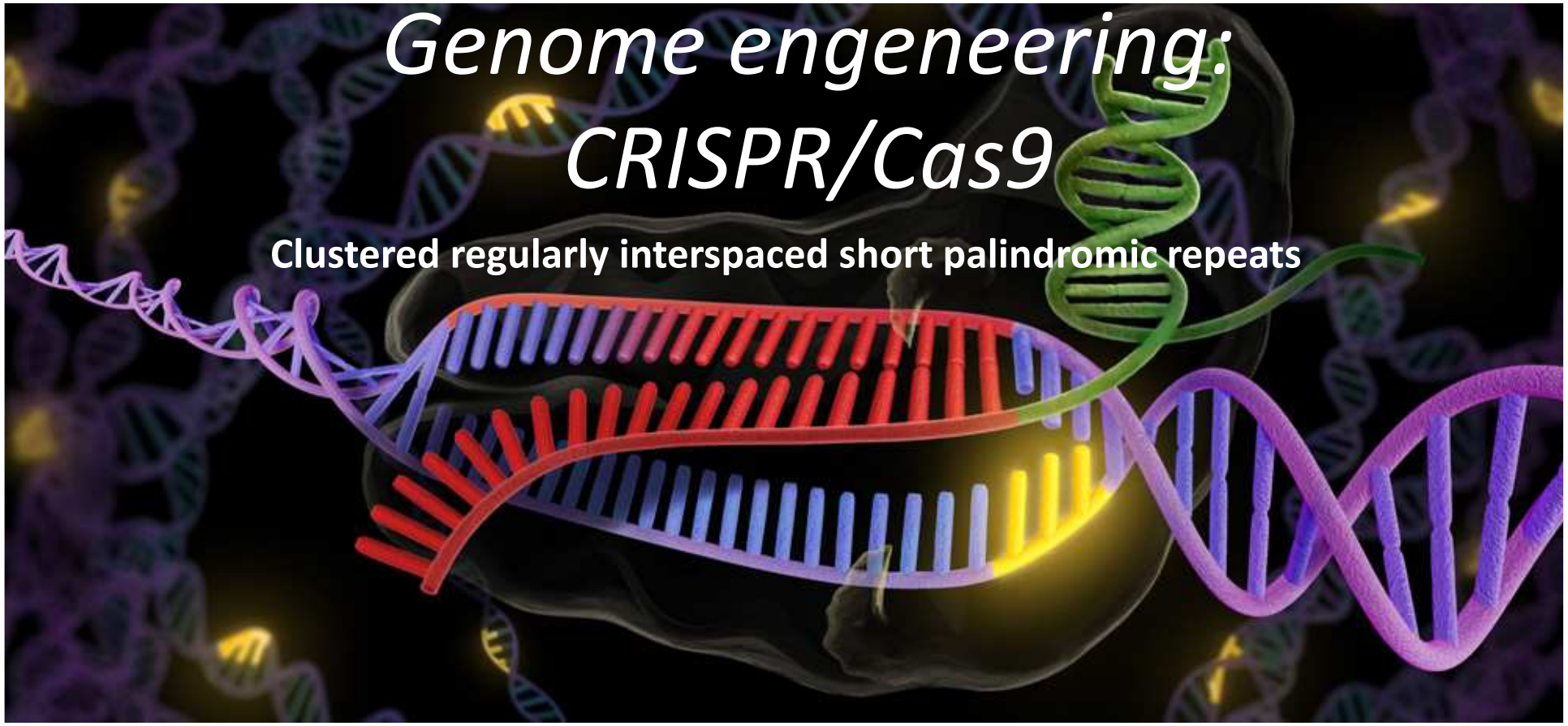
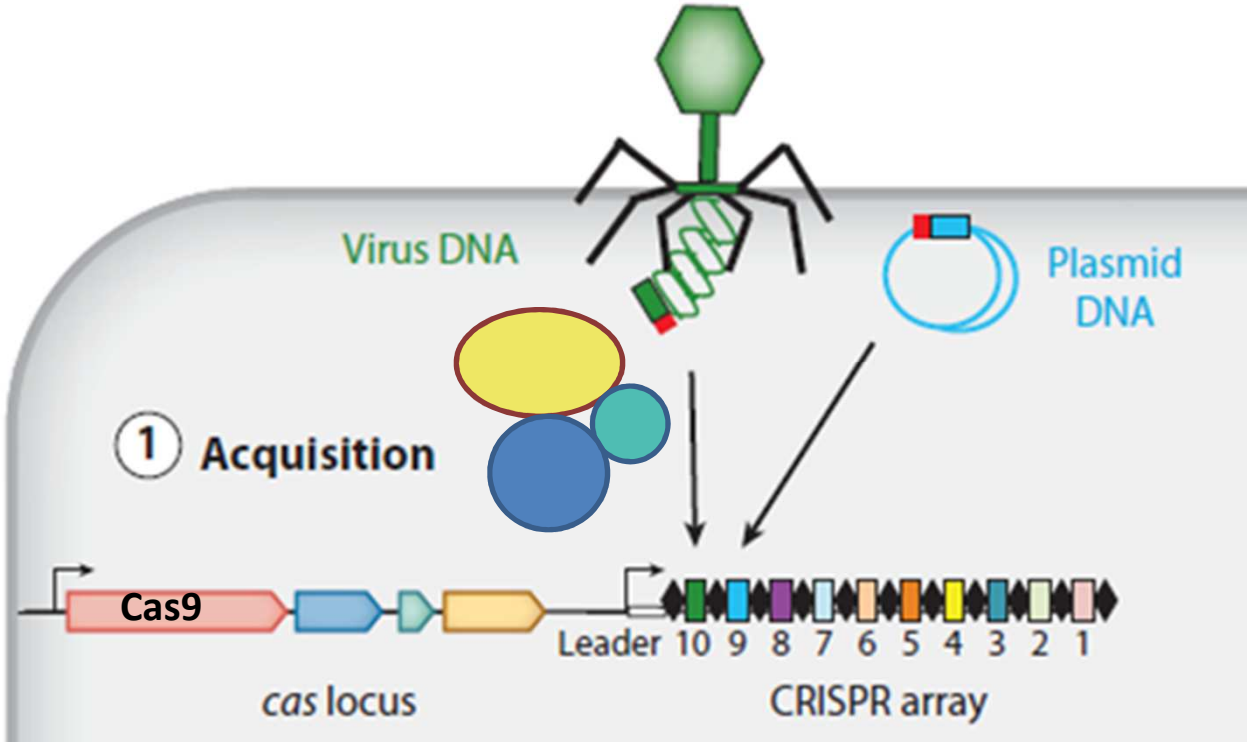


Laurea Magistrale Scienze Biomolecolari dell'Evoluzione

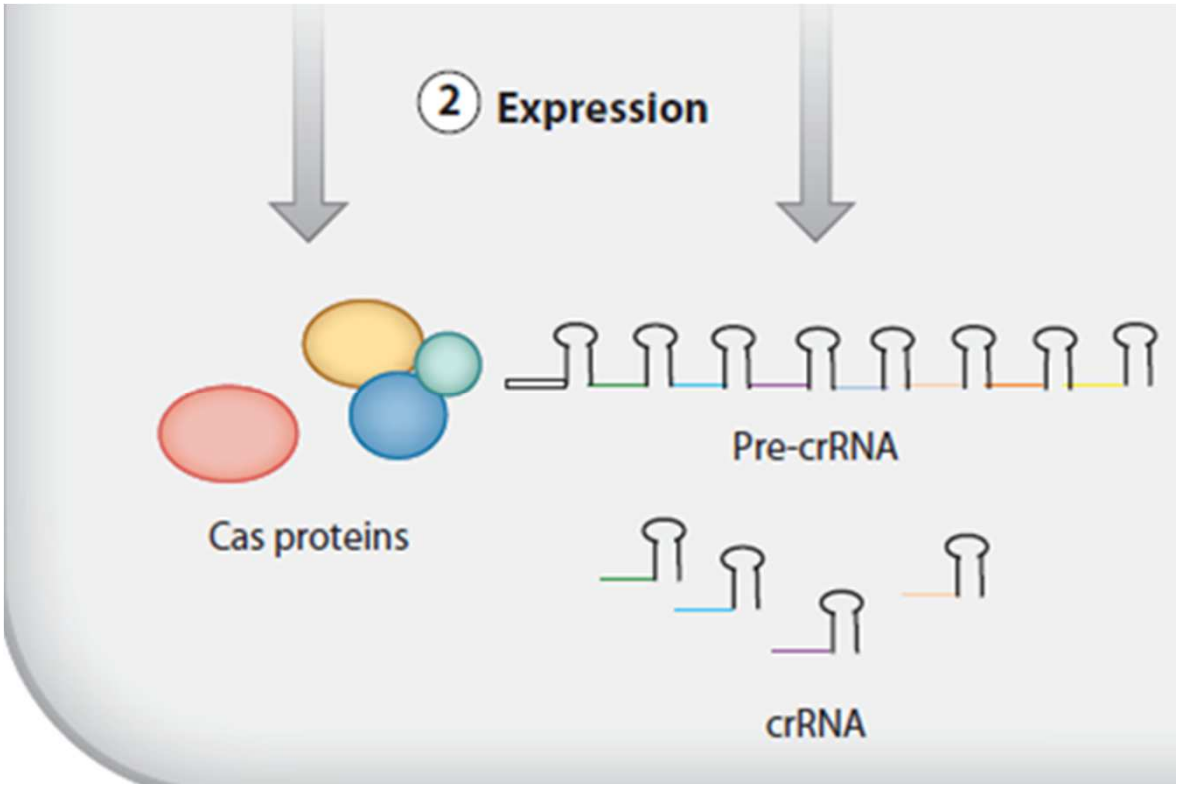
Corso di Macromolecole Biologiche

Immune Adaptative System



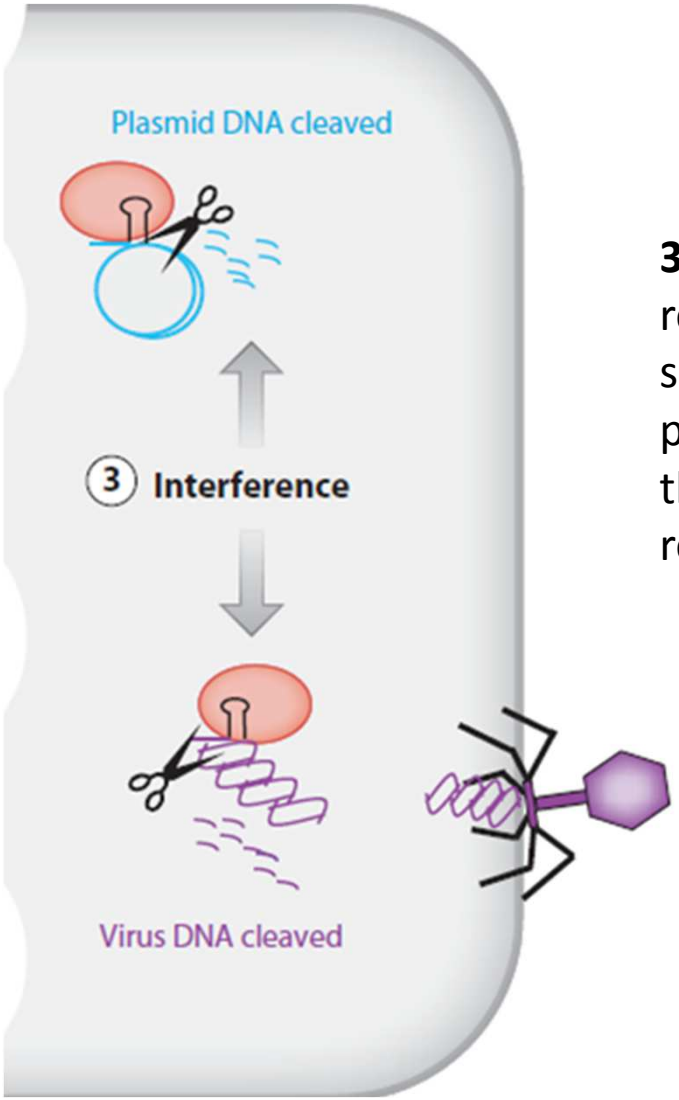
1. **Acquisition:** Invasive **Virus** or **Plasmid** DNA is cleaved by Cas proteins and it is inserted in the CRISPR array between crispr **repeats.** This cut is made before a sequence named **PAM** that is naturally present Viral or Plasmid DNA.

Immune Adaptative System



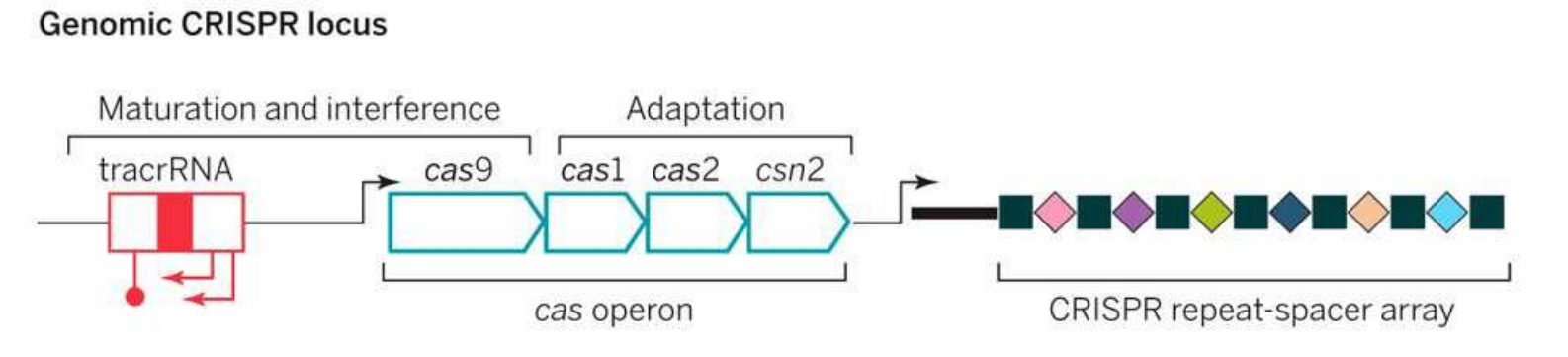
2. **Expression:** CRISPR array is transcripted and the single molecule of RNA (Pre-crRNA) maturated in different crRNA that are specific for target sequences present in Virus or Plasmidic DNA from the first contact.

Immune Adaptative System



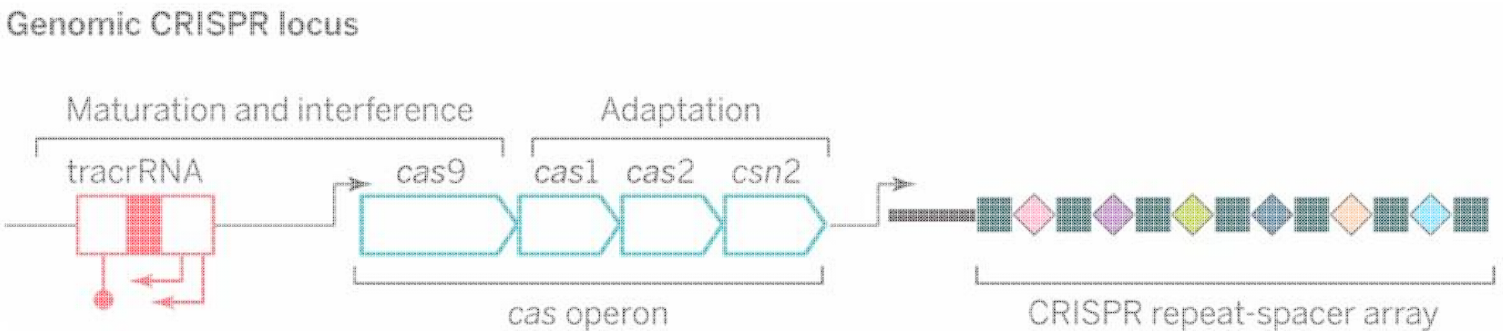
3) Interference: The crRNA recognizes the target of a second infection of the pathogen and Cas9 degradate the Viral or Plasmidic DNA after recognition

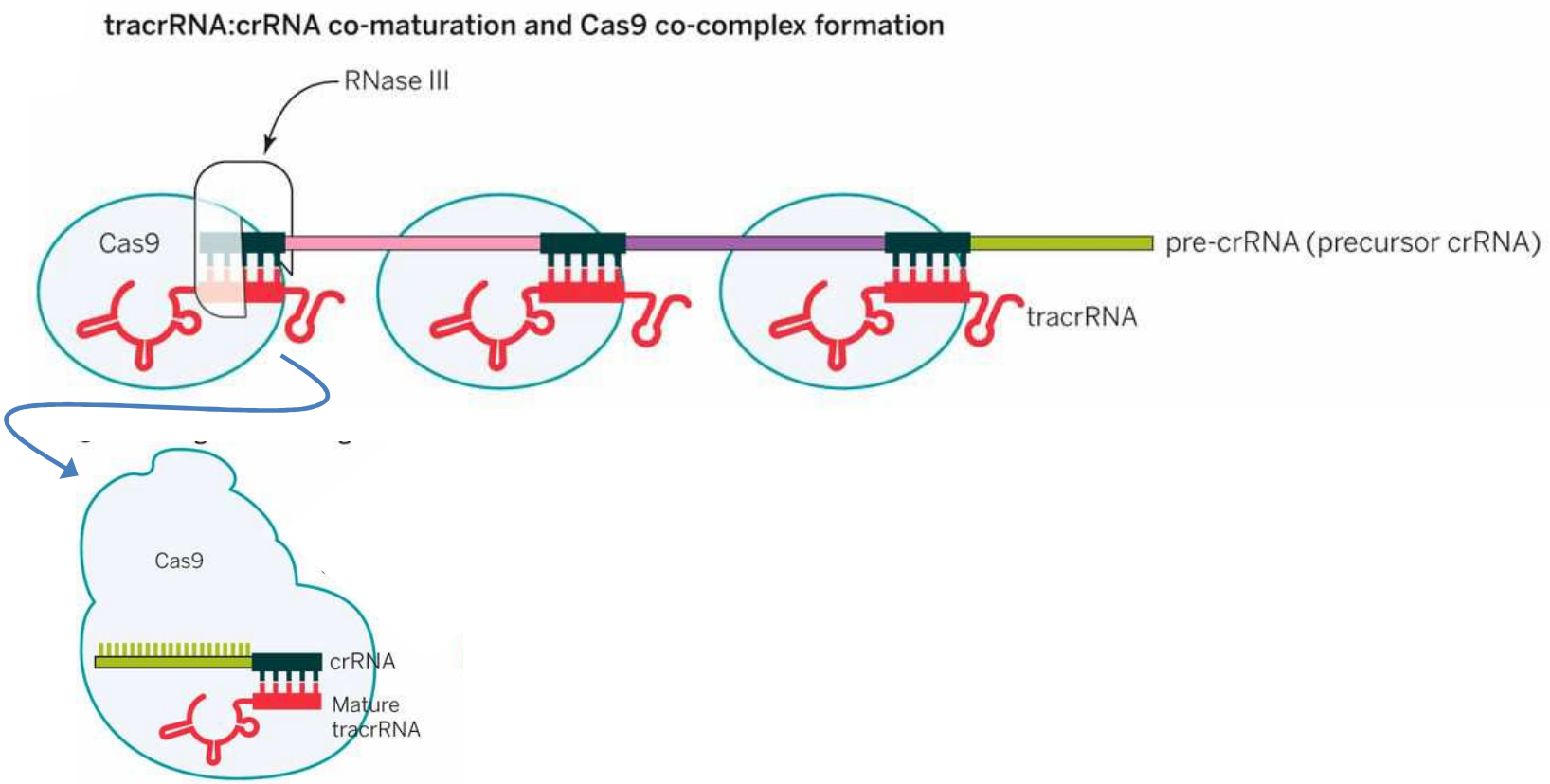
Inside the mechanism:



Viral DNA from previous infections

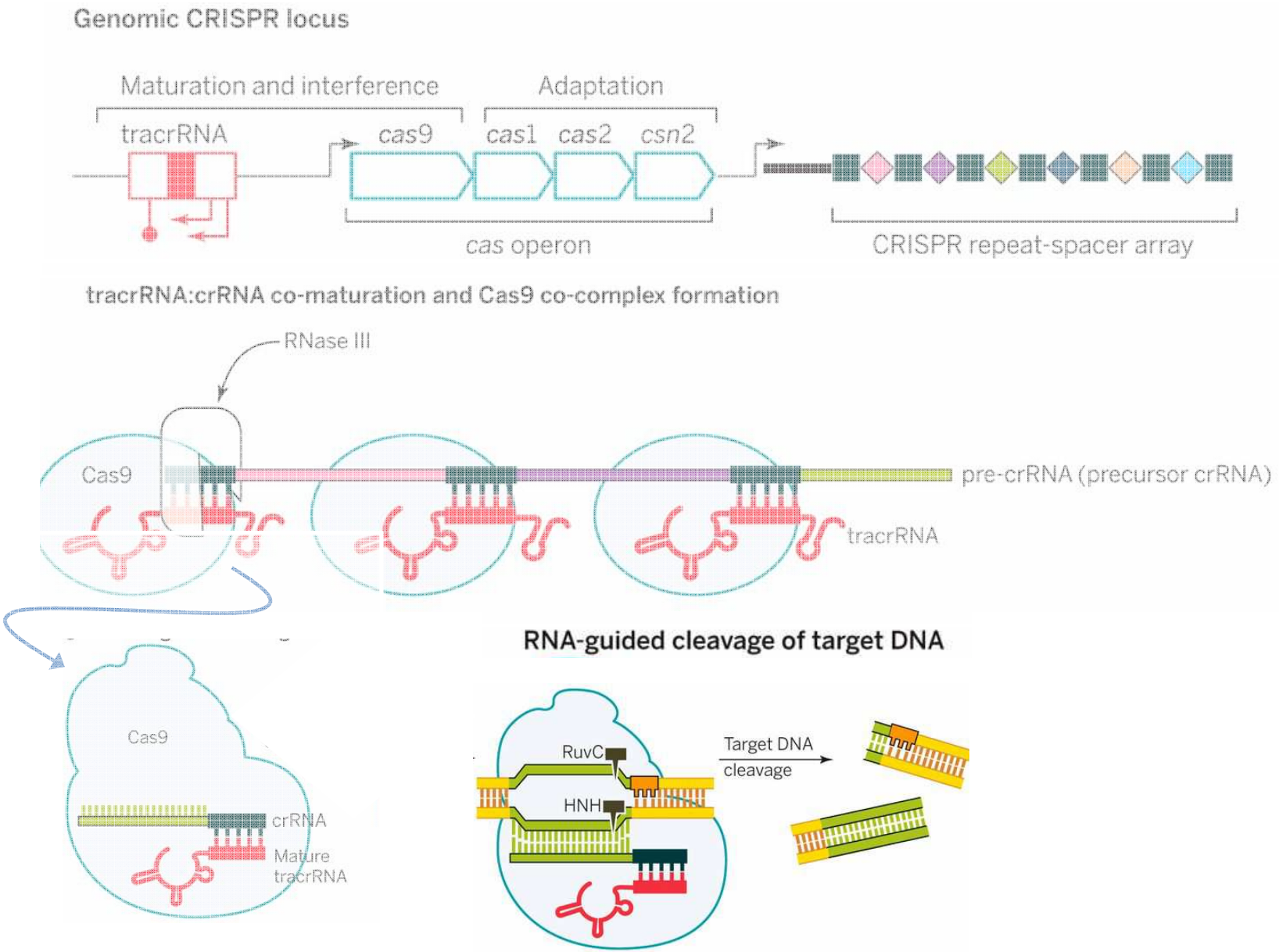
Inside the mechanism:



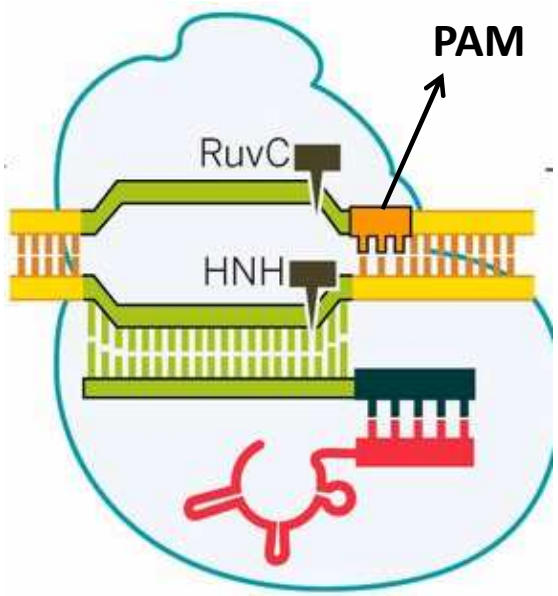


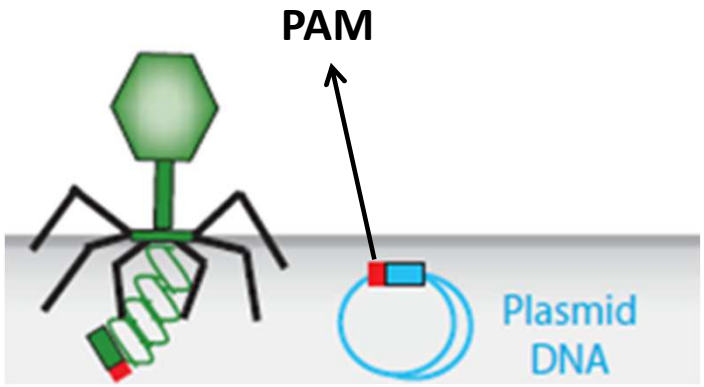
Mature complex

Inside the mechanism:



Protospacer Adjacent Motif

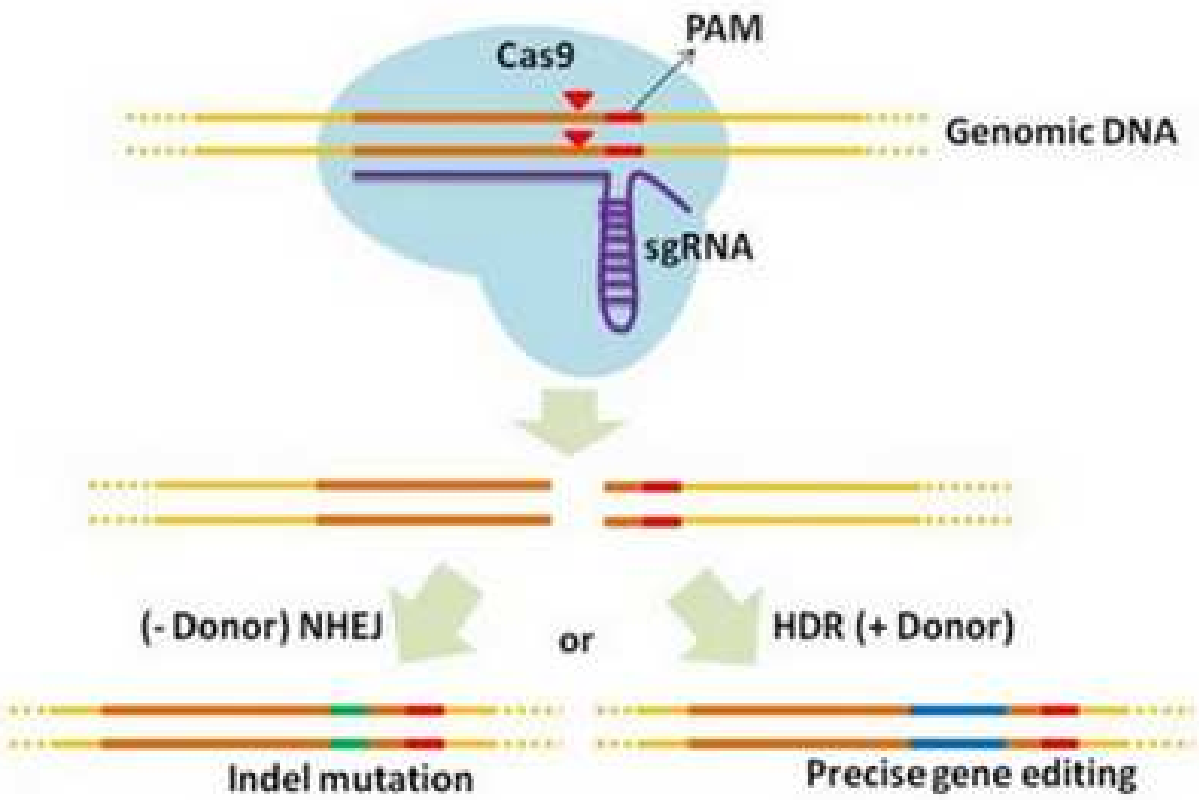




Protospacer adjacent motif (**PAM**)
PAM is a component of the invading virus or plasmid, but is not a component of the bacterial CRISPR locus.

Is an essential targeting component which distinguishes bacterial *self* from *non-self DNA*, thereby preventing the CRISPR locus from being targeted and destroyed by nuclease.
The canonical PAM of *S. Pyogenes* is the sequence **5'-NGG-3'.**

Engineered system



Doudna et al., 2014

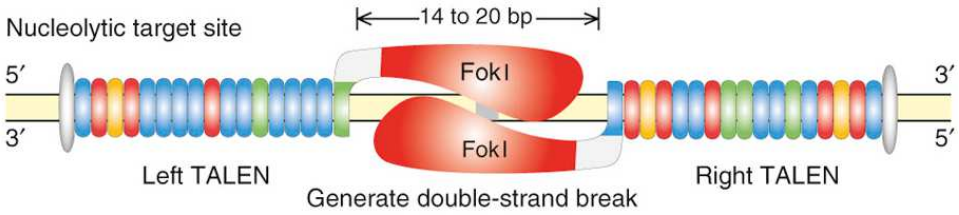
Genome Engeneering and Disease

Disease type	Nuclease platform	Therapeutic strategy
Hemophilia B	ZFN	HDR-mediated insertion of correct gene sequence
HIV	ZFN and CRISPR	NHEJ-mediated inactivation of CCR5
Duchenne muscular dystrophy (DMD)	CRISPR and TALEN	NHEJ-mediated removal of stop codon, and HDR-mediated gene correction
Hepatitis B virus (HBV)	TALEN and CRISPR	NHEJ-mediated depletion of viral DNA
SCID	ZFN	HDR-mediated insertion of correct gene sequence
Cataracts	CRISPR	HDR-mediated correction of mutation in mouse zygote
Cystic fibrosis	CRISPR	HDR-mediated correction of CFTR in intestinal stem cell organoid
Hereditary tyrosinemia	CRISPR	HDR-mediated correction of mutation in liver

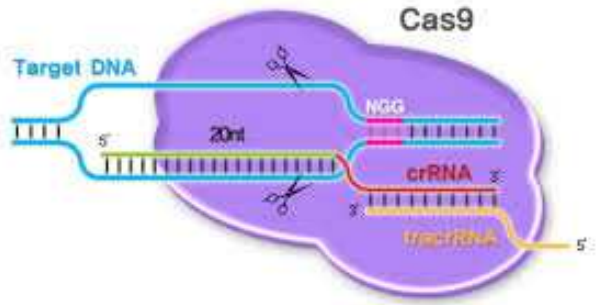
Cox, Platt and Zhang, 2015

Genome Editing

Trascription Activator Like Effector Nucleases (TALEN)



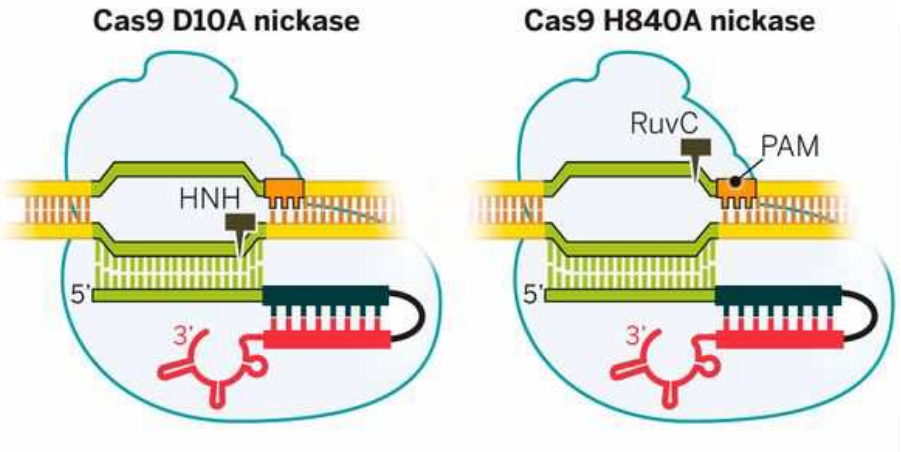
CRISPR/Cas9 system



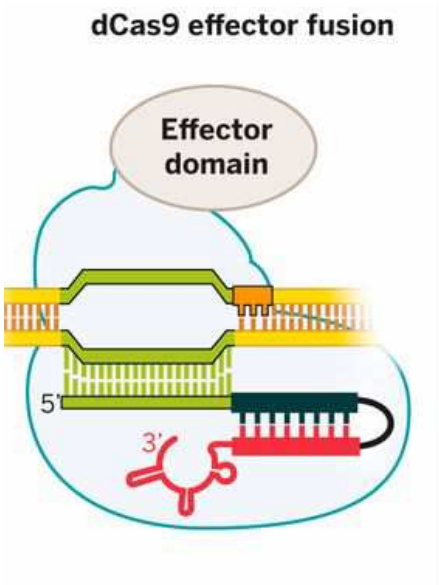
Name	Components	Mechanism	Specifity	Rapidly generation
TALEN	Non specific DNA cleaving nuclease fused to a DNA specific genome binding domain.	Induces double strand breaks in target DNA	Highly specific	Feasible but technically challenging
CRISPR/Cas9	20nt crRNA fused to tracrRNA and Cas9 endonuclease	Induce double strand breaks in target DNA (wt Cas9)	Some **off target** effect can be reduced by selection of unique crRNA sequences	Yes – requires simple 20nt sequence targeting a gene

Cas9 can be engeneered:

To be able to create nicks in a single strand



Can be fused to a Transcriptional Activator Domain



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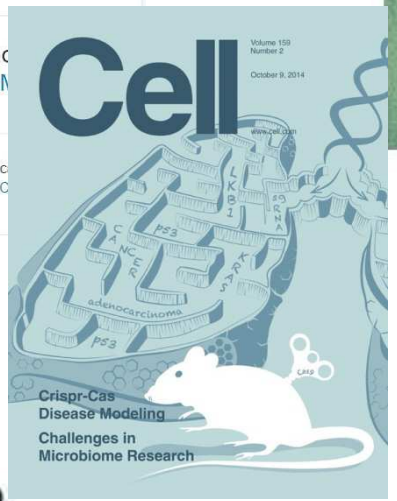

10/20/2015

THE PROMISING AND PERILOUS SCIENCE OF GENE EDITING

Dan Kedmey

REVIEW SUMMARY

GENOME EDITING

The new frontier of genome engineering with CRISPR-Cas9

Jennifer A. Doudna* and Emmanuelle Charpentier*

nature biotechnology

REVIEW

CRISPR-Cas systems for editing, regulating and targeting genomes

Jeffry D Sander[1,2] & J Keith Joung[1,2]

Targeted genome editing using engineered nucleases has rapidly gone from being a niche technology to a mainstream method used by many biological researchers. This widespread adoption has been largely fueled by the emergence of the clustered, regularly interspaced, short palindromic repeat (CRISPR) technology, an important new approach for generating RNA-guided nucleases, such as Cas9, with customizable specificities. Genome editing mediated by these nucleases has been used to rapidly, easily and efficiently modify endogenous genes in a wide variety of biomedically important cell types and in organisms that have traditionally been challenging to manipulate genetically. Furthermore, a modified version of the CRISPR-Cas9 system has been developed to recruit heterologous domains that can regulate endogenous gene expression or label specific genomic loci in living cells. Although the genome-wide specificities of CRISPR-Cas9 systems remain to be fully defined, the power of these systems to perform targeted, highly efficient alterations of genome sequence and gene expression will undoubtedly transform biological research and spur the development of novel molecular therapeutics for human disease.

Ethics?

CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes

Puping Liang , Yanwen Xu , Xiya Zhang , Chenhui Ding , Rui Huang, Zhen Zhang, Jie Lv, Xiaowei Xie, Yuxi Chen and 7 more

nature International weekly journal of science

NATURE | NEWS

Chinese scientists genetically modify human embryos

Rumours of germline modification prove true — and look set to reignite an ethical debate.

David Cyranoski & Sara Reardon

22 April 2015

nature OUTLOOK

3 December 2015
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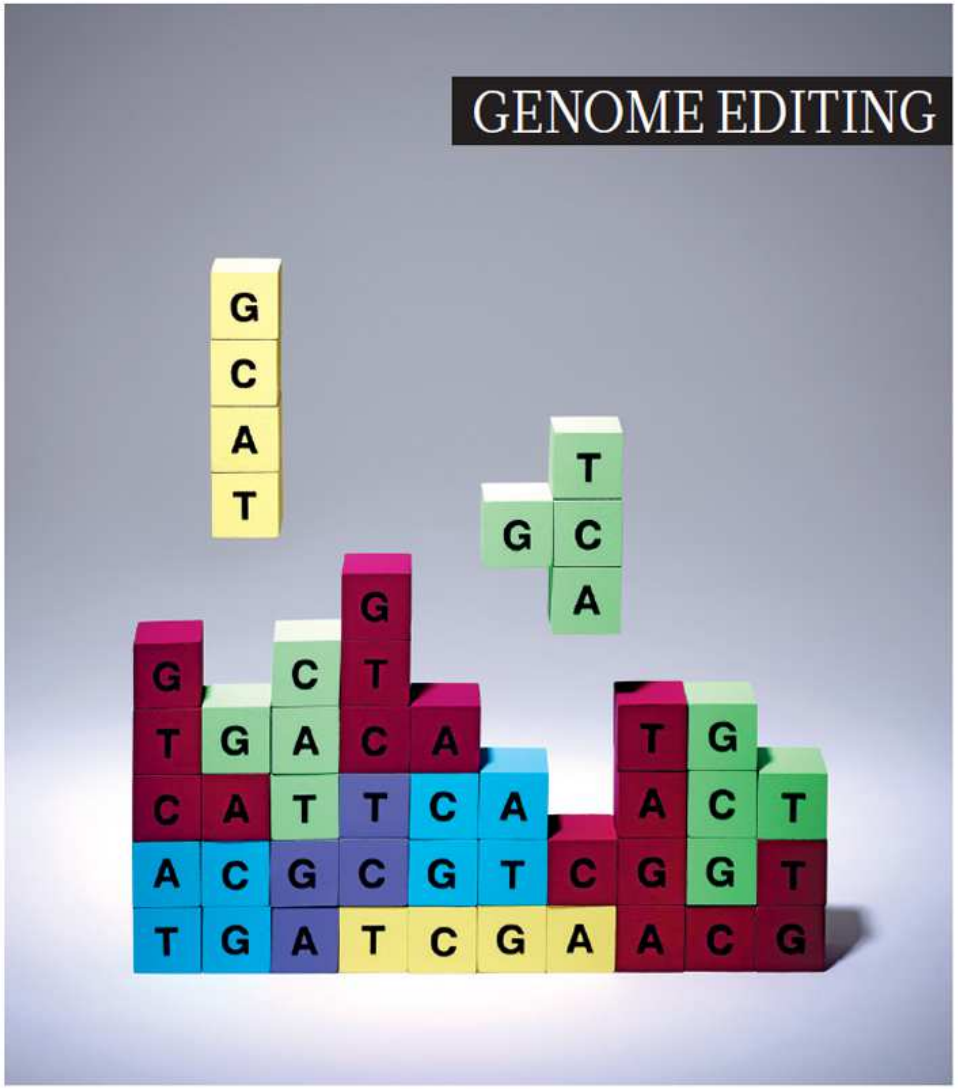
GENOME EDITING



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