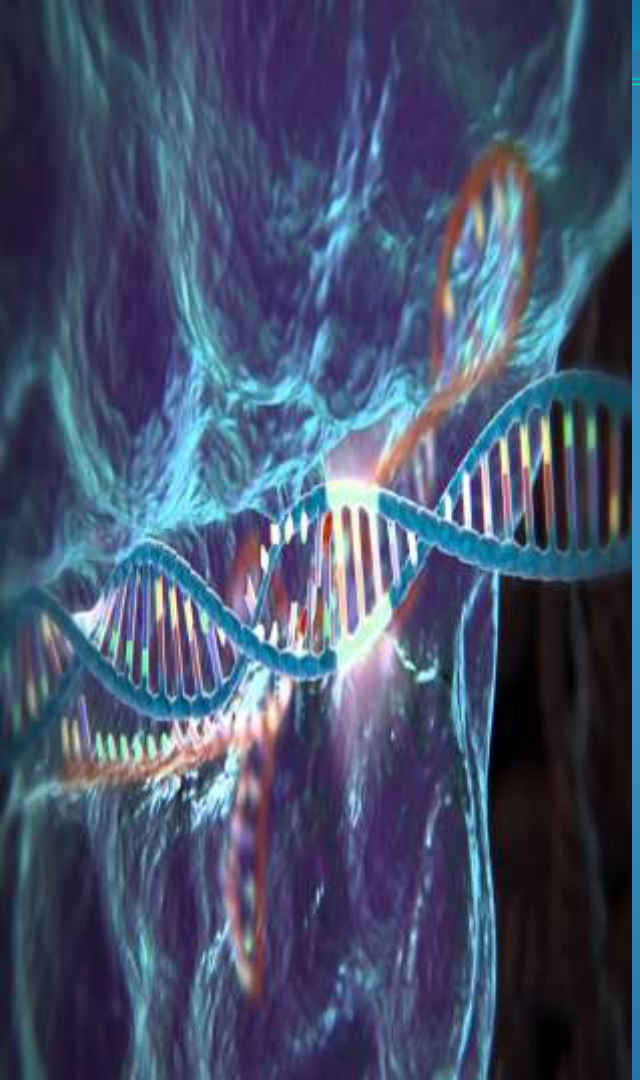


CRISPR- Cas9

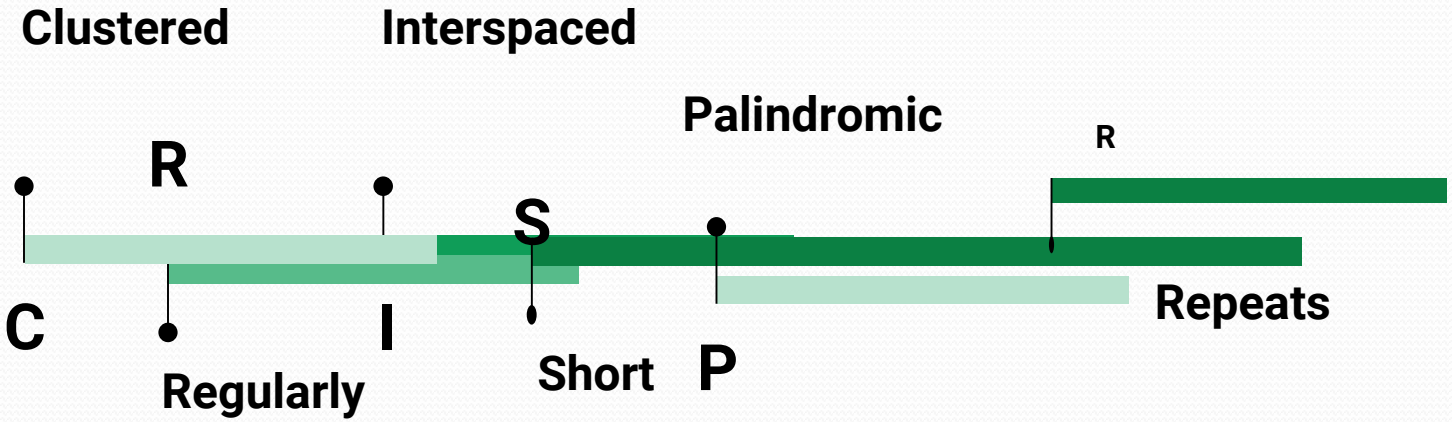
-presented by
Dr.Neha
Baldeep Chani Talwar &
Dr.Remya Pushparajan Subha

DOES YOUR HEART CRY WHEN YOU SEE THESE?

IS IT SO TODAY?

CRISPR & CAS-9 are here…

What is CRISPR?



Clustered Regularly Interspaced Short Palindromic Repeats

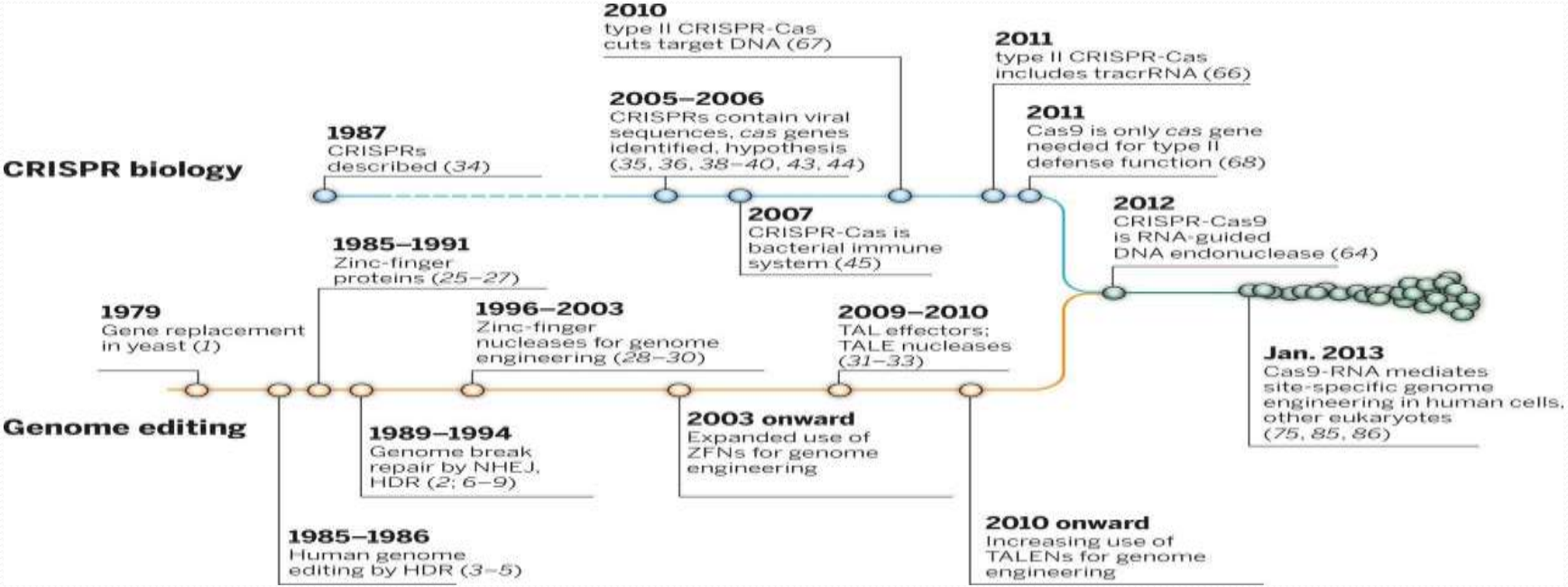
CRISPR

- **CRISPR** (/ˈkrɪspər/) is a family of DNA sequences in bacteria.
- The sequences contain snippets of DNA from viruses that have attacked the bacterium.
- These snippets are used by the bacterium to detect and destroy DNA from further attacks by similar viruses

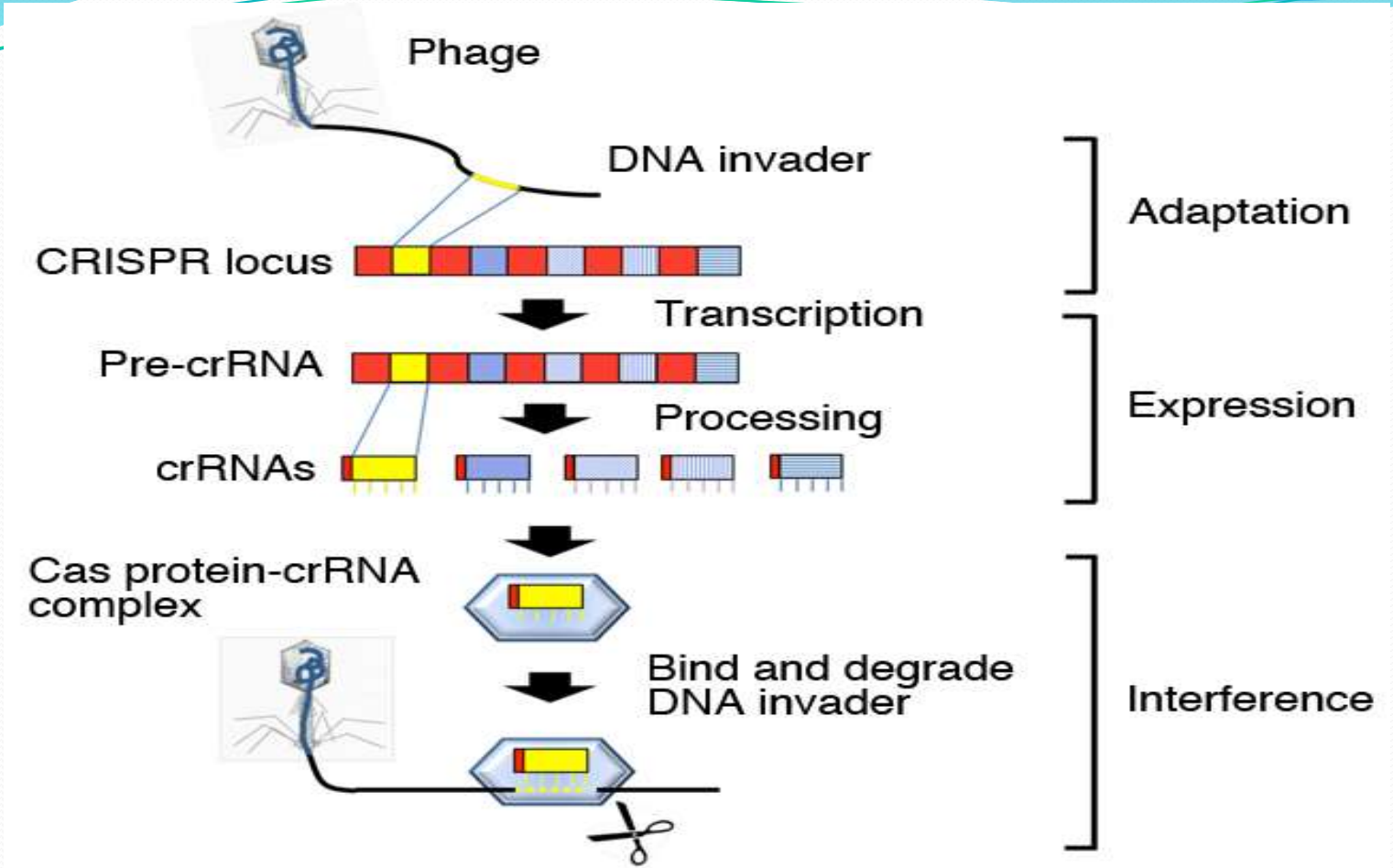
- a palindromic repeat, the sequence of nucleotides is the same in both directions.
- Each repetition is followed by short segments of spacer DNA from previous exposures to foreign DNA (e.g., a virus or plasmid).
- Small clusters of *cas* (CRISPR-associated system) genes are located next to CRISPR sequences.

- The CRISPR/Cas system is a prokaryotic immune system that confers resistance to foreign genetic elements such as those present within plasmids and phagesthat provides a form of acquired immunity.

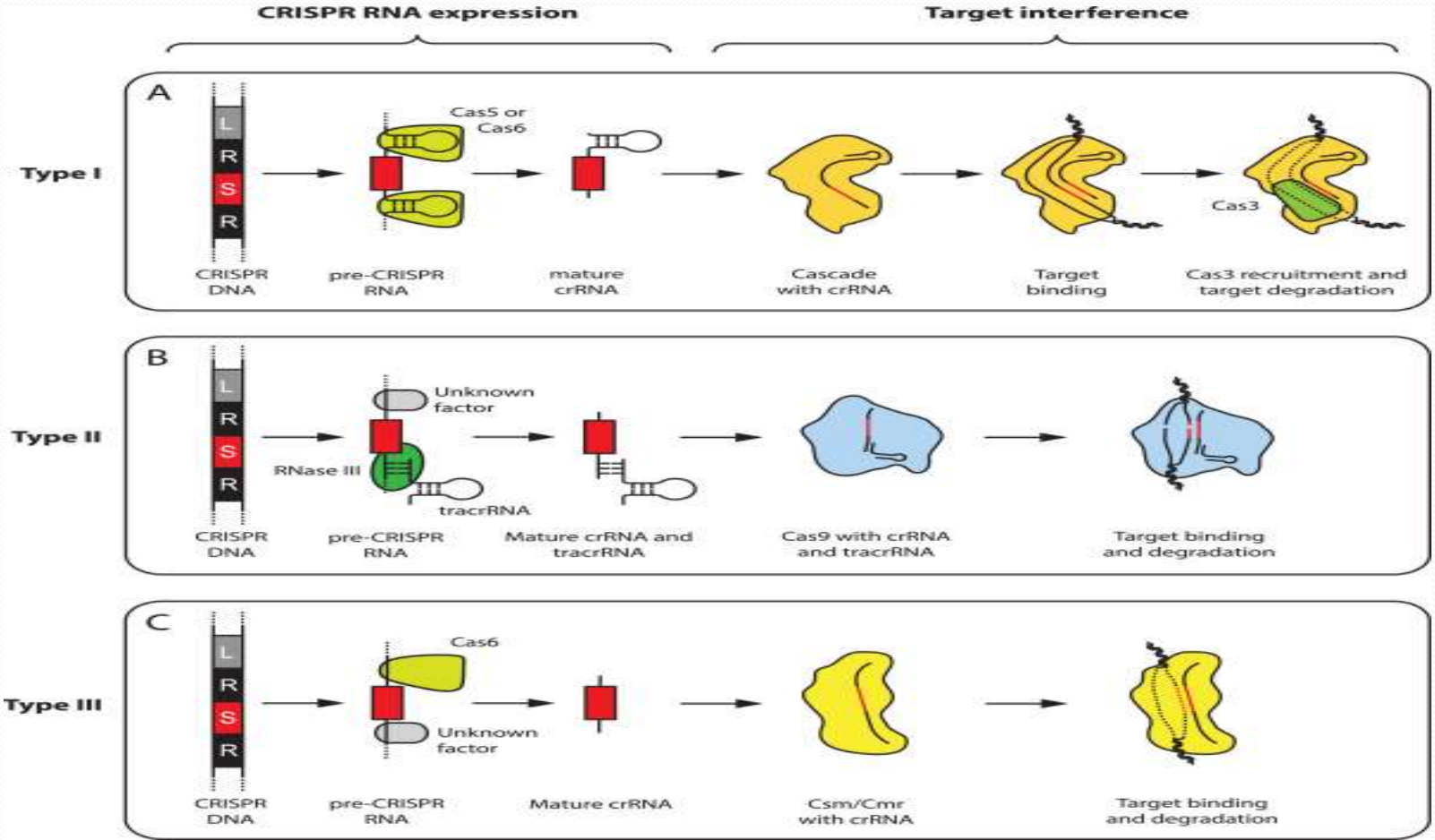
Evolution of CRISPR



CRISPR mode of action

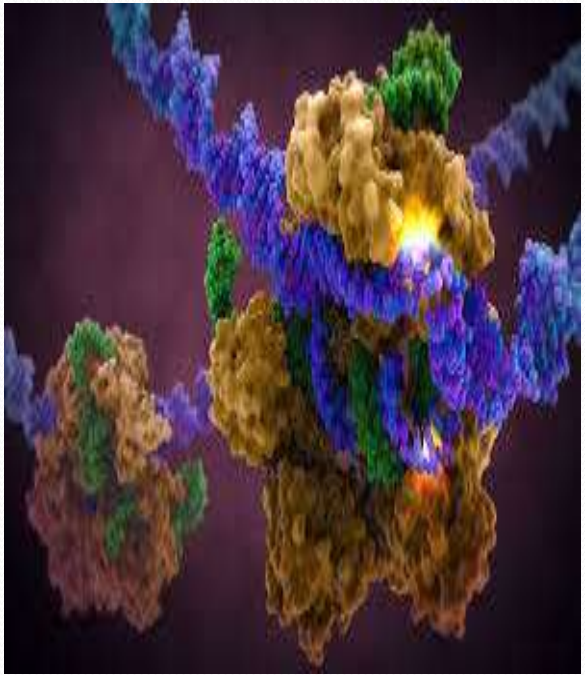


Three types of CRISPR systems

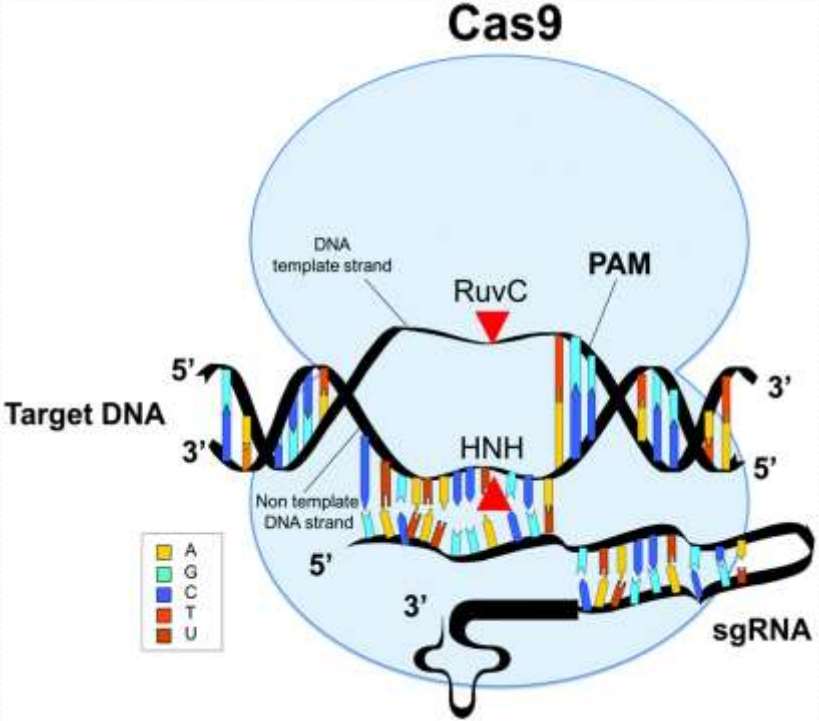


Cas-9 (**c**RISPR **a**ssociated **p**rotein 9)

- **is an RNA guided DNA endonucleases enzyme.**
- **associated with CRISPR**
- **which plays an role in adaptive immunity system, found in bacteria Streptococcus Pyogenes.**
- **involved in Type II CRISPR mechanism**



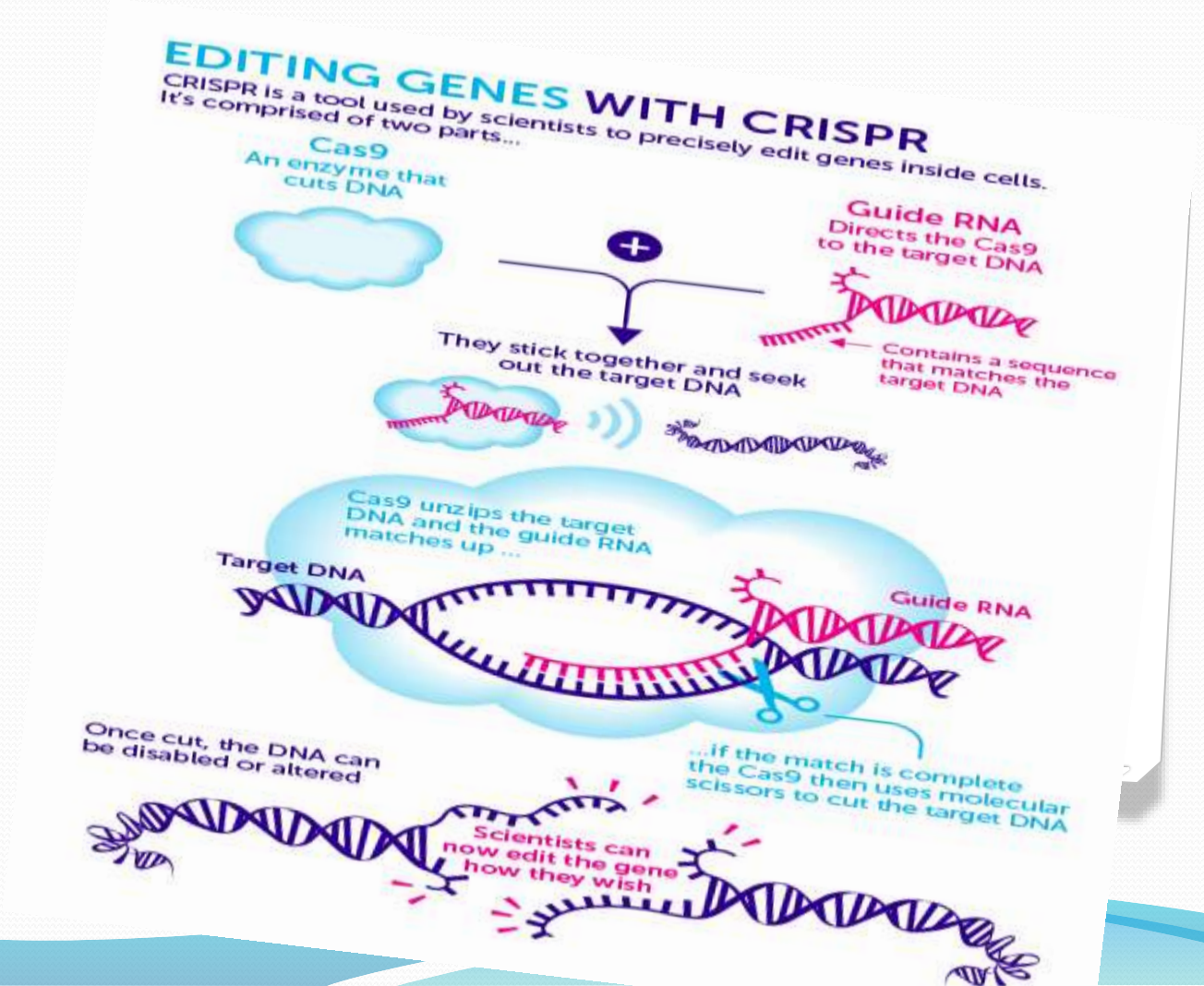
Biological structure of Cas-9



Cas9 protein has six domains-

1. **REC I-responsible for binding guide RNA**
2. **REC II-not yet well understood**
3. **Bridge Helix-(arginine-rich) is crucial for initiating cleavage activity upon binding of target DNA**
4. **PAM Interacting domain-confers PAM specificity;responsible for initiating binding to target DNA**
5. **HNH and RuvCdomains -are nuclease domains that cut single-stranded DNA. They are highly homologous to HNH and RuvC domains found in other proteins**

COMMON MODE OF ACTION:



3 types of Cas-9nucleases

Wild-type Cas9

-can site-specifically cleave double-stranded DNA, resulting in the activation of the double-strand break (DSB) repair machinery.

-insertions and/or deletions

- precise replacement mutations

Cas9D10A

- it cleaves only one DNA strand

-only nickase activity

-- target specificity when loci are targeted by paired Cas9 complexes designed to generate adjacent DNA nicks

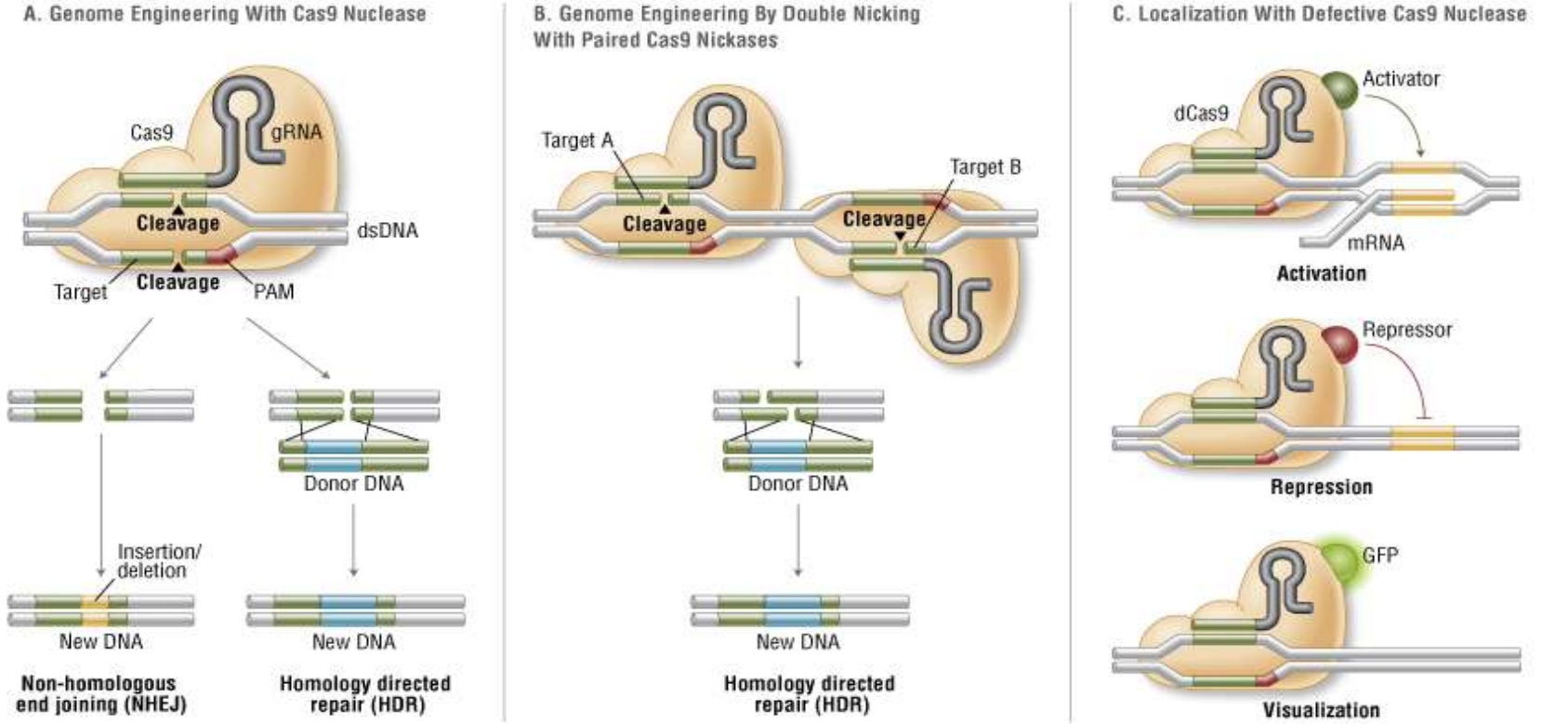
dCas9

-nuclease-deficient Cas9

-inactivate cleavage activity, but do not prevent DNA binding

-a gene silencing or activation tool

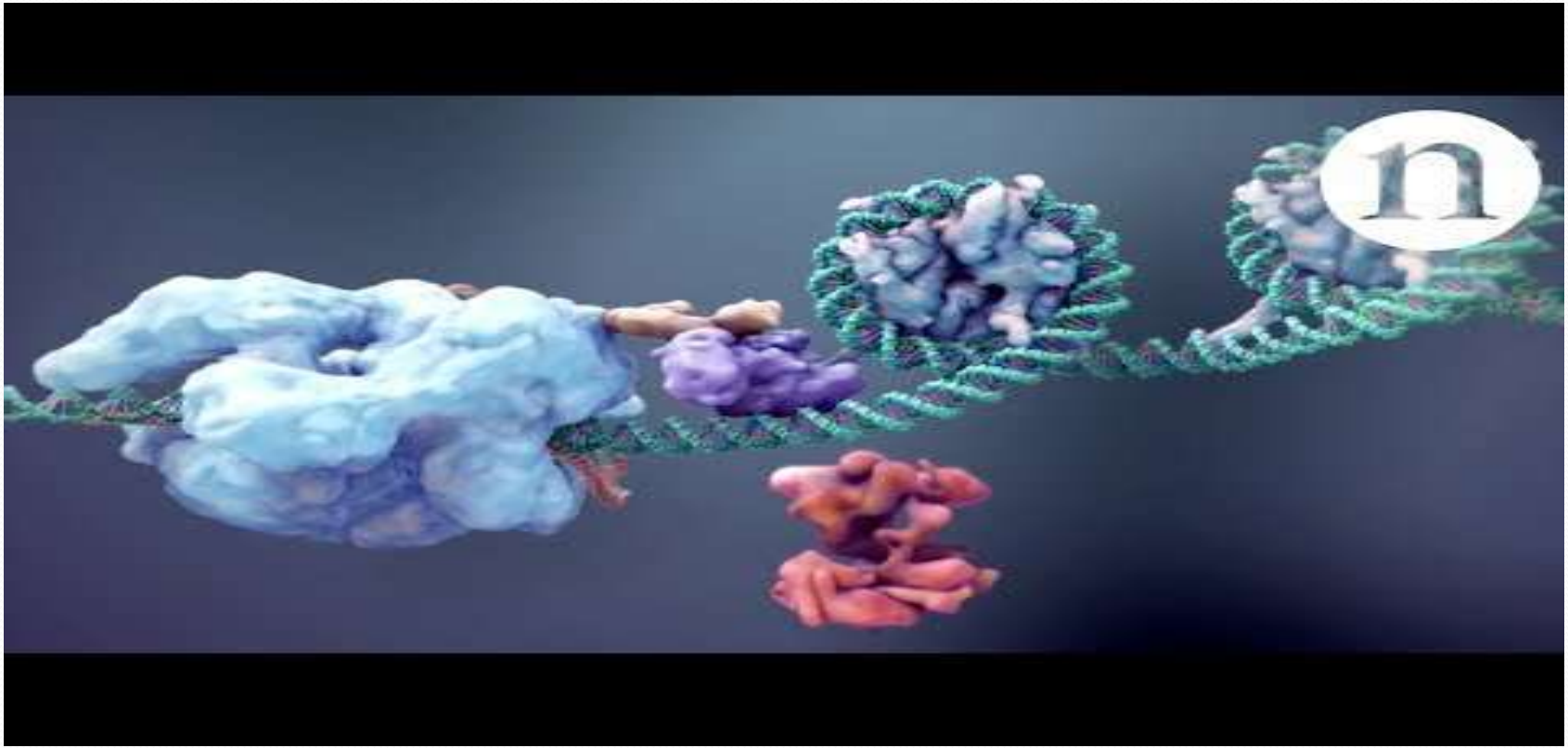
Biologic Mechanism of action of Cas9



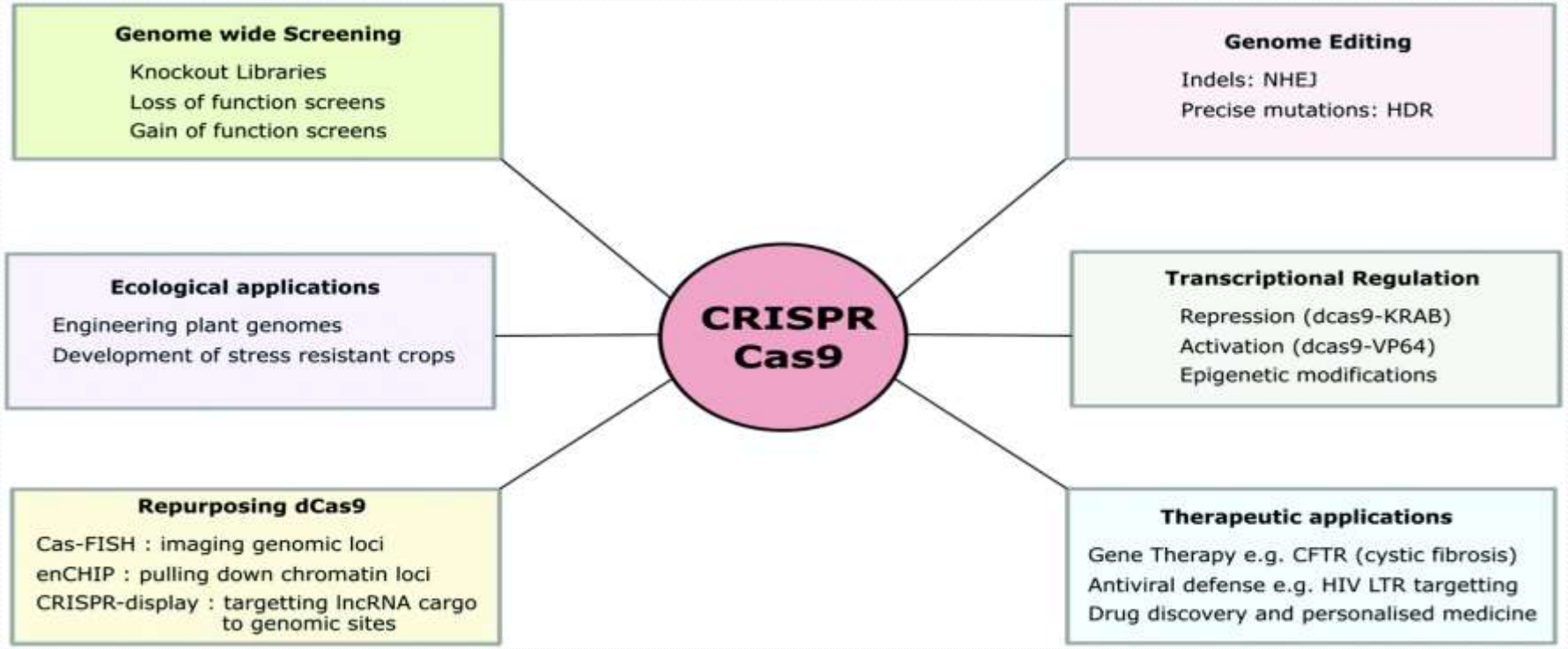
Applications:

- **Gene silencing**
- **DNA-free CRISPR-Cas9 gene editing**
- **Homology-directed repair (HDR)**
- **Transient gene silencing or transcriptional repression (CRISPRi)**
- **Transient activation of endogenous genes (CRISPRa or CRISPRon)**
- **Embryonic stem cell and transgenic animals**
- **Pooled genome-scale knockout screening**

Be clearer



Implications:



Clinical trials :

The first clinical trial involving CRISPR started in 2016.

It involved removing immune cells from people with lung cancer, using CRISPR to edit out the gene expressed PD-1, then administrating the altered cells back to the same person.

20 other trials were under way or nearly ready, mostly in China, as of 2017.

Limitations:

Targeting efficiency, or the percentage of desired mutation achieved, is one of the most important parameters by which to assess a genome-editing tool.

-T7 Endonuclease I mutation detection assay

- incidence of off-target mutations ???

-Recent improvements to the CRISPR system for reducing off-target mutations have been made through the use of truncated gRNA (truncated within the crRNA-derived sequence) or by adding two extra guanine (G) nucleotides to the 5´ end. Another method is use D10A Cas9 and two sgRNAs complementary

-How far is targeting efficiency in humans???

-CRISPR Design Tool-webbased tools to facilitate the identification of potential CRISPR target sites and assess their potential for off-target cleavage.

- Potential to edit germline? (reproductive) cells.
- Because any changes made in germline cells will be passed on from generation to generation.
- Ethical implications???

CRISPR PATENT DISPUTE HIGHLIGHTS

August 2012 — Jinek et al. (2012) Doudna and Charpentier's CRISPR-Cas9 paper was published (available online - June 2012)

December 2012 — provisional patent filed by Zhang based on work began in 2011

February 2013 — Cong et al. (2013) Zhang's CRISPR-Cas9 paper was published (available online - January 2013)

March 2013 — Doudna and Charpentier filed for a patent with a priority date - May 2012

16th March 2013 — 'first to invent' rule changed to "first to file' by the AIA

October 2013 — Zhang filed for accelerated examination of a patent for the use of CRISPR-Cas9 in eukaryotic cells; priority date referring to the provisional patent application from December 2012

April 2014 — USPTO granted patent to Zhang

2014/2015 — Doudna revised the initial patent application, broadening the claims

February 2015 — Shuailiang Lin's email to Doudna

January 2016 — patent interference proceedings started

Berkeley
UNIVERSITY OF CALIFORNIA

BROAD
INSTITUTE

What's the future of CRISPR-Cas9?


WELCOME TO
DOUBT
DONT STAY LONG


THANK
YOU!