

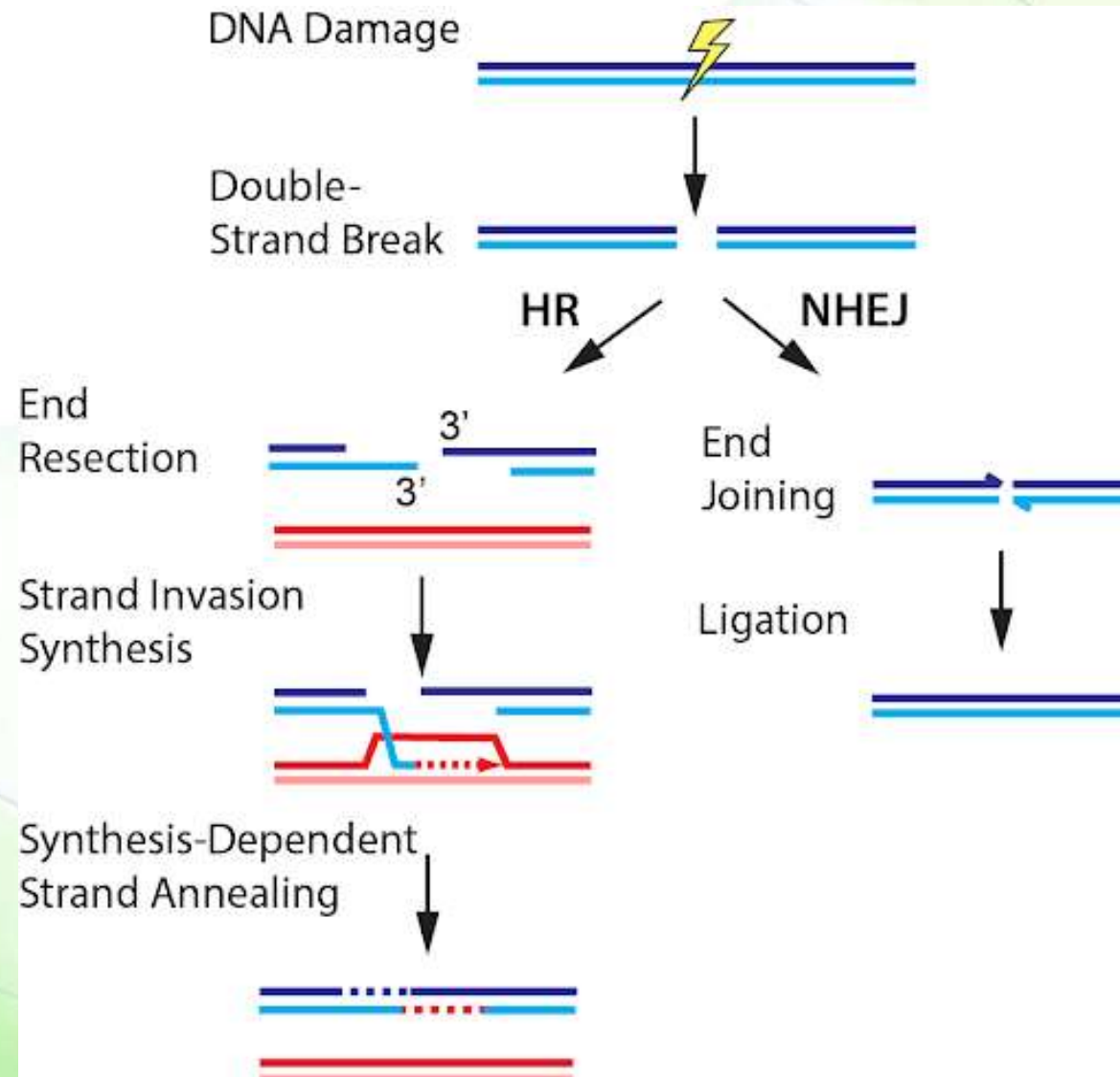


Recombinant DNA-based molecular techniques (part III)

CRISPR-CAS9 and gene editing

Prof. Mamoun Ahram

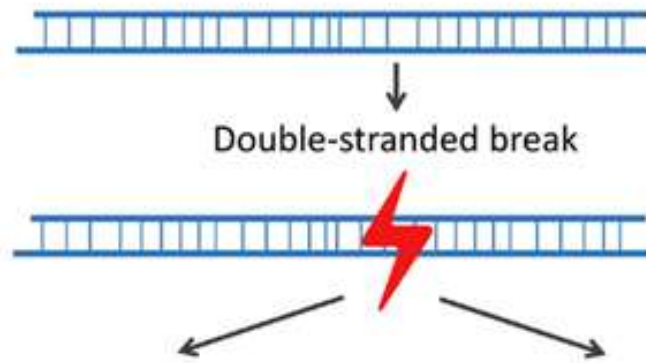
DNA repair mechanisms in human cells



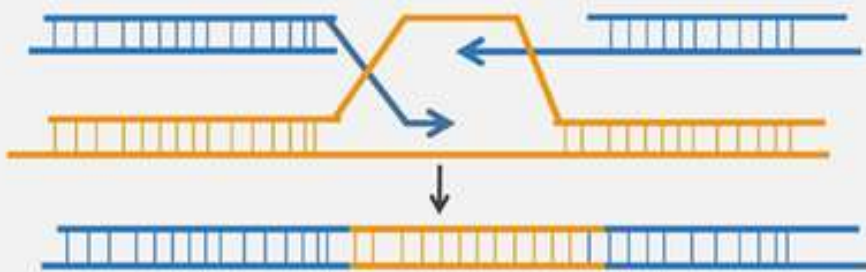
The consequences of DNA damage repair



Genome editing: harnessing natural repair mechanisms to modify DNA



Homology-directed repair: template with specific alterations

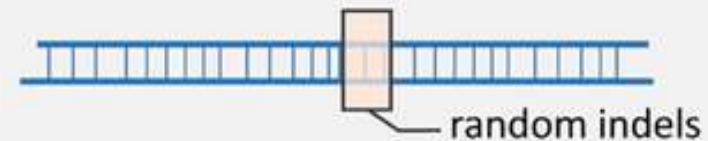


Correct mutation

Introduce mutation

Insert gene

Non-homologous end-joining: error-prone



Knock out gene

In 2020...



Emmanuelle Charpentier and Jennifer Doudna



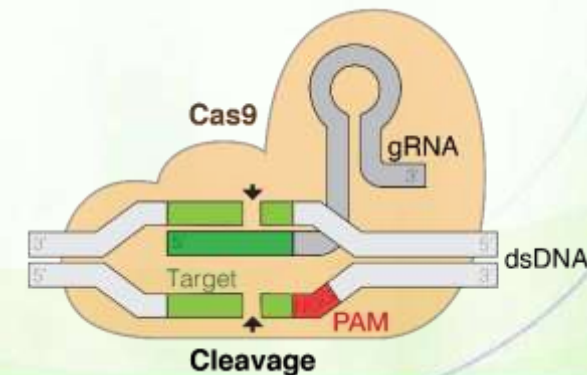
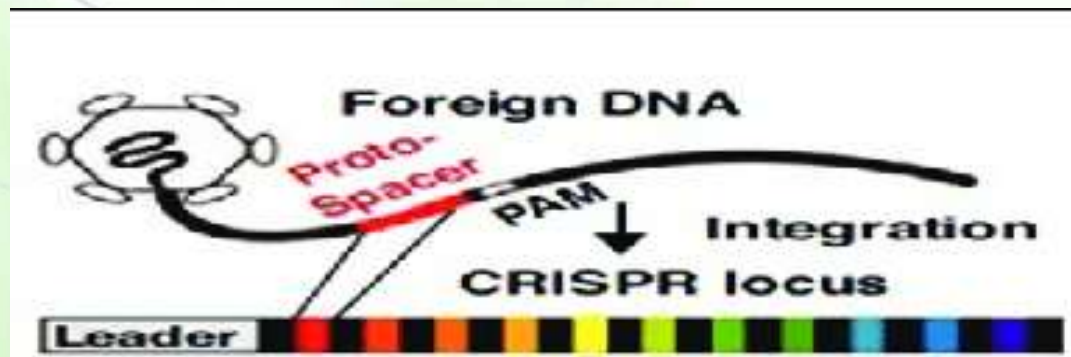
Modest men do not make history.

Cynthia Pando

What is CRISPR/Cas9?



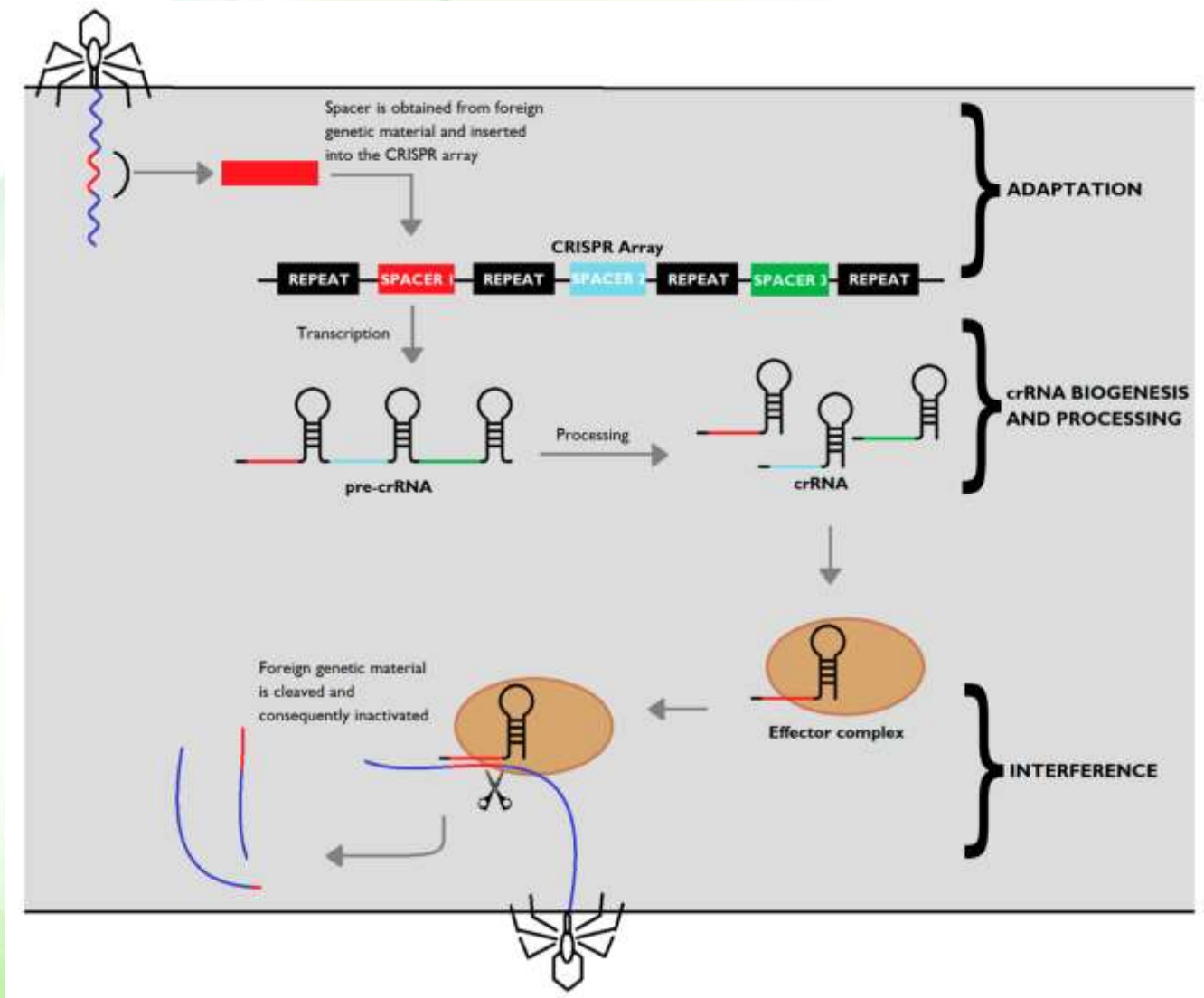
- CRISPR is clustered regularly interspaced short palindromic repeats
 - It is a bacterial genetic system that constitutes the immune system of bacteria against phages.
- Cas9 is a RNA-guided nuclease that can either create single or double strand breaks
 - The nuclease is directed to its target sequence by a short RNA fragment known as a guide RNA (gRNA) or single guide RNA (sgRNA), which is complementary to the target segment of the genome.



The concept



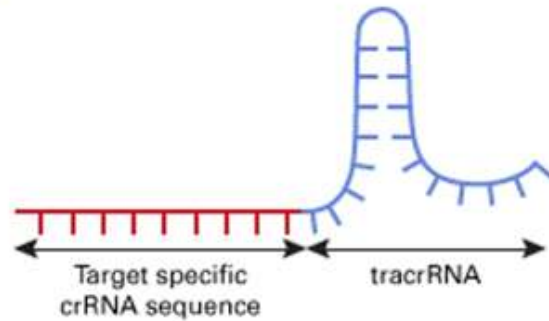
- When a phage infects a bacterial cell, the cell degrades the phage DNA into smaller pieces and integrates one of these fragments into the CRISPR cluster.
- When the phage infects the cell again, the cell transcribes the DNA into RNA (guide RNA or gRNA), which is integrated into the Cas9 nuclease and guides it to the phage DNA to degrade it.



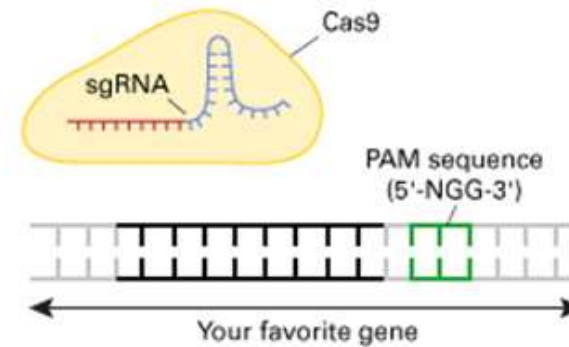
Targeting genes by Cas9



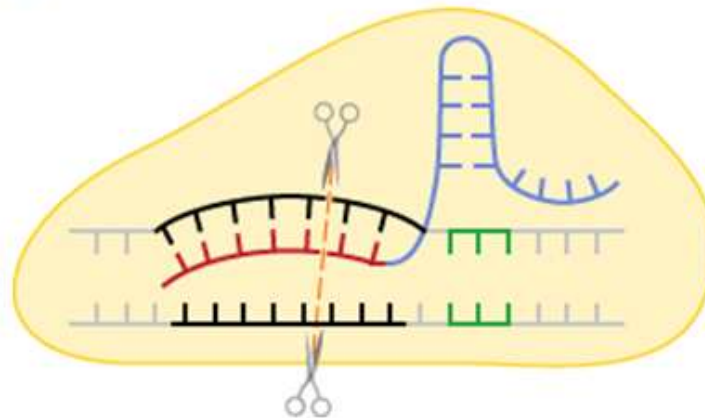
1 sgRNA (single guide RNA)



2 sgRNA + Cas9 protein

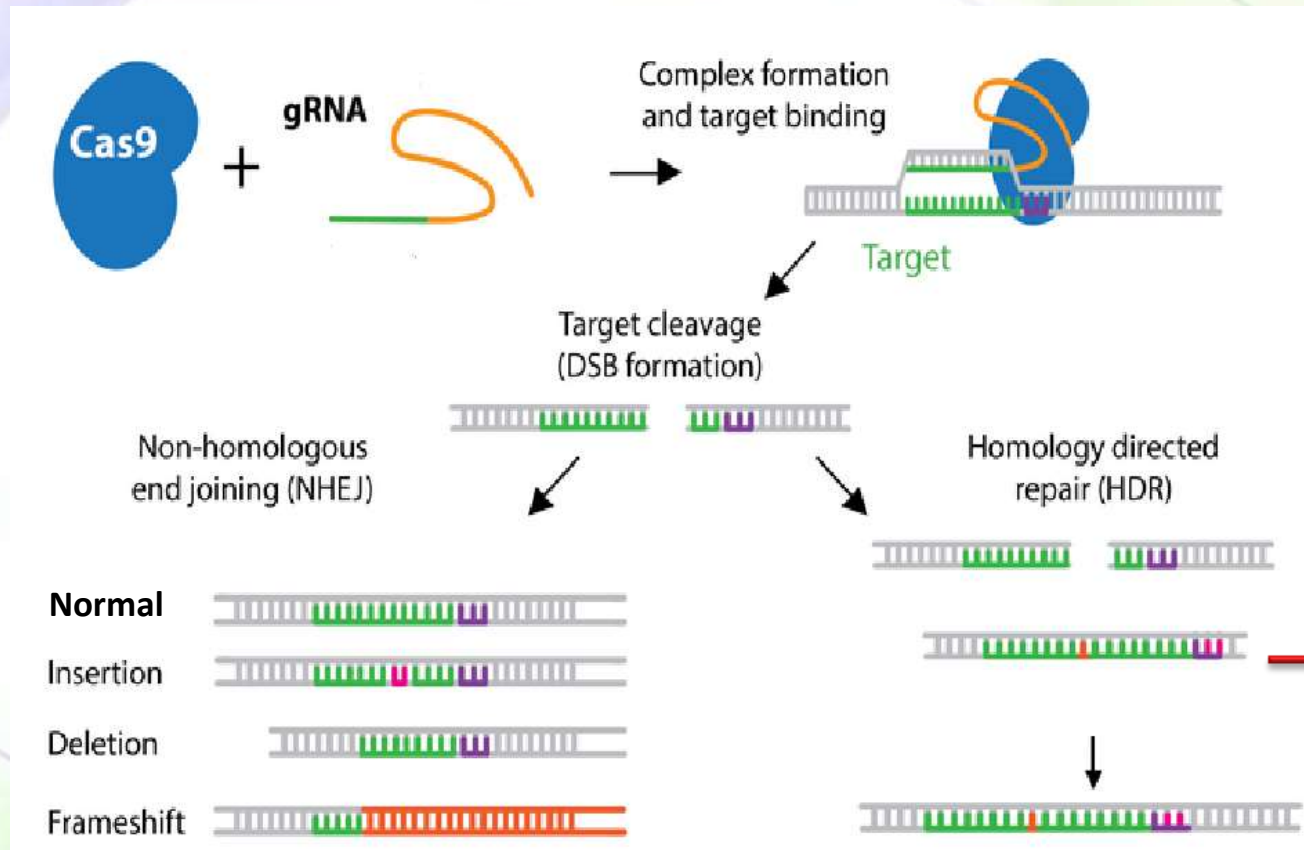


3 Target specific cleavage



Both the gRNA and Cas9 gene can be introduced into human cells as genes cloned into plasmid vectors.

Gene editing



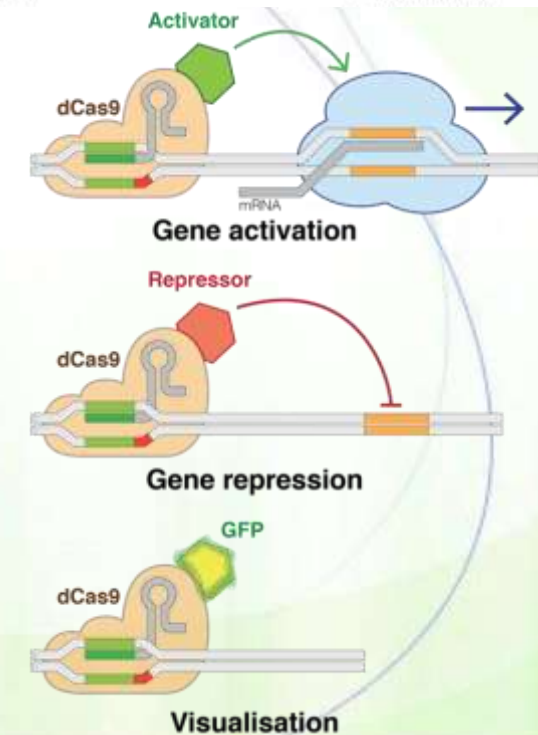
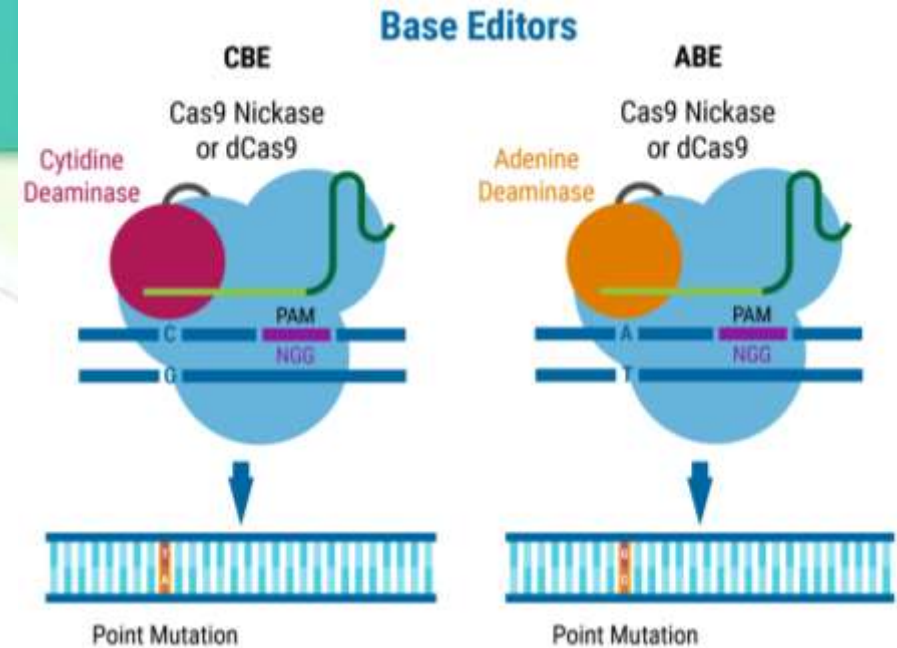
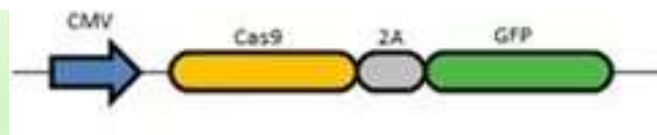
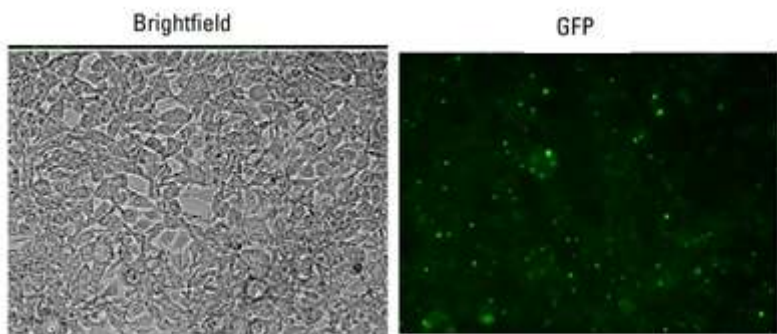
This DNA is introduced into cells so the DNA repair mechanism uses it for recombination.

Through either mechanism, the function of a gene can be studied by mutating it.

Specifically in this mechanism, a mutated gene is replaced by a normal one (or the opposite).

Other creative uses of Cas9

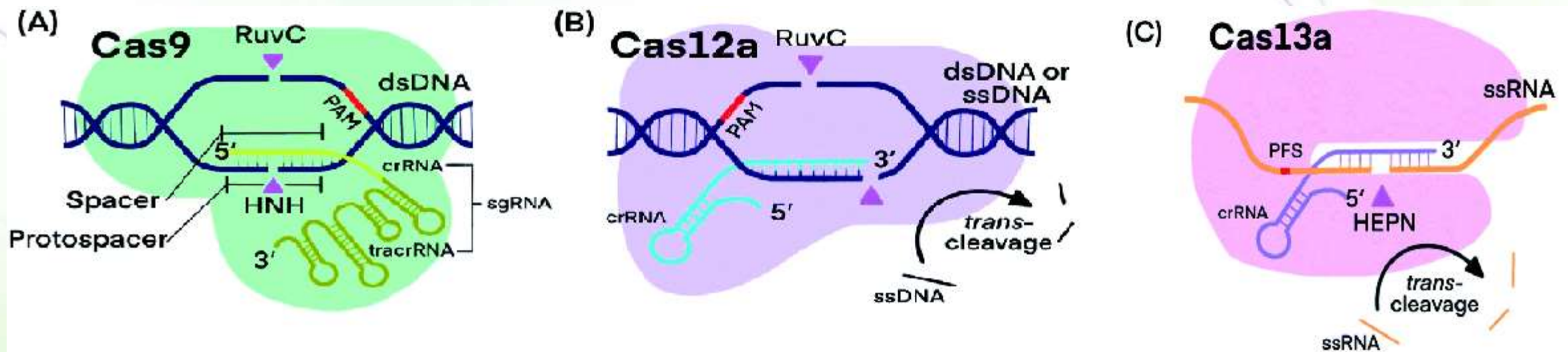
- Base editing
 - If deaminated, C is converted to U, which is read by the DNA polymerase as T changing CG into TA.
 - If deaminated, A is converted into inosine, which is read by the DNA polymerase as G changing AT into GC.
- Transcriptional regulatory factors can be added to a “dead” Cas9 (dCas9), enabling them to turn genes on or off or adjust its level of activity.
- GFP can be added to visualize genes that contain the Cas9 gene.

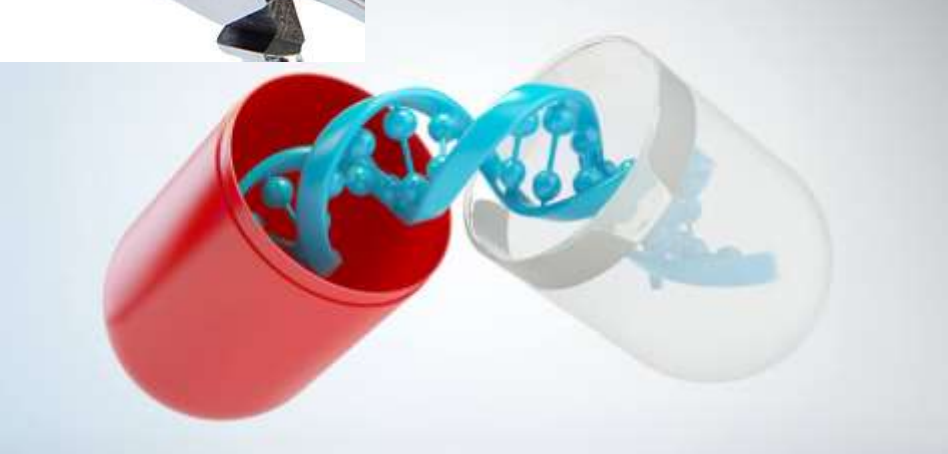
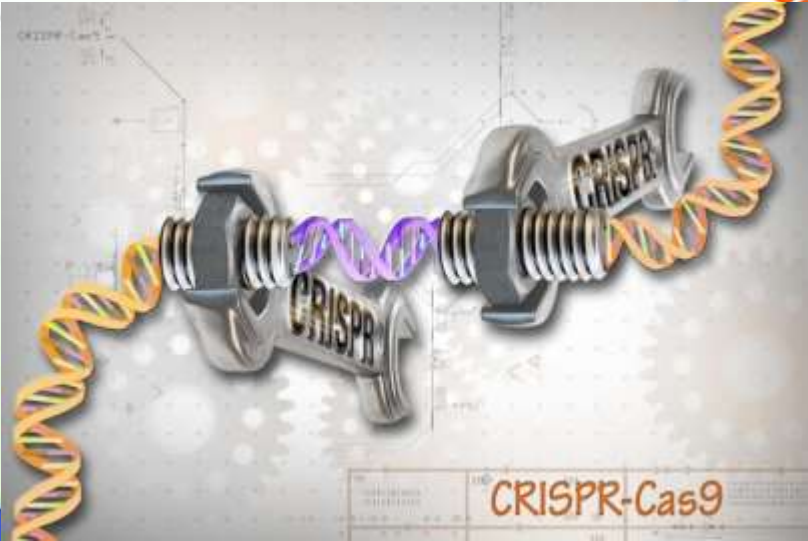
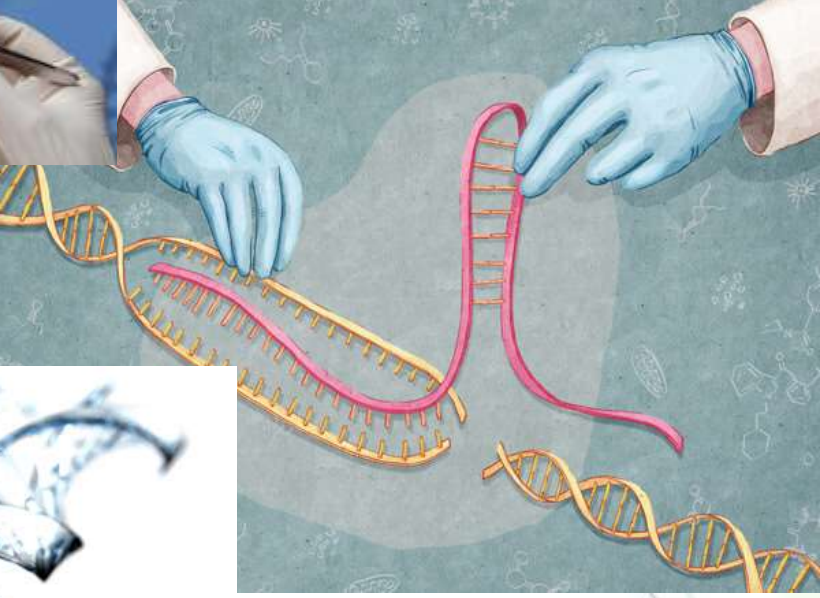
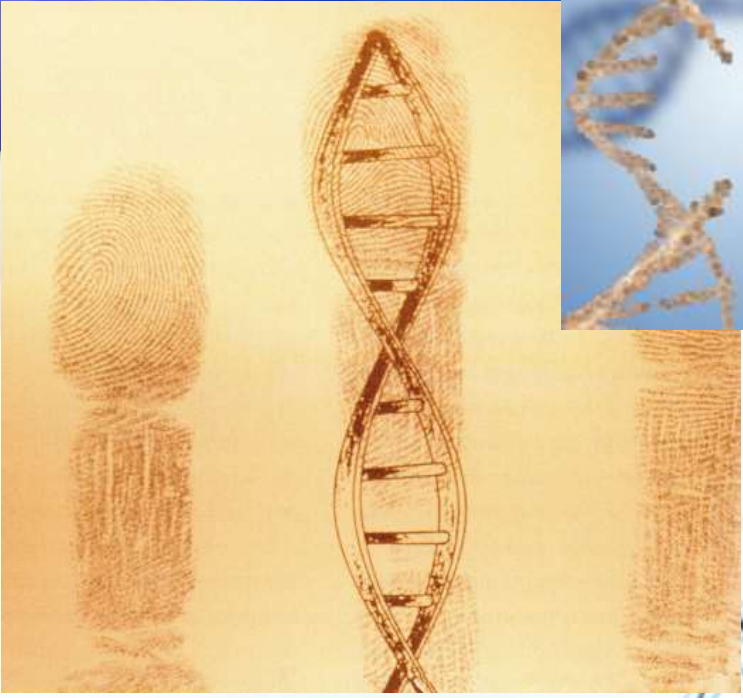


Other Cas enzymes



- Cas12a: A smaller enzyme that introduces staggered cut.
- Cas13a: A RNA endonuclease





Controversial issue

Gene repair



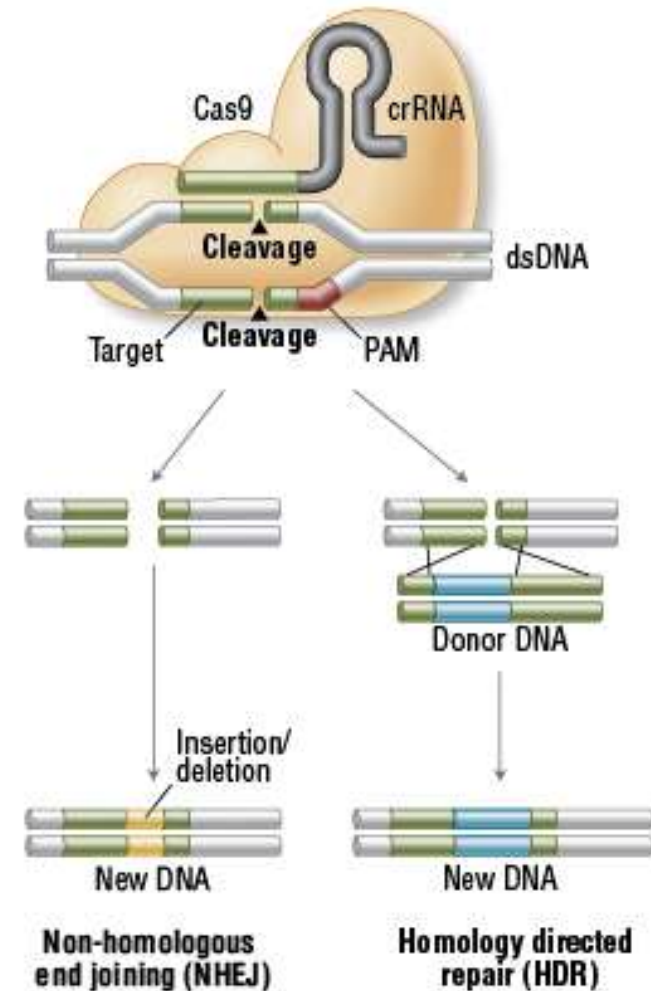
UK scientists ready to genetically modify human embryos

Researchers awaiting approval to use gene editing in embryos, which they hope will help them understand early stage life and improve fertility treatment



<https://www.theguardian.com/science/2016/jan/13/uk-scientists-ready-to-genetically-modify-human-embryos>

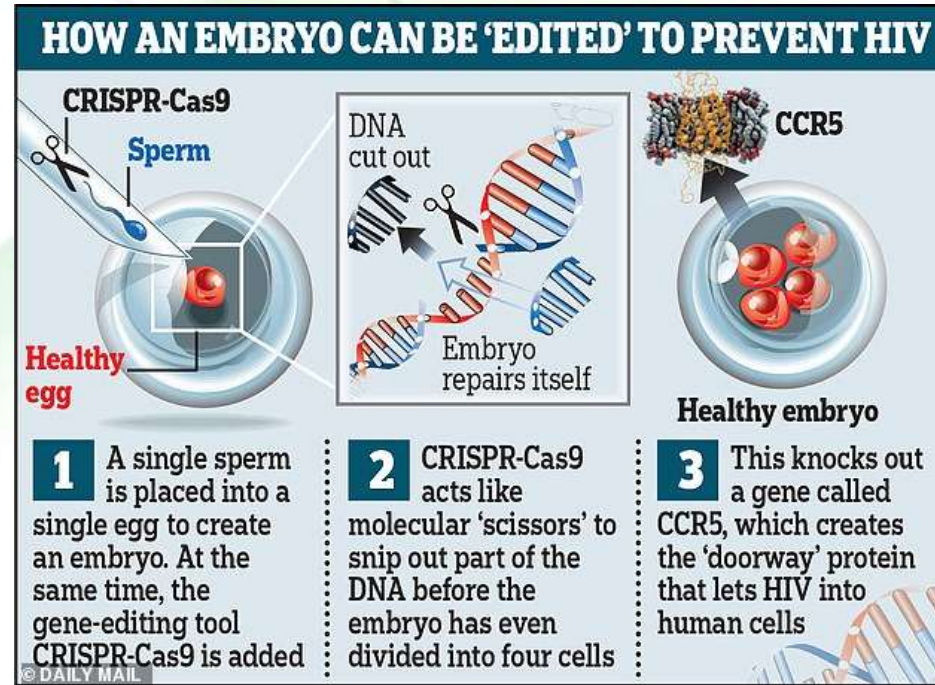
A. Genome Engineering With Cas9 Nuclease



The dark side of science



<https://www.theguardian.com/world/2019/dec/30/gene-editing-chinese-scientist-he-jiankui-jailed-three-years>



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China This article is more than 3 months old

Chinese scientist who edited babies' genes jailed for three years

He Jiankui was guilty of illegal practices in trying to alter the genetic makeup of twin girls

Lan Sample Science editor
@lansample
Tue 31 Dec 2019 00:23 GMT

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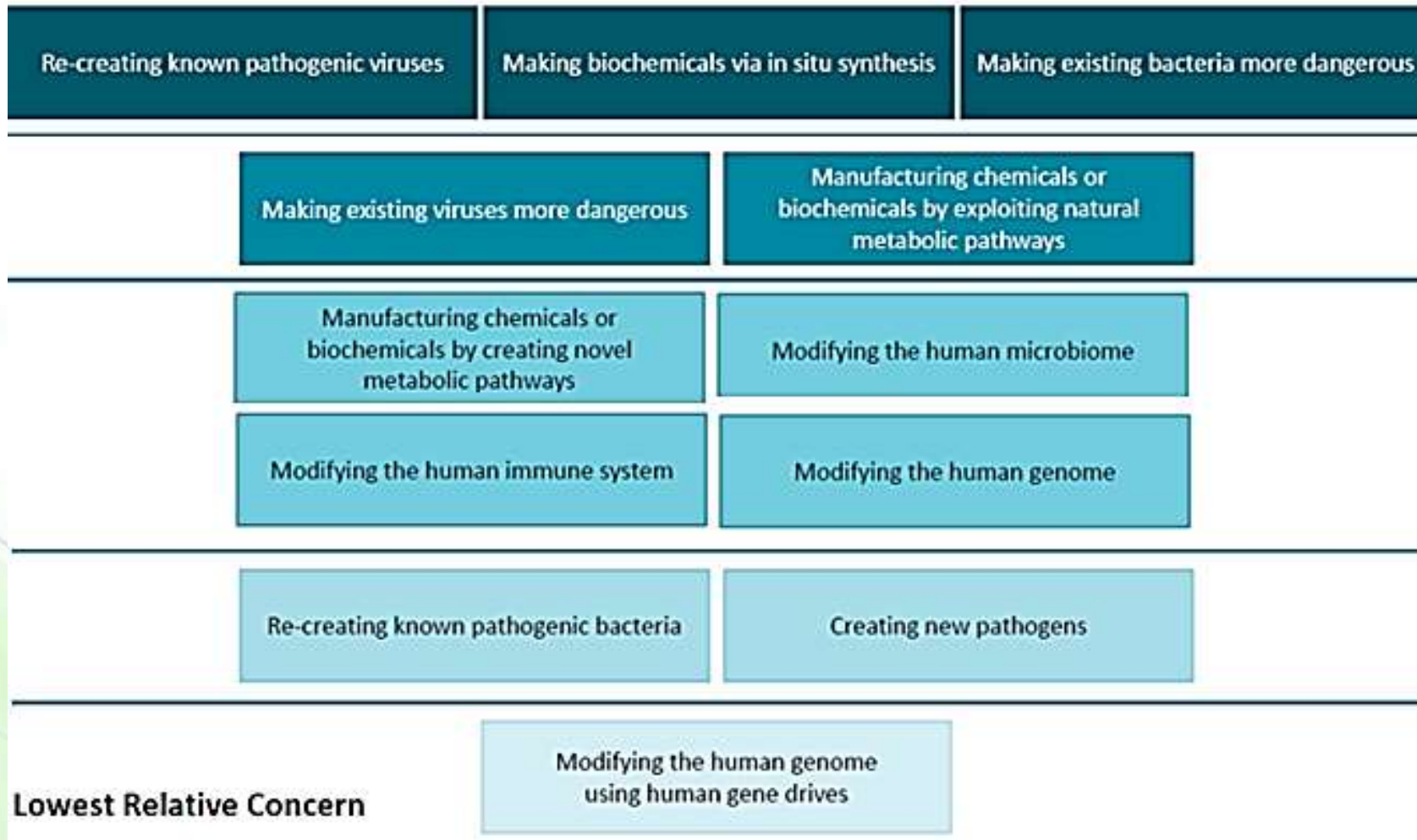
China's CRISPR twins might have had their brains inadvertently enhanced



Bioterrorism



Highest Relative Concern



Bioterrorism

