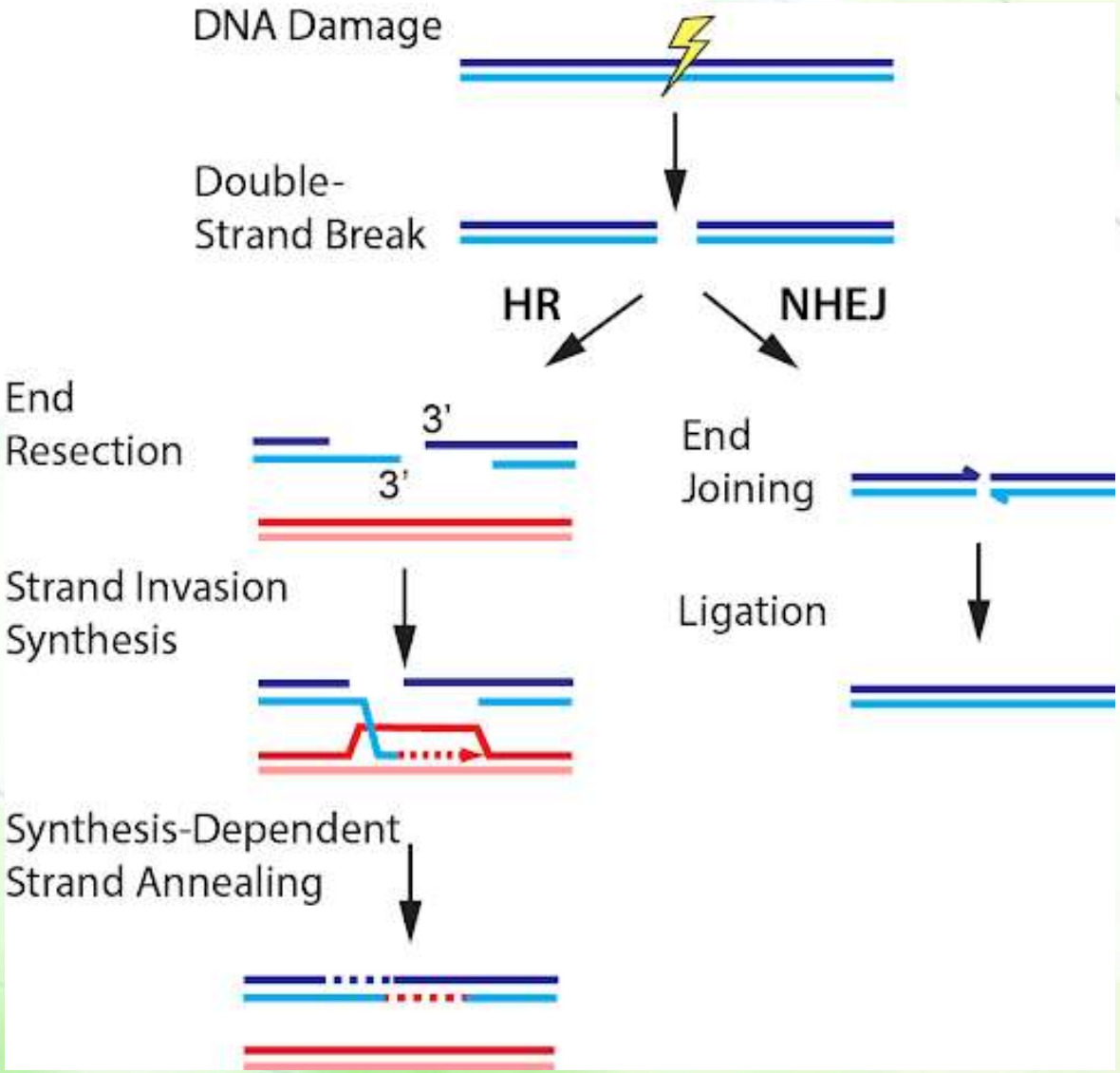
Recombinant DNA-based molecular techniques (part III)

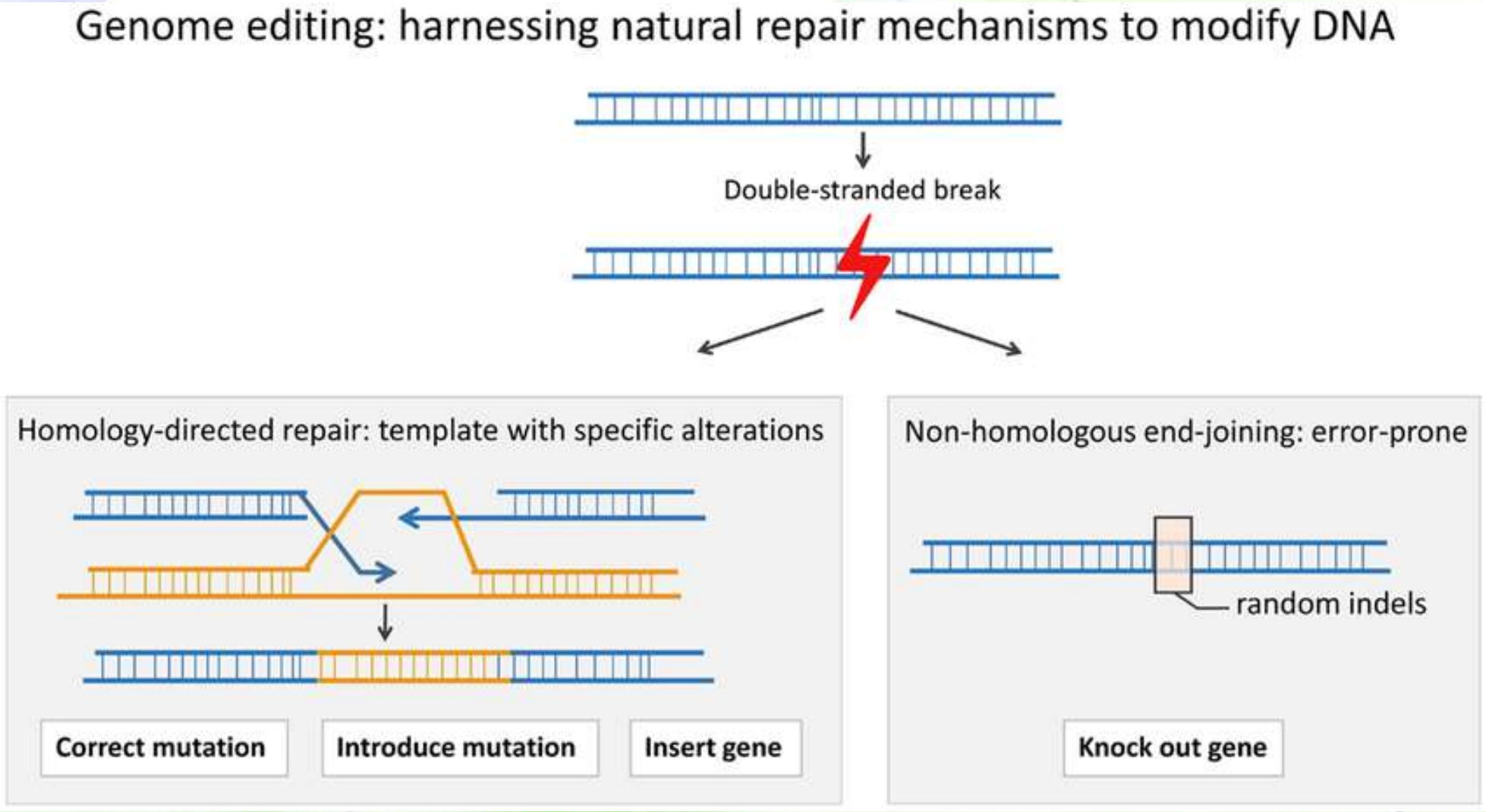
CRISPR-CAS9 and gene editing

Prof. Mamoun Ahram

DNA repair mechanisms in human cells

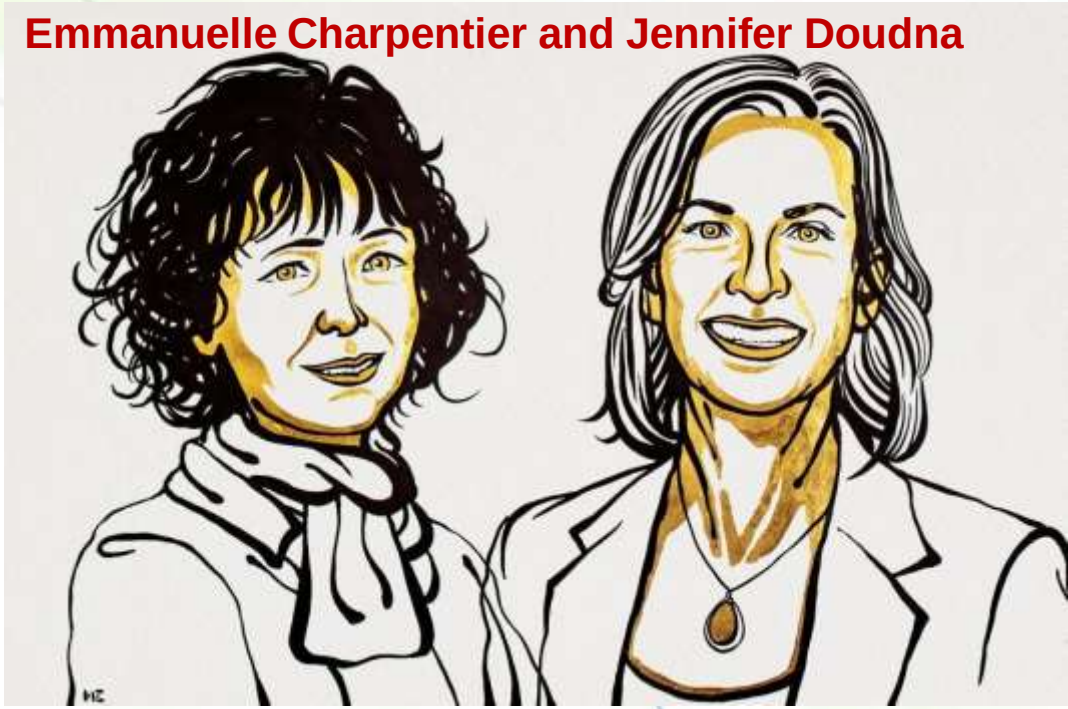


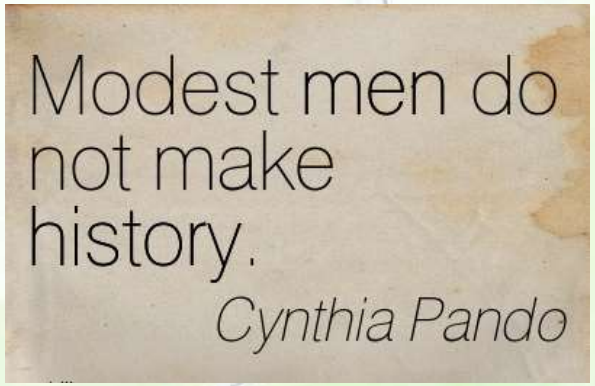
The consequences of DNA damage repair



In 2020…

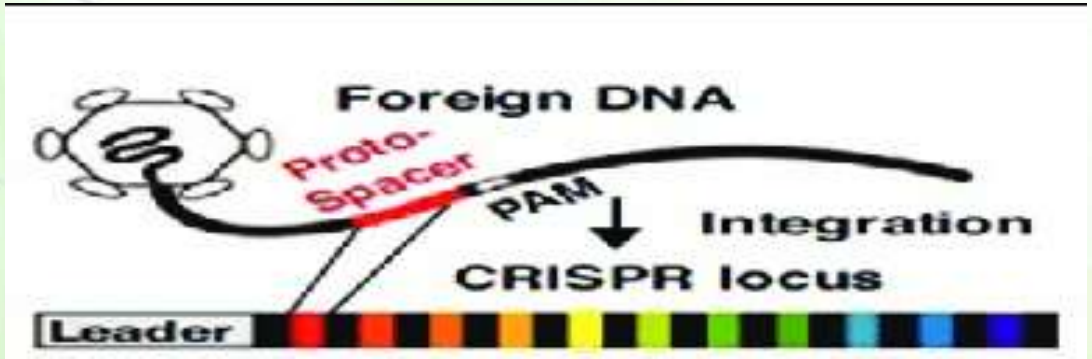
Emmanuelle Charpentier and Jennifer Doudna

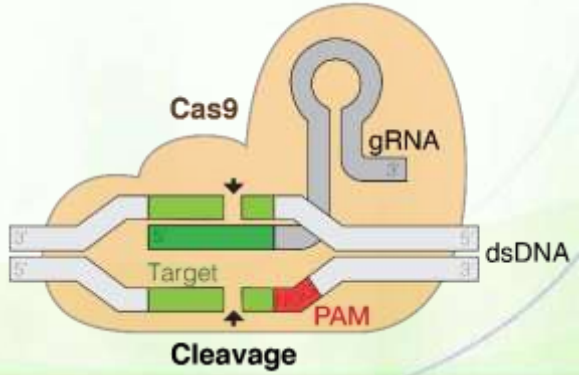




What is CRISPR/Cas9?

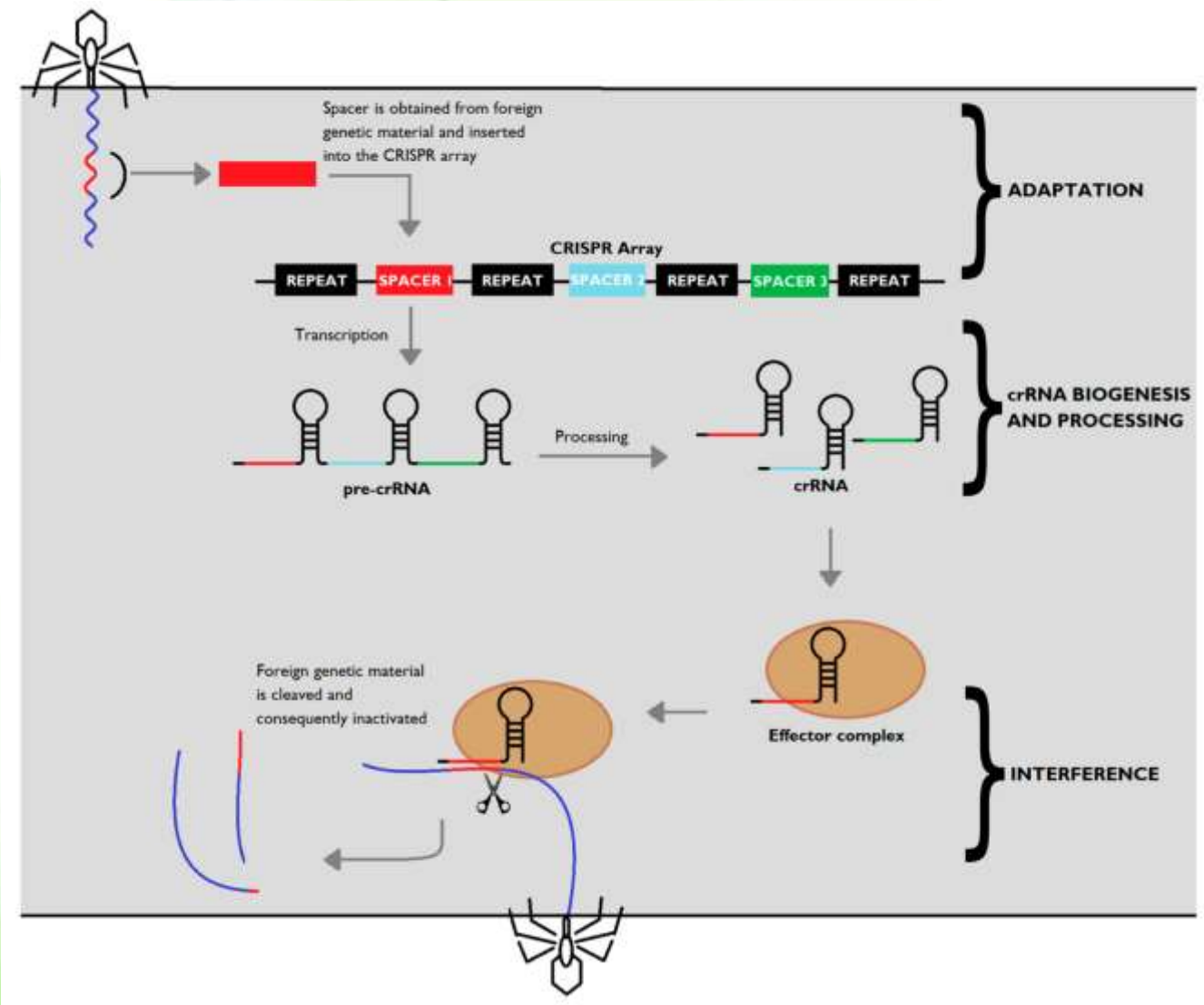
- CRISPR is clustered regularly interspaced short palindromic repeats
  - It is a bacterial genetic system that constitutes the immune system of bacteria against phages.
- Cas9 is a RNA-guided nuclease that can either create single or double strand breaks.
  - The nuclease is directed to its target sequence 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.



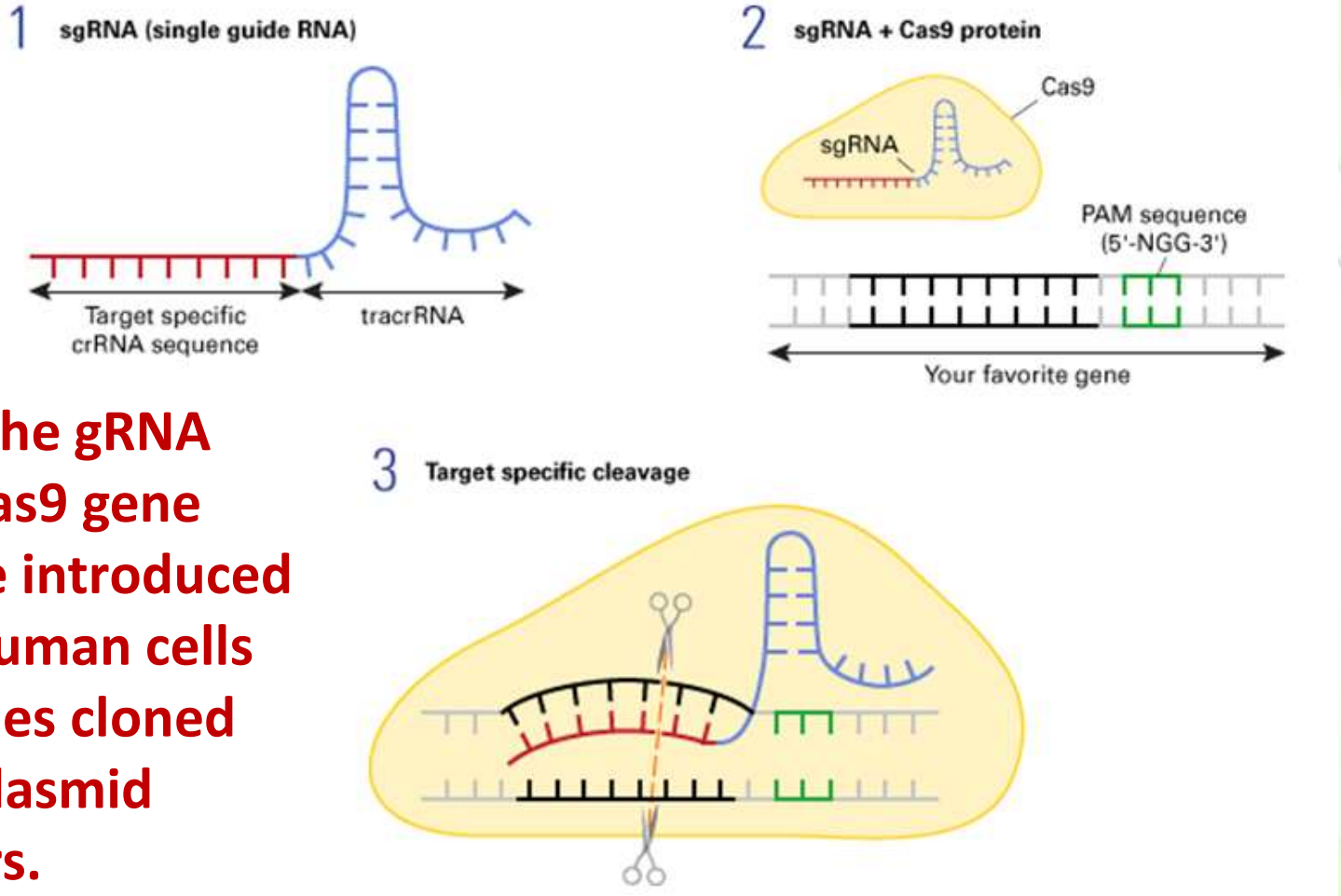


The concept

- 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.

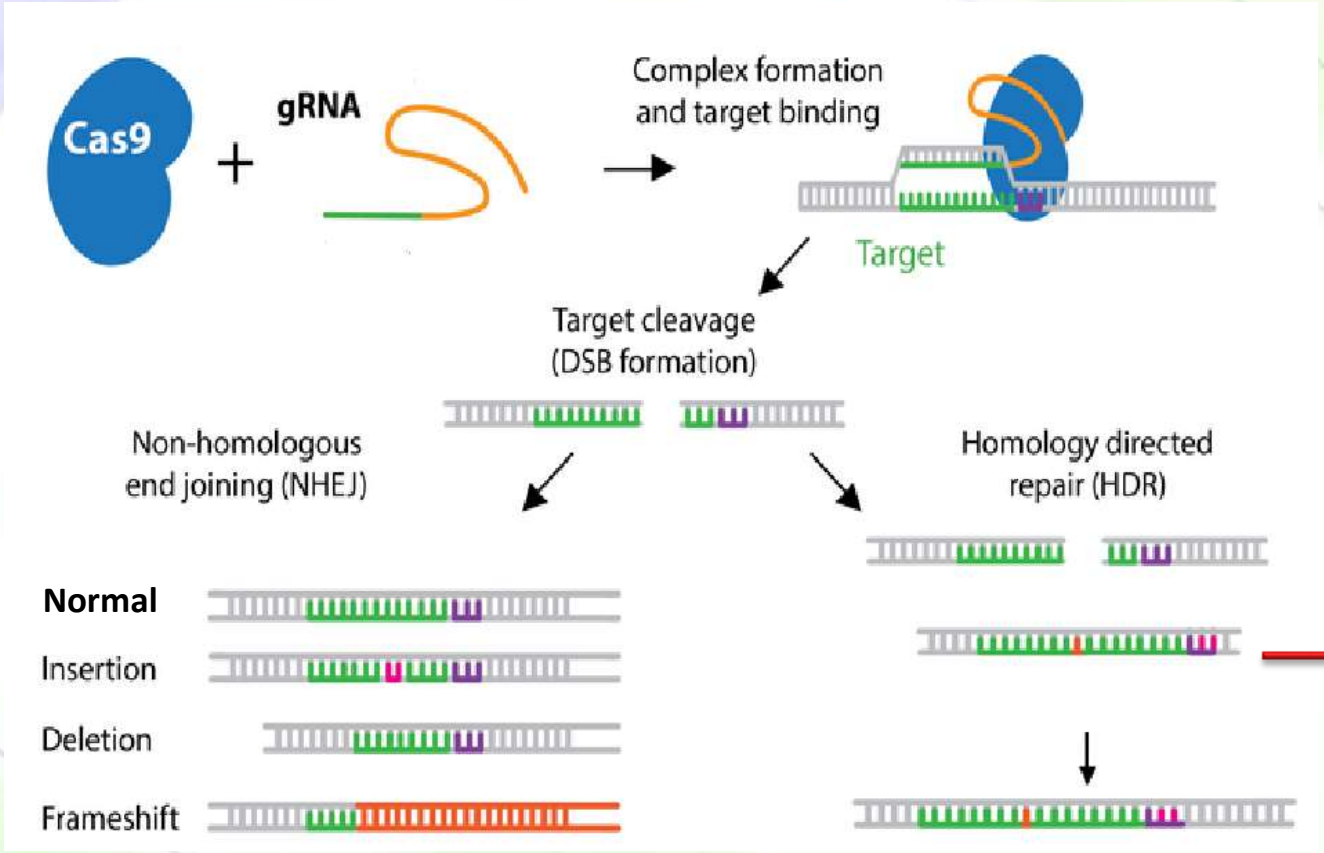


Targeting genes by Cas9



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

Gene editing



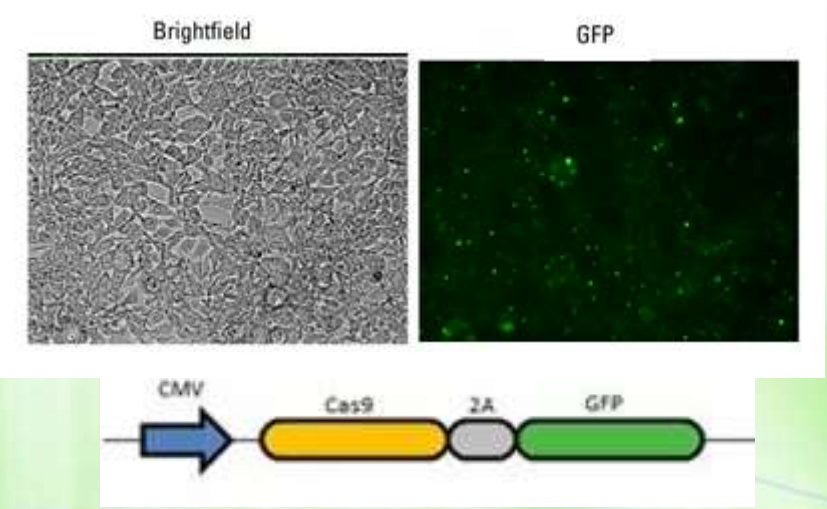
This DNA is introduced into cells so the DNA repair mechanism uses it for recombination.

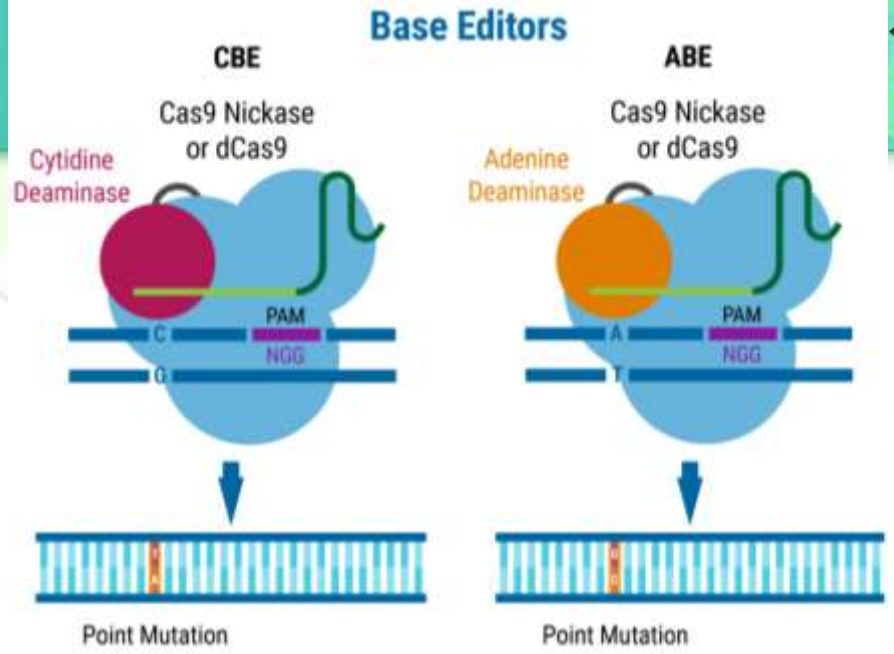
Through either mechanism, the function of a gene can be studied by mutating it.

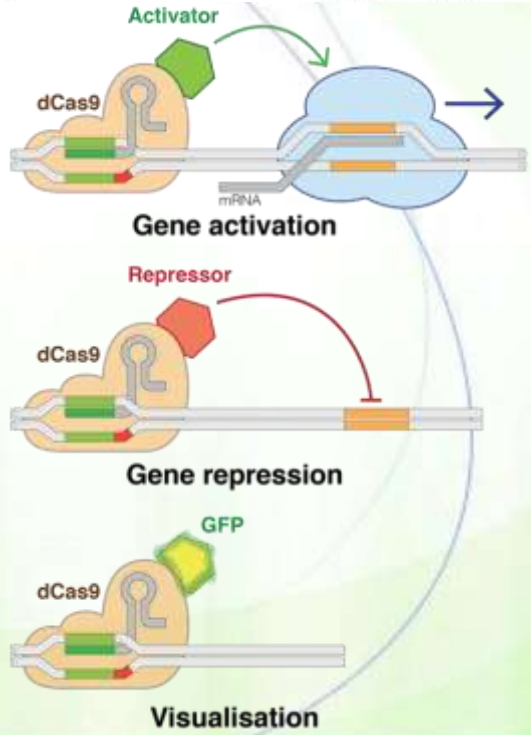
Specifically in this mechanism, a mutated gene is replaced by a normal one (or the opposite).

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.

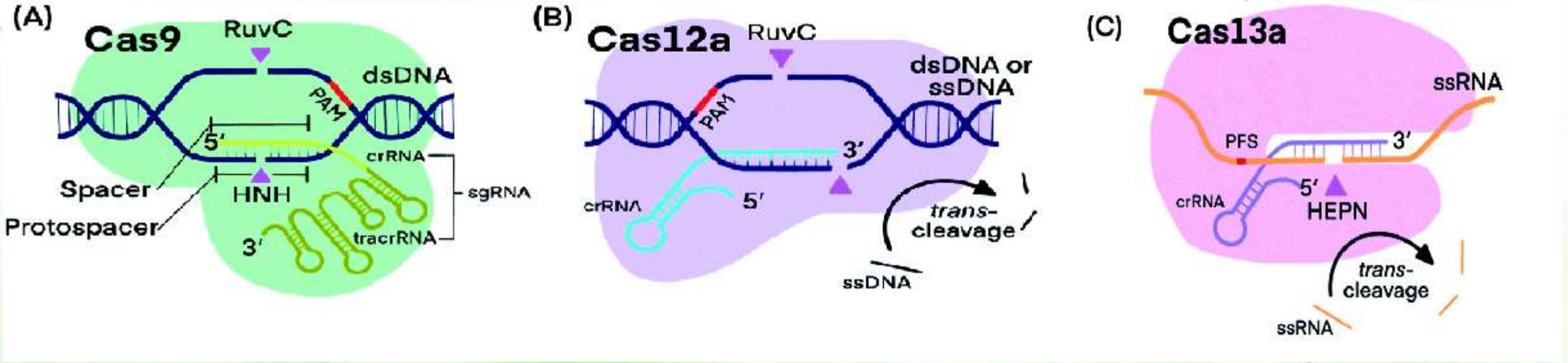


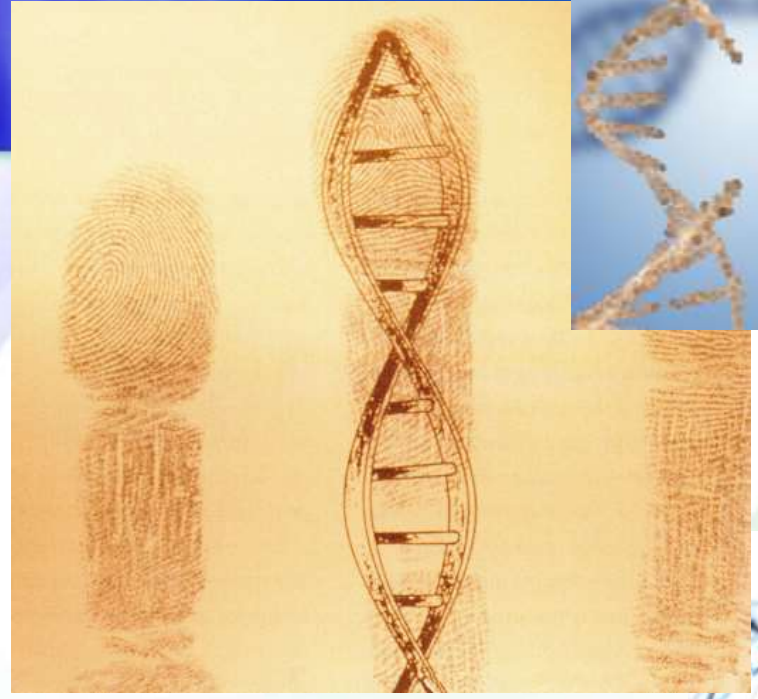


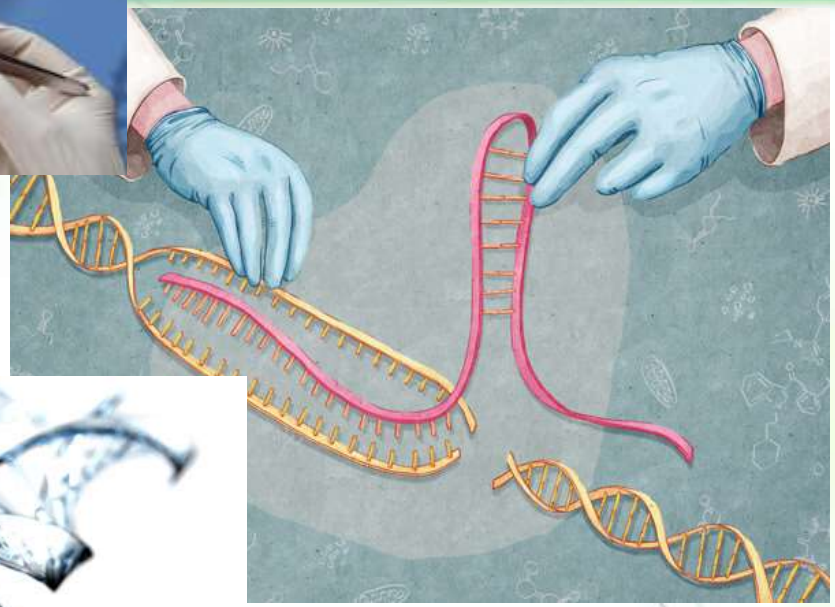


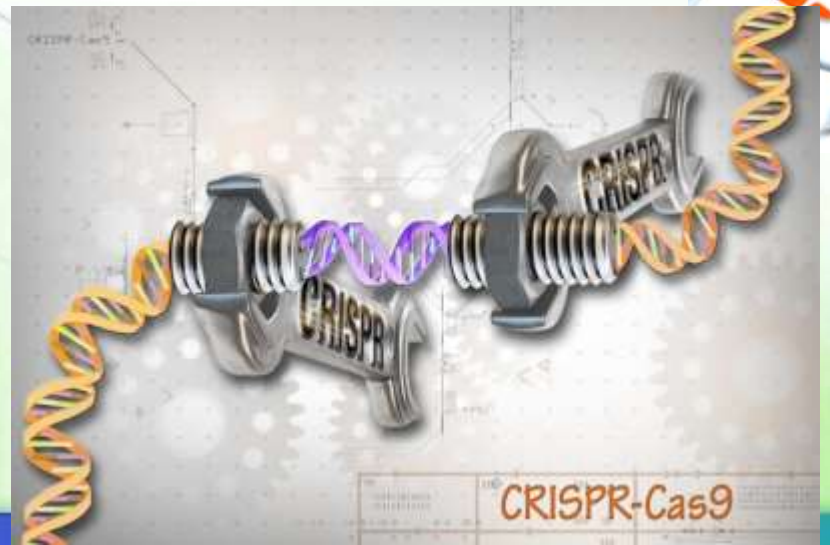
Other Cas enzymes

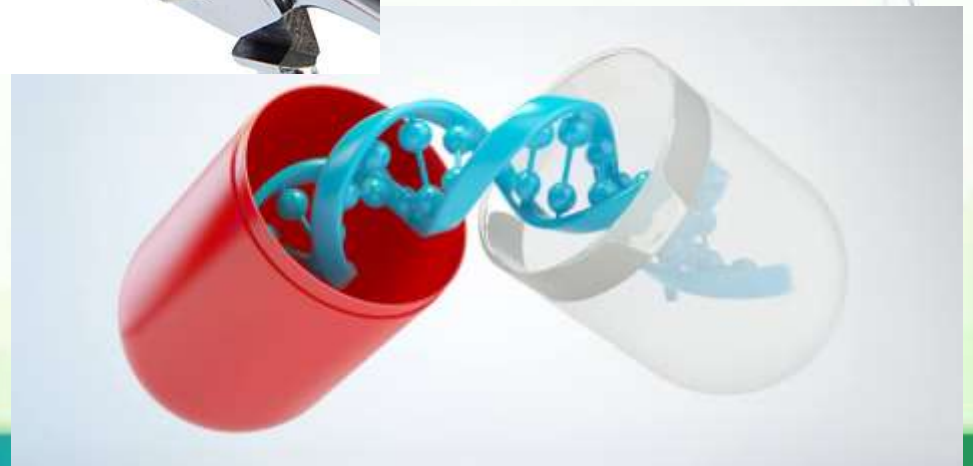
- Cas12a: A smaller enzyme that introduces staggered cut.
- Cas13a: A RNA endonuclease








CRISPR
CRISPR
CRISPR-Cas9



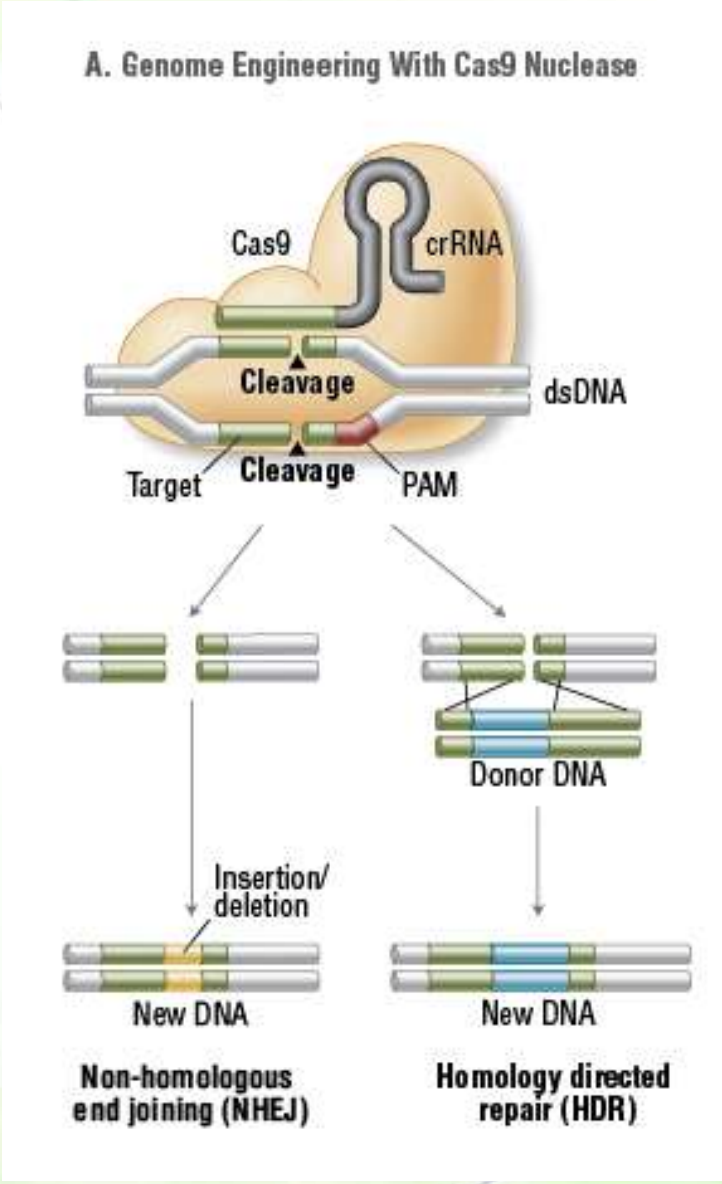
Controversial issue

Gene repair

UK scientists ready to genetically modify human embryos

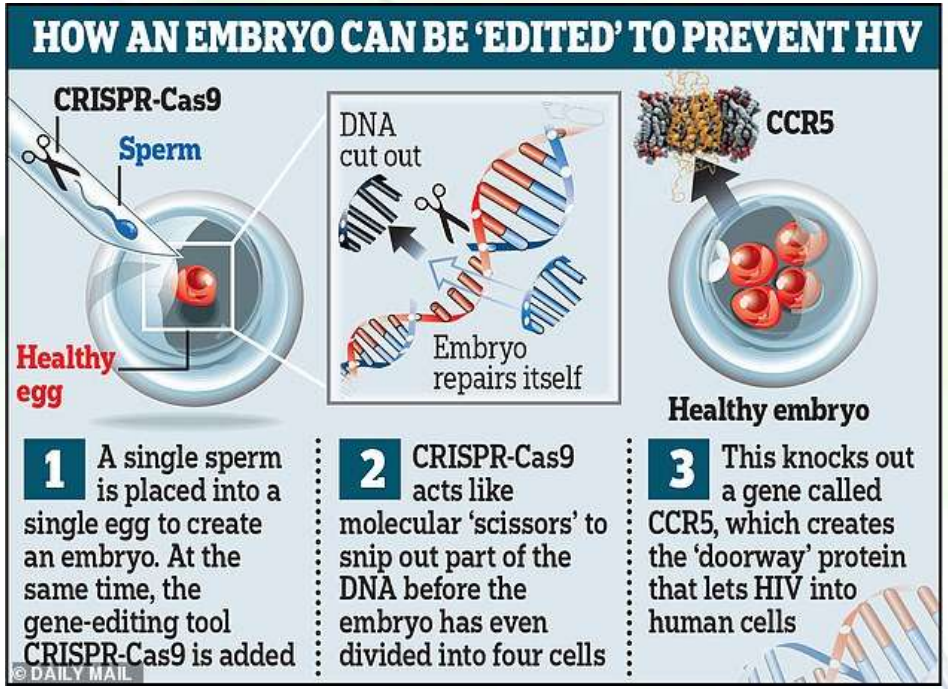
Researchers awaiting approval to use gene editing in embryos, which they hope will help them understand early stage life and improve fertility treatment

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



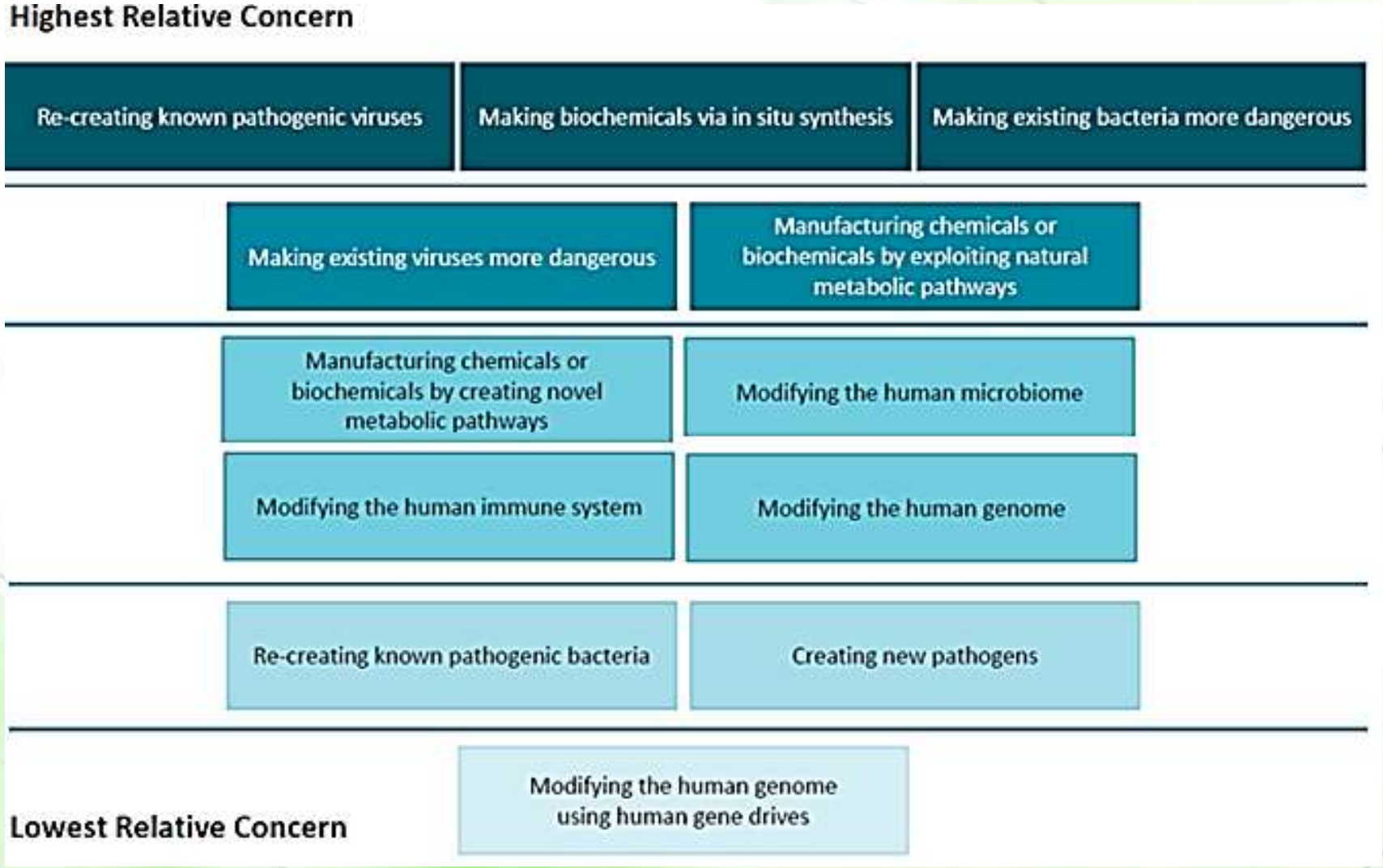
The dark side of science

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



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

Bioterrorism



Bioterrorism