بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Cytology & Molecular Biology | FINAL 22

DNA mutations & Repair pt.2

Last Modified

Written by : DST
NST

Reviewed by : Batool Zayed
Shorouq Matalkah

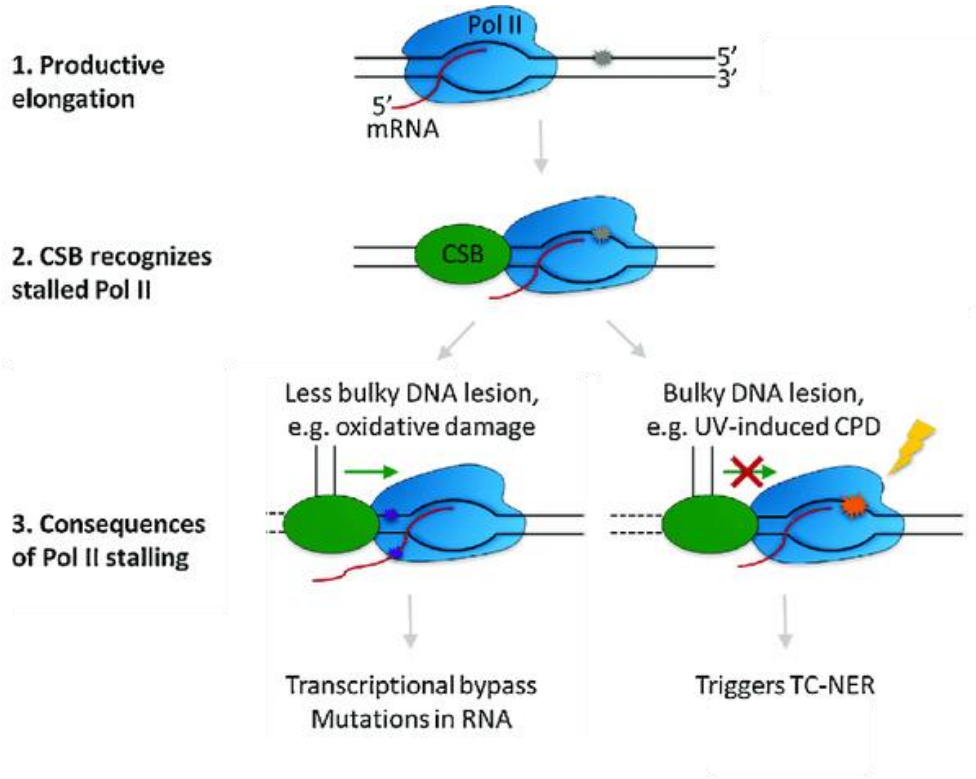
Transcription-coupled repair

During mRNA synthesis, RNA polymerase may stall upon encountering DNA damage in the transcribed strand, triggering transcription-coupled repair to address the lesion.

Transcription-coupled repair

- There is a preferential repair of the transcribed strand of DNA for actively expressed genes.
- RNA polymerase pauses when it encounters a lesion such as a pyrimidine dimer.
- CSB protein recognizes the stalled enzyme and it either allows it to continue or activates DNA repair by nucleotide excision repair.
- The general transcription factor TFIIH and other factors involving XP proteins carry out the incision, excision, and repair reactions.
- Then, transcription can continue normally.

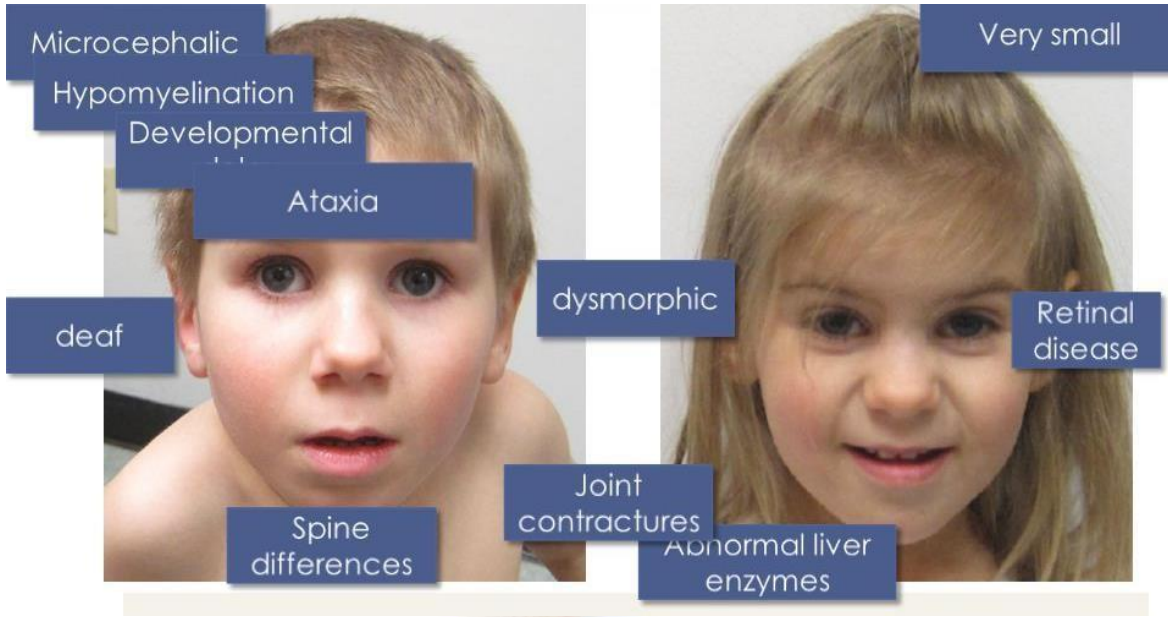
Association between transcription and DNA repair



Cockayne's syndrome

Somehow like xeroderma pigmentosum (recall the previous lecture) but with different effects

- Cockayne's syndrome: a condition caused by defects in XP proteins, but predominantly CSB.
- They recognize the RNA polymerase pausing at a site of mutation.
- It is caused by a defect in preferential DNA repair of transcriptionally active DNA genes.
- The genes affected by Cockayne's syndrome are not known.
- Patients are characterized by short stature, an abnormally small head (microcephaly), and neurologic abnormalities that can lead to intellectual disability and may have skin that is sensitive to light (photosensitivity).

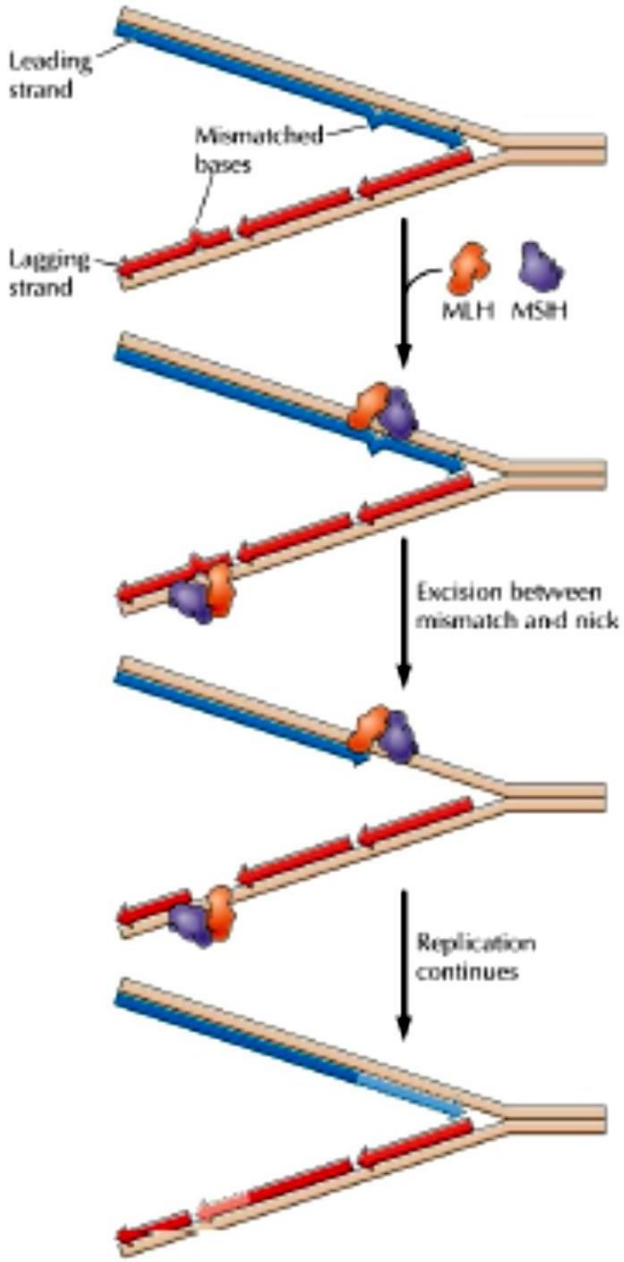


The doctor doesn't care about clinical features

Mismatch repair and replication-related repair

Mismatch repair in humans

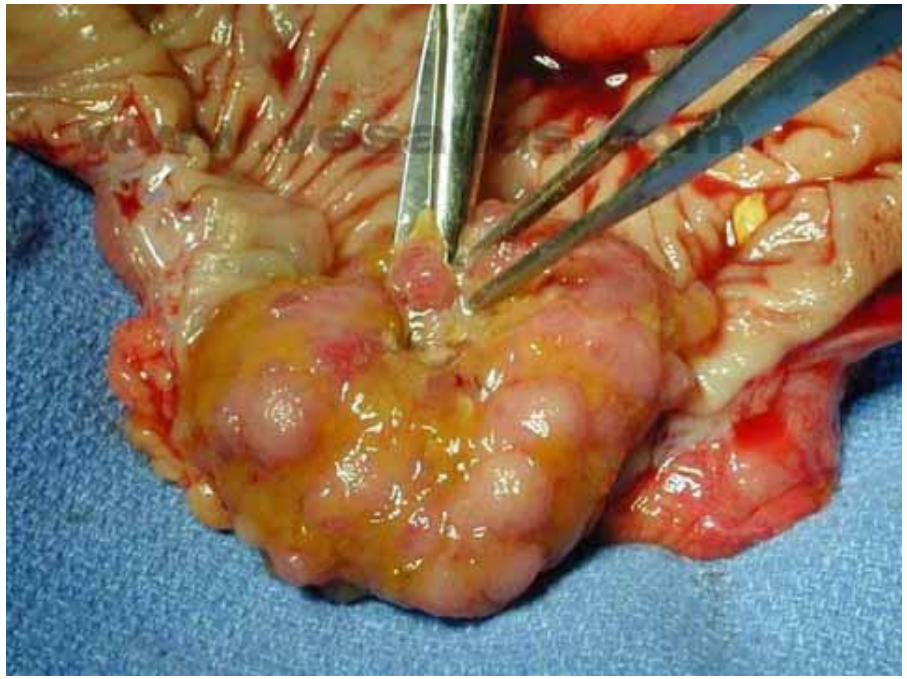
- During replication, MSH and MLH bind to mismatched bases within the lagging strand (Okazaki fragments) and the leading strand.
- DNA is excised and replication continues.
- Mismatch repair is 3-4 times more effective on the lagging strand than the leading strand, but DNA polymerase ε (synthesizes the leading strand) is more accurate than DNA polymerase δ (synthesizes the lagging strand).
- The increased effectiveness of mismatch repair in the lagging strand addresses the higher frequency of errors introduced during discontinuous synthesis.
- MSH and MLH proteins recognize mismatched base pairs during DNA replication. Upon detecting an error caused by DNA polymerase, they remove the mismatched section. DNA polymerase fills the gap with the correct sequence, and DNA ligase seals the strand.

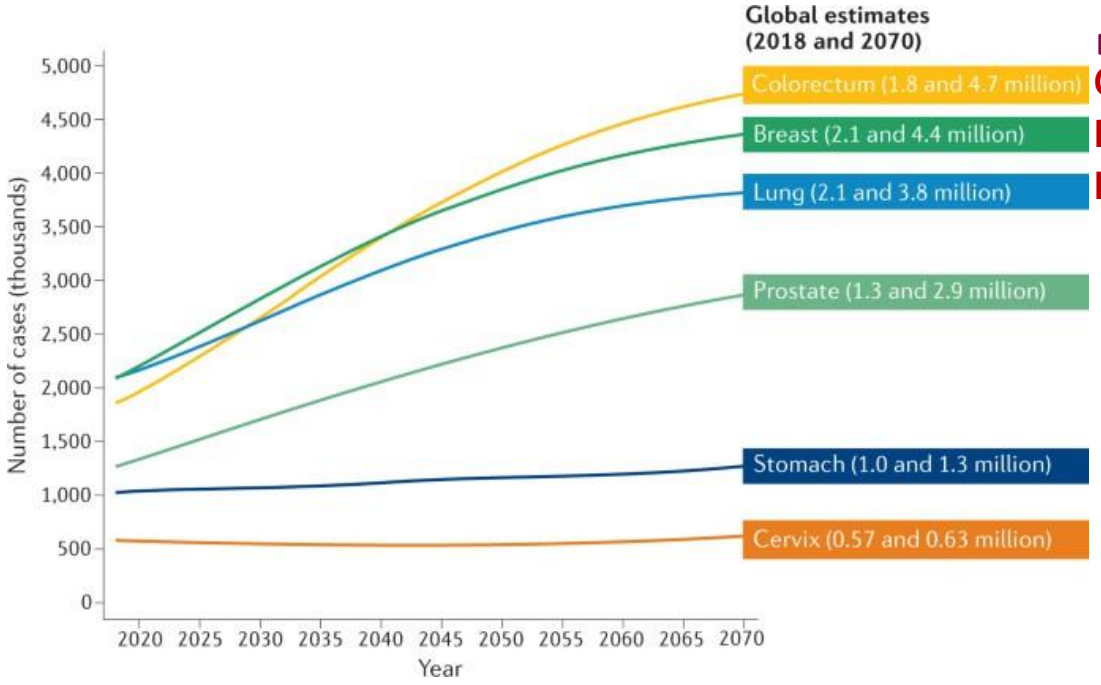


Hereditary nonpolyposis colon cancer (HNPCC)

- It affects as many as one in 300 people.
- 15% of colon cancer cases.
- It is mainly caused by mutations in MSH and MLH.

COLON CANCER is expected to be the no.1 cancer globally !





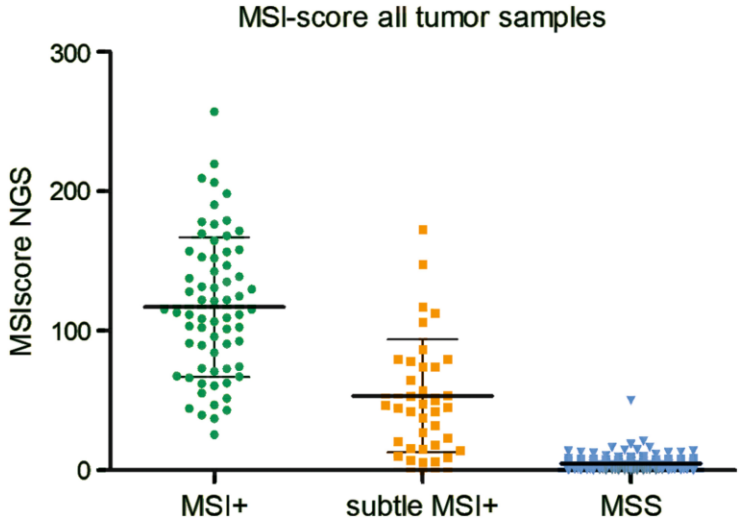
Diet & lifestyle **Colorectal**

Breast Hormones

Lung Smoking

Microsatellite instability (MSA)

- • Microsatellites are segments of 1-6 base pairs (e.g., CAGCAGCAG) repeats. These repeated BPs make DNA more susceptible to mutations and mistakes
- • They are prone to errors (insertions/deletions) during DNA replication, due to a defective mismatch repair system leading to a phenomenon called mismatch instability . (increasing the number of repeats)
- • MSI is a biomarker, especially for colorectal (Lynch syndrome) and endometrial cancers, indicating a specific pathway of tumor development.
- • (MSI-H) tumors in colorectal cancer often have better prognoses and respond differently to treatment than Microsatellite Stable (MSS) tumors.
- • MSA can be confirmed by immunohistochemistry and/or PCR ing and/or sequencing specific microsatellite regions.



Basically , they take a tissue from the tumor and from the surroundings to study the MSI , the more the MSI (more repeats) the less aggressive the tumor is , so its treatment strategy and prognoses will be better and vise versa. HOW ? Because MSI tumors create antigens (foreign proteins) that stimulate the immune system .

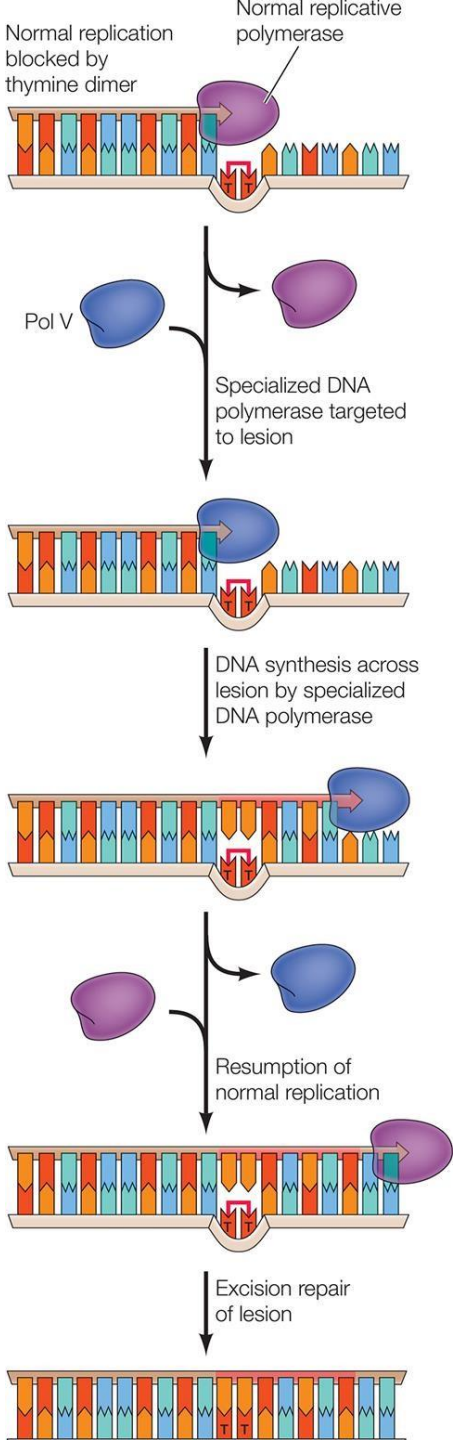
Translesion DNA synthesis

A lesion is a defect or distortion in the DNA structure, which can sometimes be bulky, such as thymine dimers or interstrand crosslinks. Translesion synthesis (TLS) refers to a process where specialized DNA polymerases replicate over the lesion, allowing DNA synthesis to continue despite the damage.

Translesion DNA synthesis

- There are many more DNA polymerases beyond those primarily involved in DNA synthesis. These additional polymerases play crucial roles in DNA repair processes, such as translesion synthesis and mismatch repair.
- During normal DNA synthesis, if standard DNA polymerases encounter a site of damage, such as thymine dimers, they stall and dissociate from the DNA. Specialized DNA polymerases then take over.
- Specialized DNA polymerases (not the typical replicative enzymes) can then synthesize DNA over the lesions.
- But they have low fidelity and lack proofreading mechanisms, and, hence, are error-prone .
- But they are selective toward the introduction of A nucleotides, so that TT dimers are often replicated correctly, through an "educated guess".

  - The advantage of this : no occurrence of sever mutations afterwards .



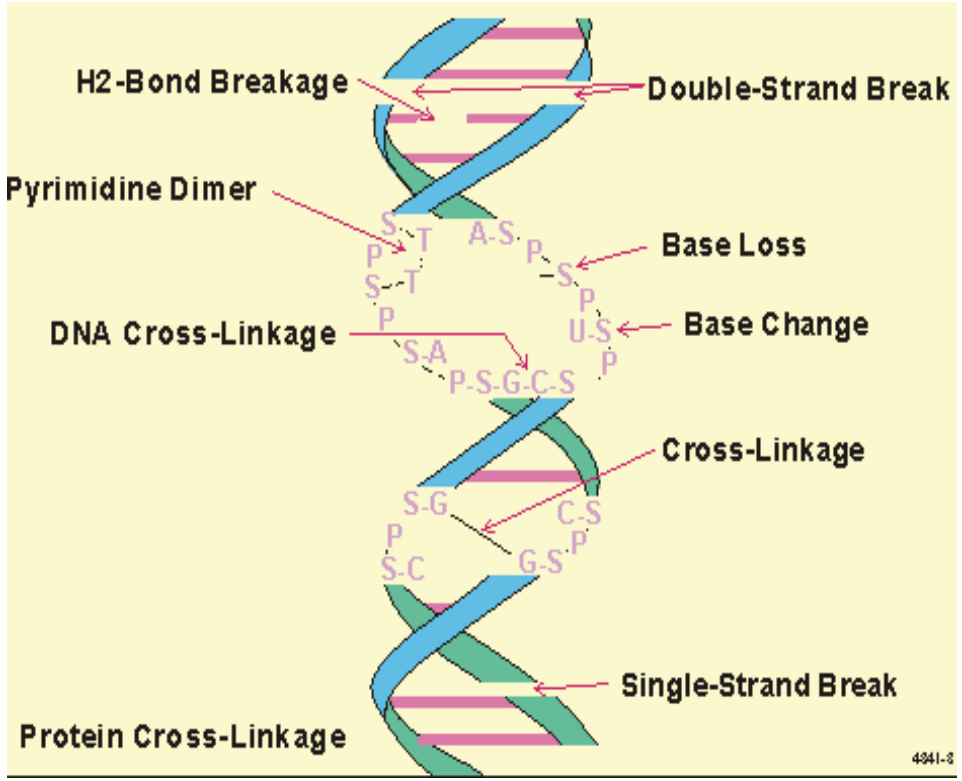
Recombinationalrepair

Recombinational repair involves using a segment from another chromosome to repair the damaged chromosome. This process is similar to the homologous recombination mechanism that occurs during metaphase, where aligned chromosomes exchange genetic material.

Ionizing radiation

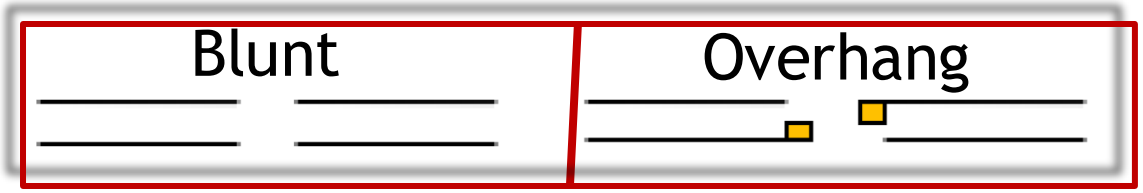
- Ionizing radiation can cause different type of DNA damage:
  - Creation of AP sites, by loss of nitrogenous base.
  - Base damage, repaired by DNA glycosylases.
  - Strand breaks due to phosphodiester bonds breaking, either single (SSB) or double (DSB), which is severe.
- Repair of DSBs depends on the severity and nature of the break (e.g., blunt ends or overhangs).
- Two repair mechanisms:
  - Non-homologous end joining (NHEJ)
  - Homologous repair

Homologous: identical

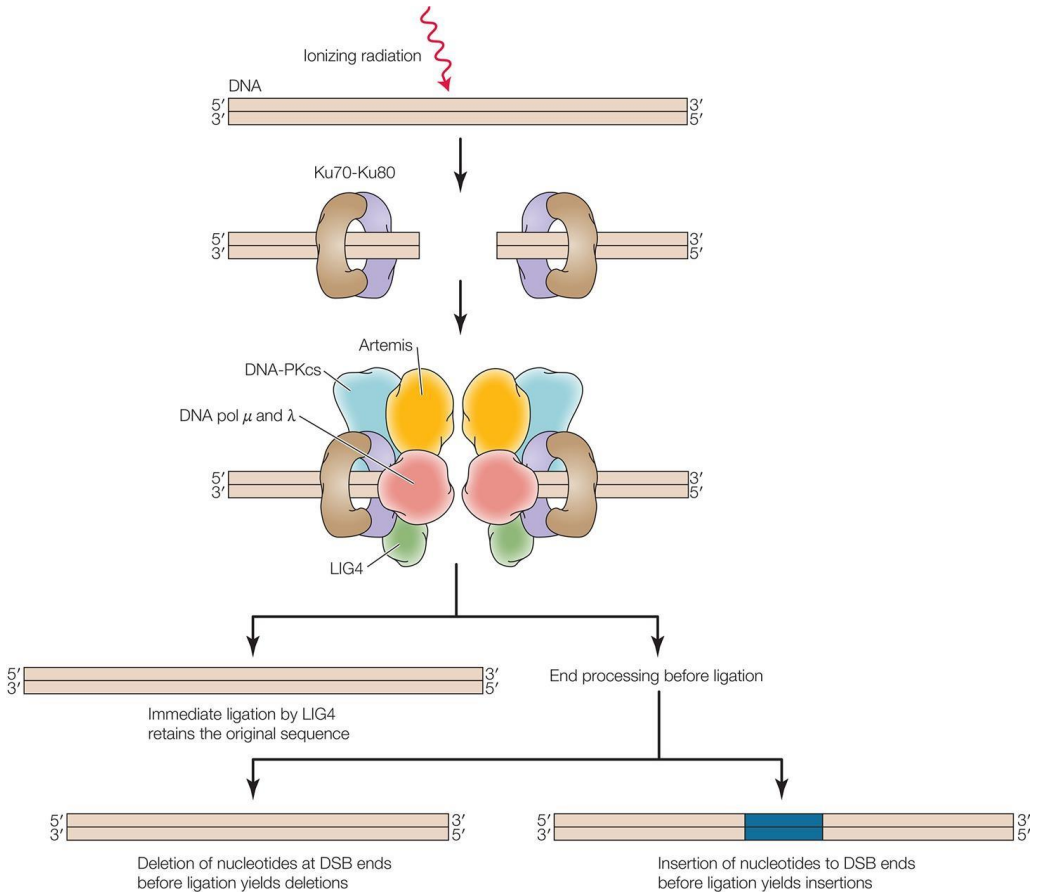


Straight cut
More severe

Single stranded DNA on both sides
Less severe because eventually there is two complementary strands that can come toward each other



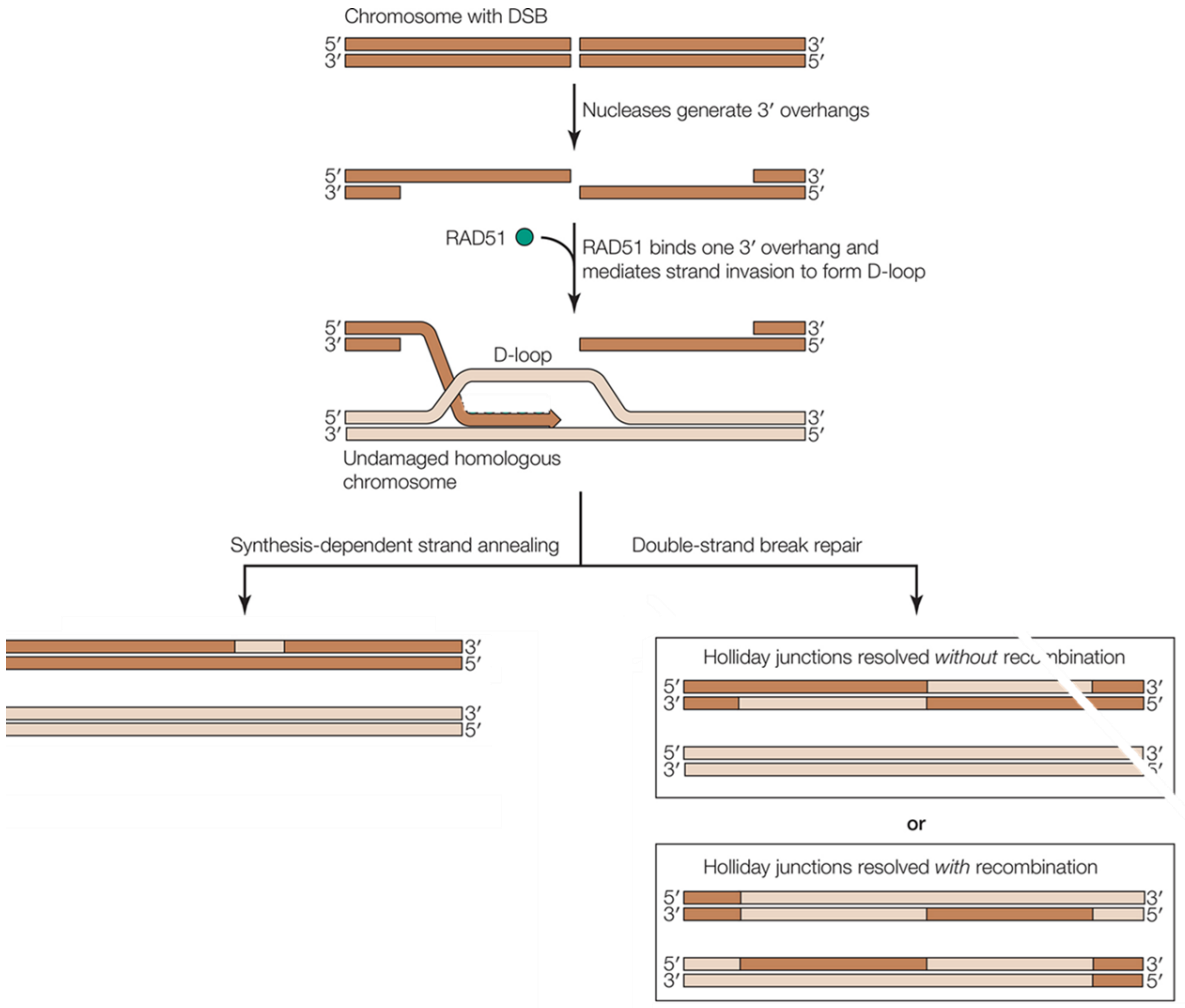
Nonhomologous end joining (NHEJ) repair



- The DSB ends are first bound by the Ku70-Ku80 complex, which recruits additional factors including a DNA ligase.
- If ligation is possible, The ligase will immediately ligate the two DNA strands and the original sequence can be retained.
- If direct ligation is not possible, additional proteins are needed but insertions or deletions (i.e., called INDELS) are introduced.
- INDELs (insertions and deletions) can cause mutations, as the addition or removal of bases is often necessary for ligation during DNA repair. This process resolves the severe issue of double-strand breaks (DSBs) at the cost of introducing less severe mutations.

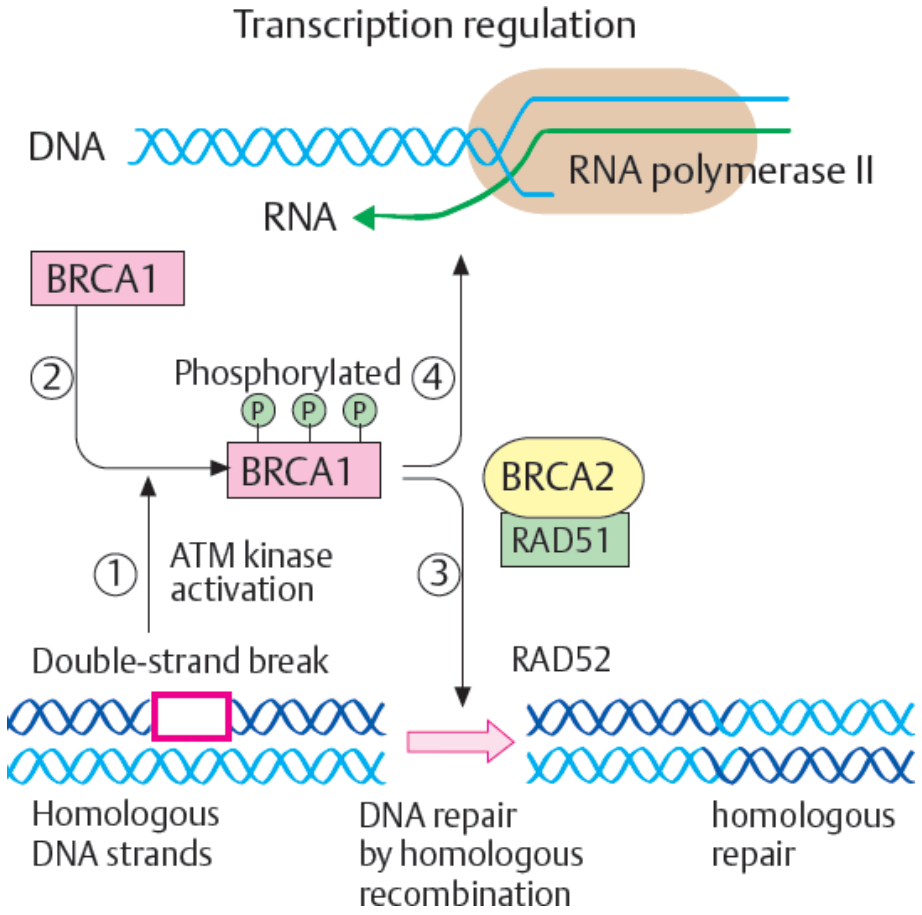
Homology-Directed Repair (HDR) Homology Repair (HR)

- Homology-directed repair (HDR), or homologous recombination, uses DNA sequences within the undamaged homologous chromosome from the other parent.
  - More accurate than NHEJ.
- In germline cells, HDR generates genetic diversity by producing different combinations of gene alleles
- Exchange between two homologous chromosomes, maternal and paternal, occurs as in recombination, involving the transfer of genetic material. The chromosome with the broken strand uses an intact strand from its homologous counterpart for repair, creating "patches" of DNA. This results in the repaired DNA incorporating a segment from the other chromosome, leading to an exchange of genetic material between the paternal and maternal chromosomes.



Breast Cancer and BRCA Genes

- 15% of breast cancer cases are hereditary. The rest are environmentally and hormonally induced.
- 10% of the 15% are caused by mutations in BRCA1 and BRCA2 genes are responsible for a portion of hereditary breast and ovarian cancers. These 2 genes are involved in DNA recombinational repair.
- BRCA1 activates homologous recombination repair of DNA double-stranded breaks.
- BRCA2 can recruit Rad51 to the ssDNA. radiation
- BRCA1 is also involved in transcription and transcription-coupled DNA repair.
- BRCA2 is also involved in ovarian cancer.



BRCA Genes: Key Players in DNA Repair and Genomic Stability

- BRCA1 and BRCA2 are essential for DNA repair. When a double-strand break occurs, the ATM kinase protein is activated, initiating a cascade of repair mechanisms. BRCA1 is activated by ATM and participates in repairing transcriptionally active genes. Additionally, BRCA1 activates BRCA2 and another protein, RAD51, both of which are involved in recombinational DNA repair.
- In summary, BRCA1 and BRCA2 are two critical genes involved in DNA repair, specifically recombinational DNA repair. BRCA1 activates BRCA2 and also plays a role in repairing transcriptionally active genes. Understanding the roles of these genes is essential, as they are key players in maintaining genomic stability.

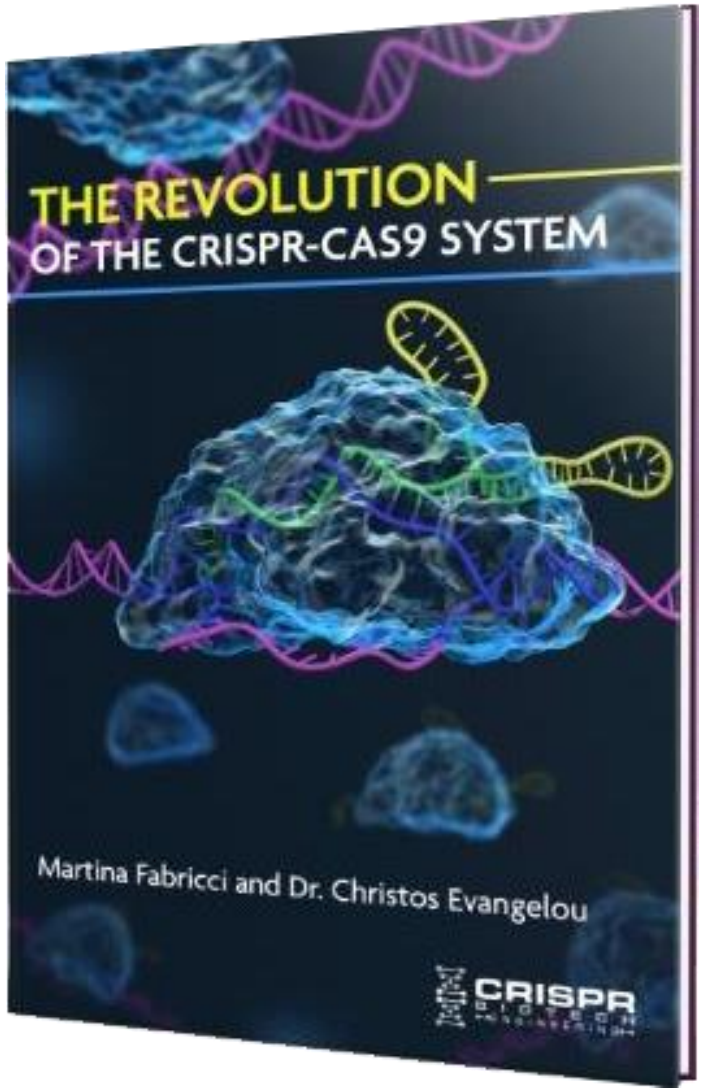
Wrap-up

Important table !

Type of DNA repair	Mechanism	Genes/proteins
Base excision repair	Removal of abnormal bases	DNA glycosylases
Nucleotide excision repair	Removal of thymine dimers and large chemical adducts	XP proteins, CSB
Mismatch repair	Correction of mismatched bases caused by DNA replication	MLH1, MSH2
Post-replication repair	Removal of double-strand breaks by HR or NHEJ	BRCA1, BRCA2

CRISPR-Cas9

casper the ghost



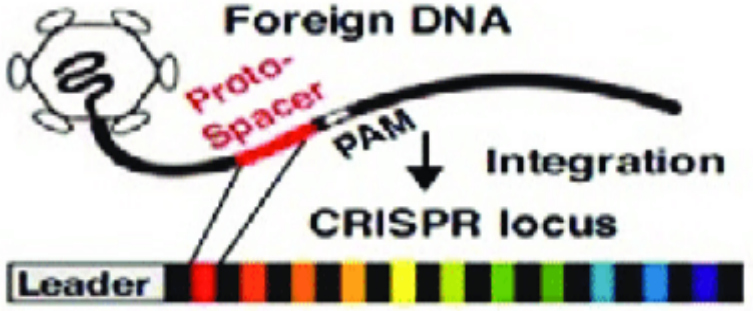
What is CRISPR/Cas9?

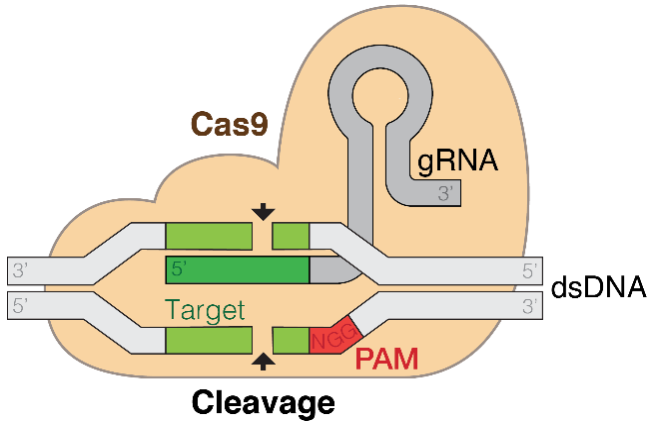
wondering where you heard this term before
Modified #3, Slide 6

متكتّلين في منطقة واحدة

spaces بينهم

- CRISPR: 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 (enzyme that cleaves neucleic acids) that can introduce double-strand breaks creating blunt-ended DNA fragments.
  - 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.

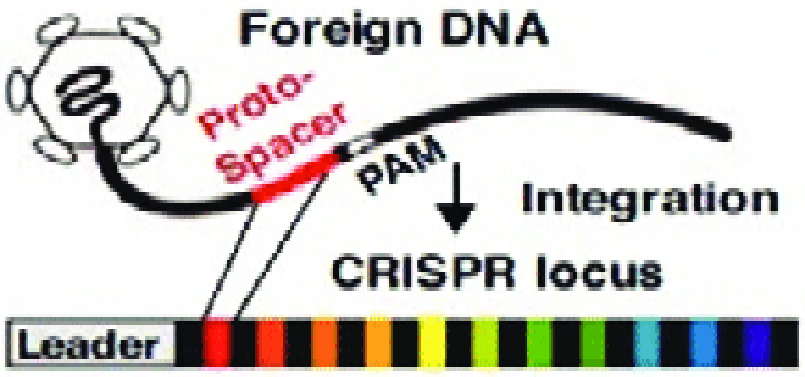




Modified #3, Slide #6 :

· The term “palindromic” means these sequences can be read the same way in the 5’ to 3’ direction on complementary strands.

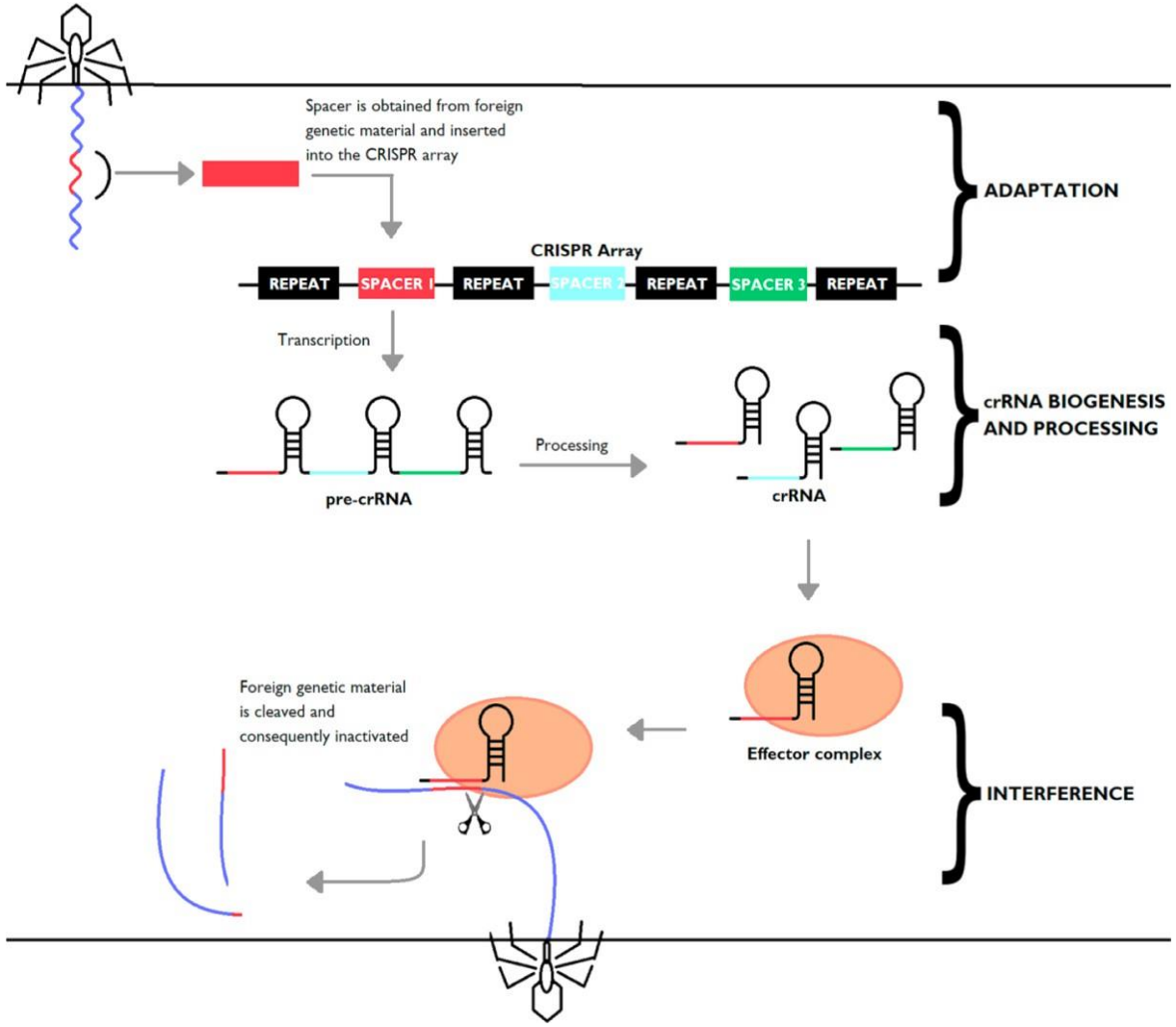
- Cas9 is an enzyme associated with CRISPR and is classified as a ribonucleoprotein. It has both protein and RNA components. The RNA part, called guide RNA (gRNA), directs Cas9 to the specific DNA sequence to be cut.



- The repeats (black regions in the image) are identical and separated by variable DNA sequences, hence “interspaced.” They are grouped together, or “clustered,” in the genome.

The biological function

- When a phage infects a bacterial cell, the cell degrades the phage DNA into smaller pieces using restriction endonucleases and integrates one of these fragments into the CRISPR cluster.
- The black repeats in the image are separated by bacteriophage DNA fragments.
- 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.



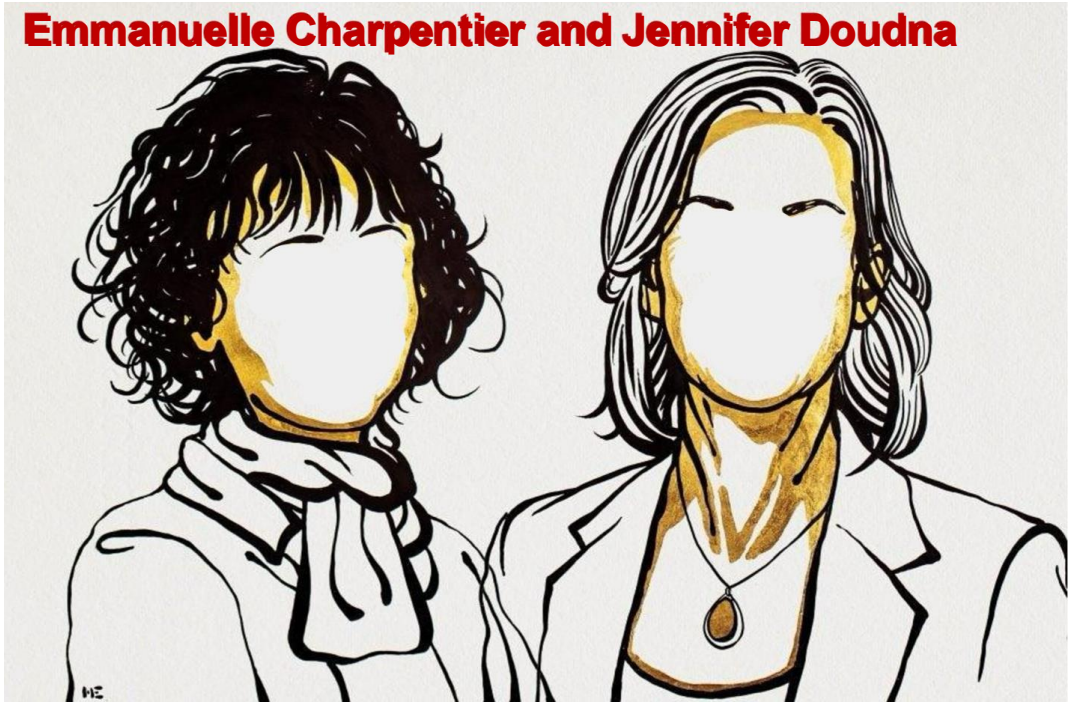
CRISPR Mechanism in Bacteria

- When a bacteriophage infects a bacterium, the bacterial CRISPR system incorporates a segment of the bacteriophage's DNA into its genome as a memory of the infection. This segment is stored within the CRISPR region of the bacterial DNA.

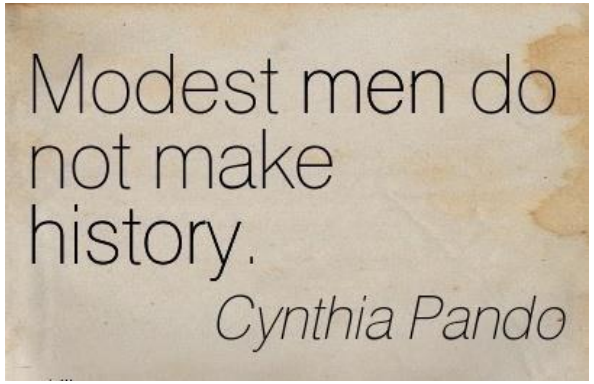
1. **Transcription and RNA Formation:**
   - The bacterium transcribes the CRISPR region into RNA, producing fragments that each contain a repeat sequence and a segment of the bacteriophage DNA.
2. **Loading onto Cas9:**
   - These RNA fragments are loaded onto the Cas9 enzyme. The guide RNA (gRNA) directs Cas9 to the complementary DNA sequence in the bacteriophage.
3. **Targeting and Cutting:**
   - When the bacteriophage reinfects the bacterium, the gRNA recognizes the matching DNA sequence in the bacteriophage genome. Cas9 binds to the target DNA and acts as a nuclease, cutting the bacteriophage DNA.
4. **Destruction of Bacteriophage DNA:**
   - The Cas9-mediated cut disables the bacteriophage DNA, preventing further infection and effectively "eliminating" the threat. This mechanism is akin to the immune system's memory cells in humans.

In2020...

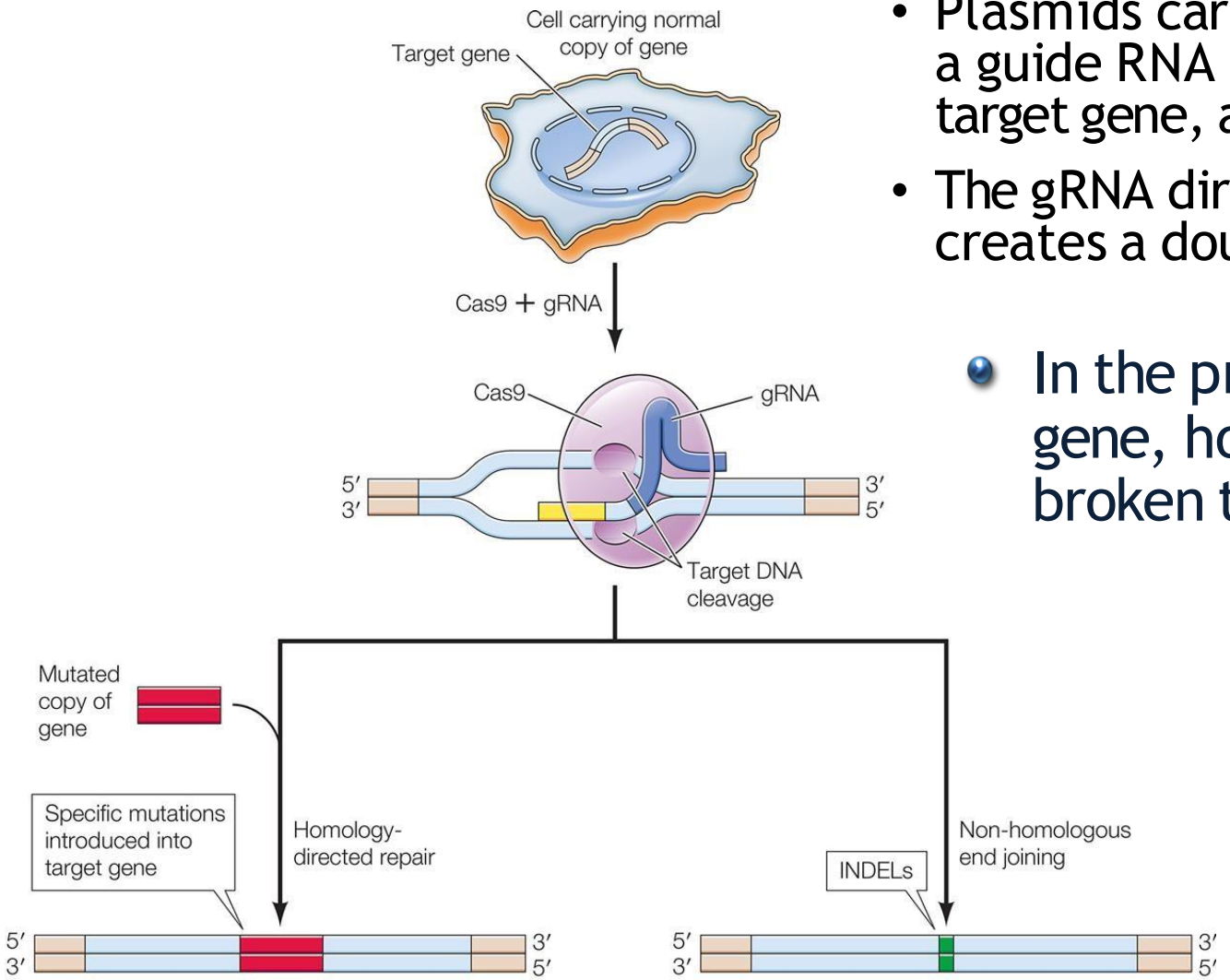

NAT·
MDCCC
XXXIII
OB·
MDCCC
XCVI
ALFR·
NOBEL


Emmanuelle Charpentier and Jennifer Doudna


TECHNOLOGY NETWORKS EXPLORES
THE
CRISPR
REVOLUTION
Francisco
Mojica
Article
Francis Mojica: The Modest Microbiologist Who Discovered and Named CRISPR
Published: October 14, 2019 | Molly Campbell


Modest men do not make history.
Cynthia Pando

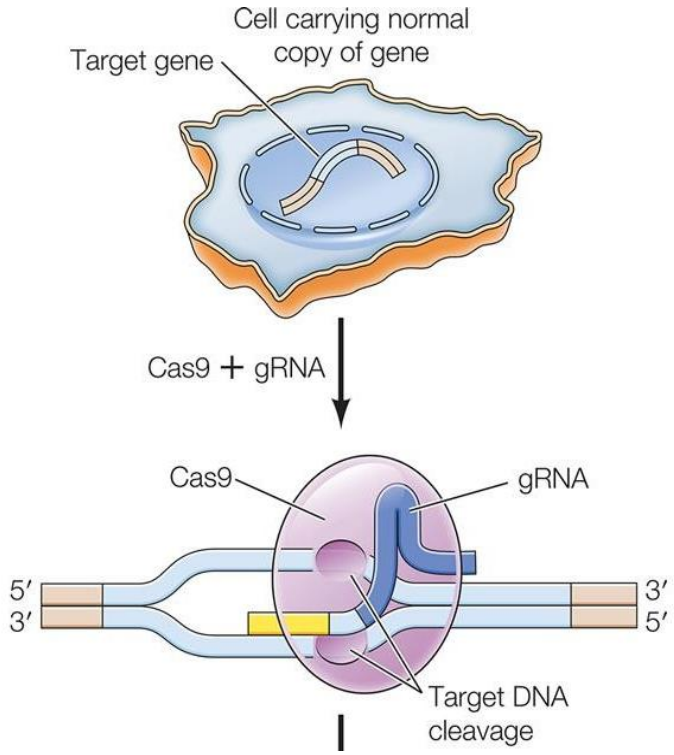
The experiment



- Start with cells.
- Plasmids carrying genes for Cas9 and a gene expressing a guide RNA (gRNA), i.e. sequences homologous to the target gene, are introduced into the cells.
- The gRNA directs Cas9 to the target gene and Cas9 creates a double-stranded break.

- In the presence of a homologous copy of the gene, homology-directed repair replaces the broken target gene with the mutated copy.
- In the absence of a homologous copy of the gene, non-homologous end joining reseals the broken DNA introducing Insertion/deletion mutations (INDELS) that make the gene nonfunctional.

The experiment (Dr's Notes)



- We start with normal human cells; خلايانا ما فيها Cas9 .
- A plasmid is introduced into the cell containing:
  - The gene encoding Cas9
  - A gene expressing the guide RNA (gRNA)
- The gRNA undergoes transcription only (**no translation**) and is complementary to the target gene

❖ **Let's assume we have a gene that causes cancer and we want to stop it from working.**

- **After plasmid entry:**

1. Cas9 is expressed
2. The complementary gRNA is expressed
3. The gRNA binds to Cas9, forming a complex.
4. This complex is directed to the target gene, where Cas9 performs cleavage.

- As a result, the gene becomes **inactive** due to a double-strand break.

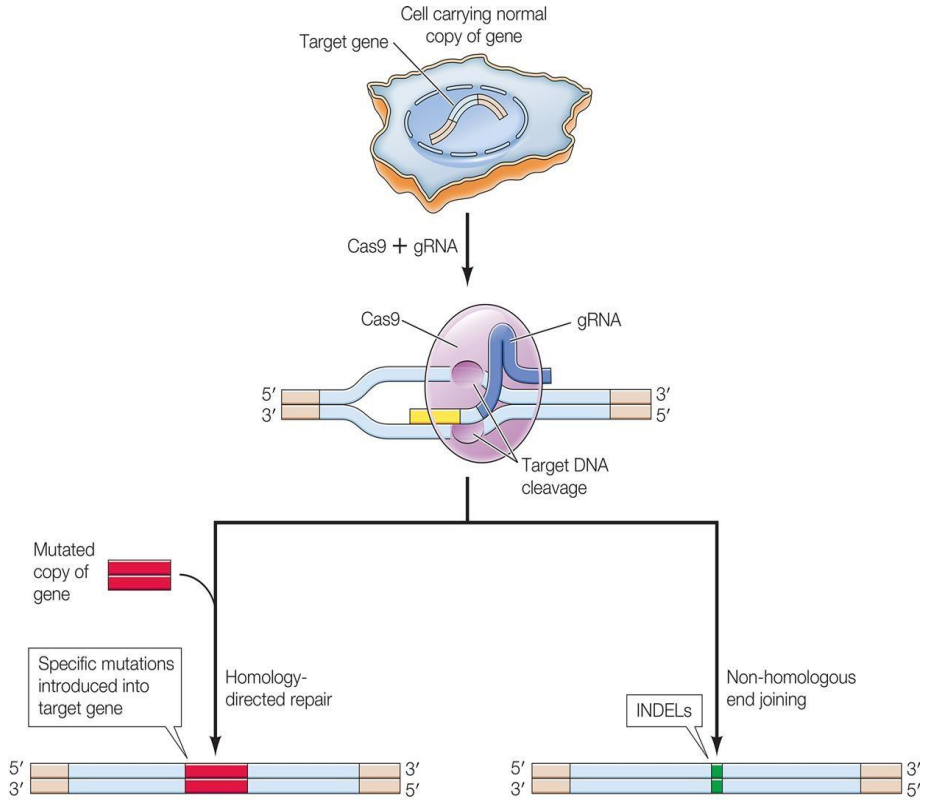
The experiment (Dr's Notes)

❖ **Assume that in the same plasmid we introduced:**

1. the Cas9 gene
2. the DNA sequence that produces the gRNA which guides Cas9 to the target gene
3. and a good (normal) copy of the same gene

❑ **What does Cas9 do?**

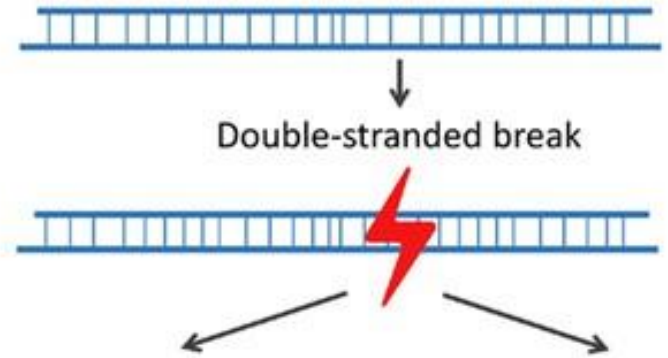
1. Cas9 is guided to the target gene in the genome and performs cleavage.
2. After this cleavage, we now have a double-strand break.
3. This leads to activation of the repair system.
4. The cell uses the homologous copy as a template and performs
5. homology-directed repair, replacing the broken gene with the correct copy.



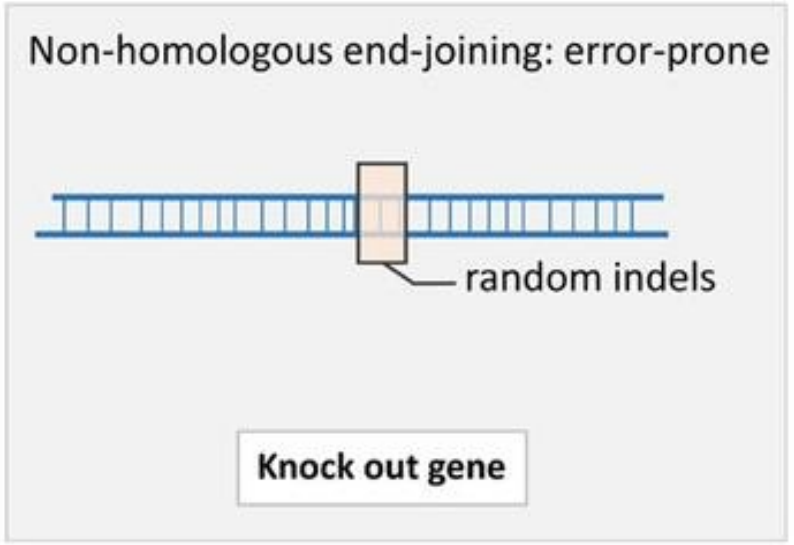
Important: listen to Dr's explanation

The consequences of DNA damage repair

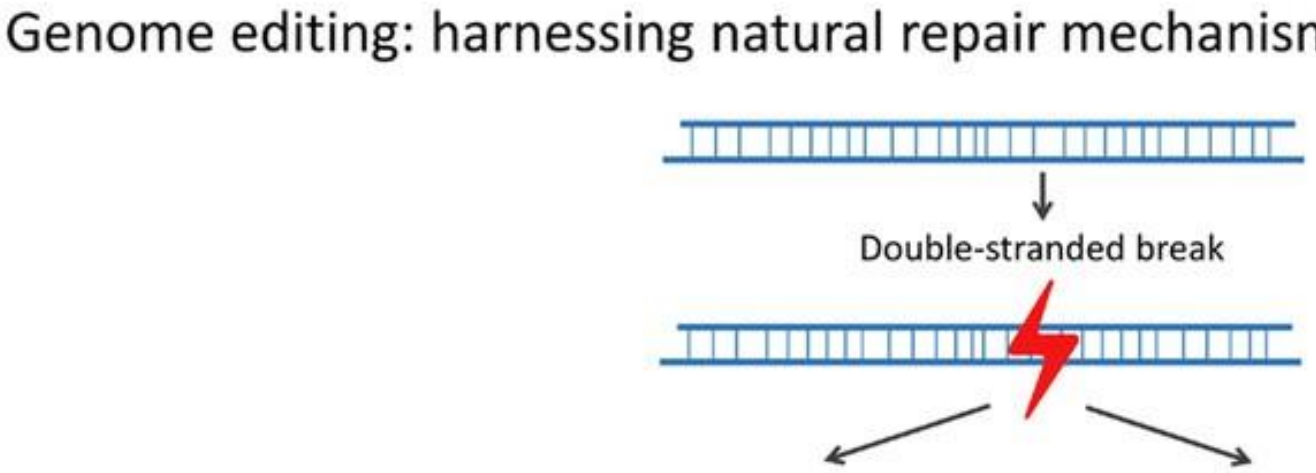
Genome editing: harnessing natural repair mechanisms to modify DNA



- double strand DNA break → non-homologous end joining might reconnect DNA (super glue), but causes **indel mutation** → so gene works but we're بنخربه



The consequences of DNA damage repair

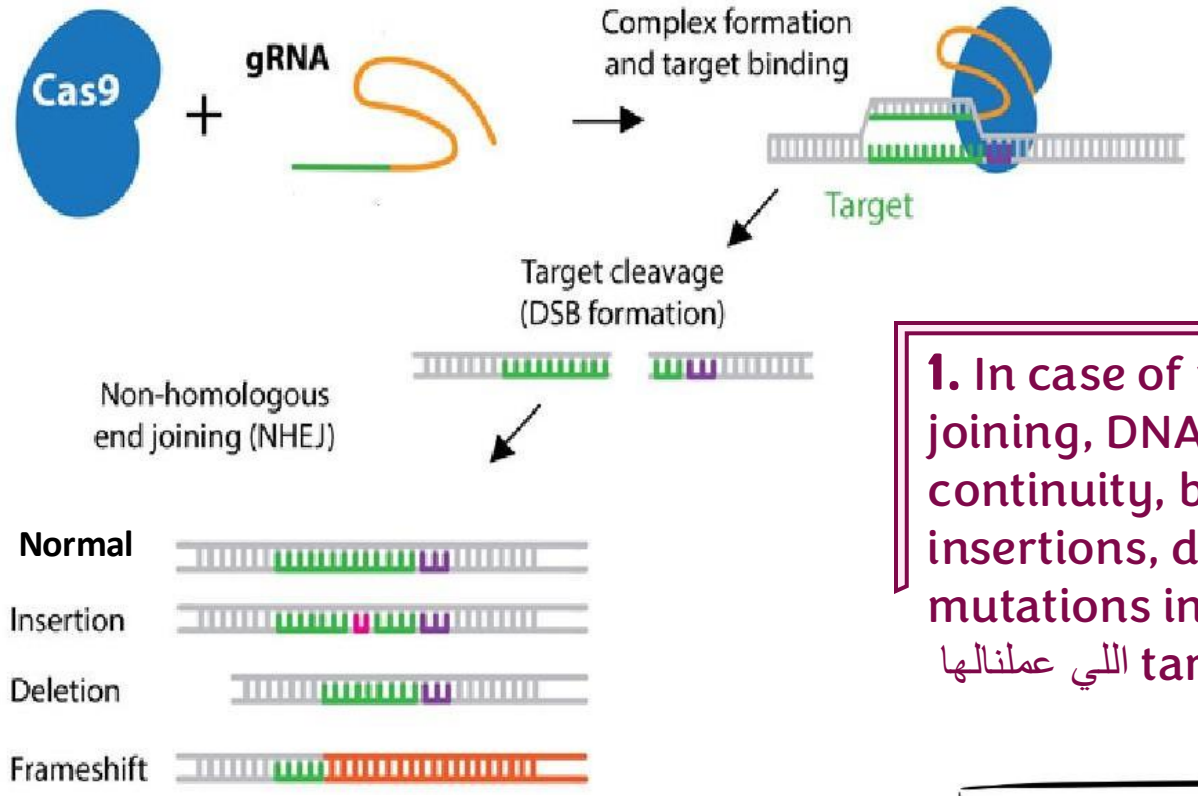


Or: activation of homology-directed repair (HDR)

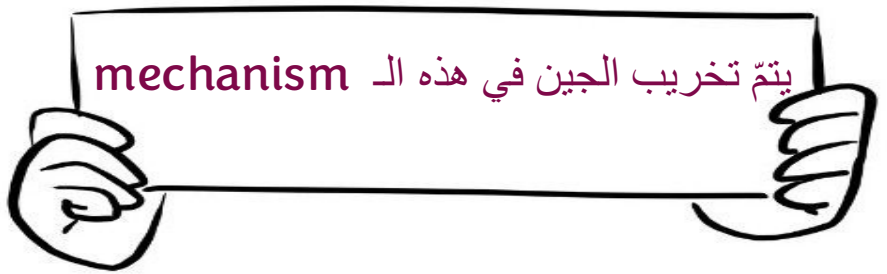
- In this case → we do replacement
- Targeted gene undergoes cleavage

→ replaced with another copy of the gene, so gene gets swapped, not just بنخربه

Gene editing

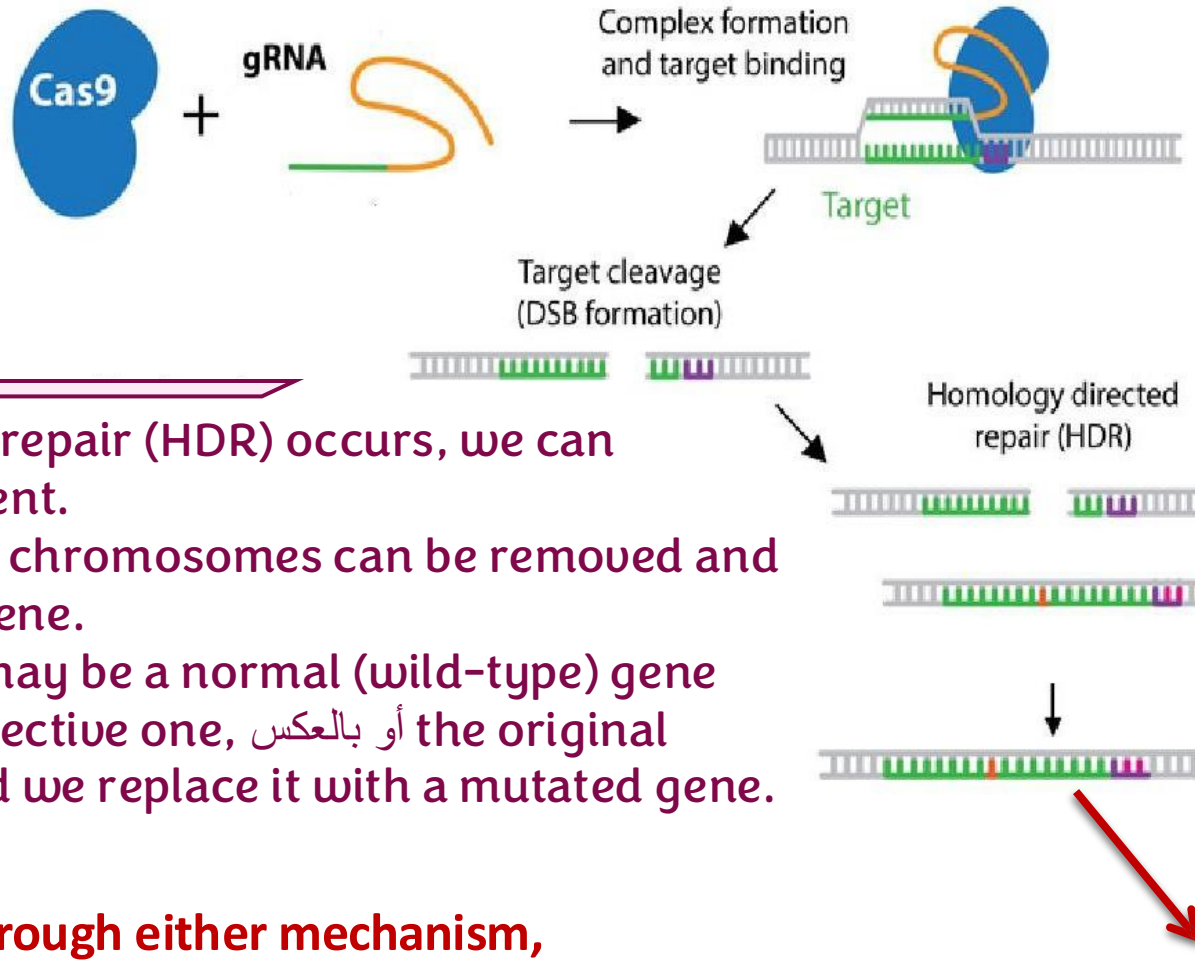


1. In case of non-homologous end joining, DNA repair may restore continuity, but it often results in insertions, deletions, or frameshift mutations in the genes اللي عملنالها targeting.



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

Gene editing



2. If homology-directed repair (HDR) occurs, we can perform gene replacement.
The gene present on our chromosomes can be removed and replaced with another gene.
This replacement gene may be a normal (wild-type) gene inserted instead of a defective one, أو بالعكس the original gene may be normal and we replace it with a mutated gene.

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).

Using CRISPR-Cas6 for Gene Editing:

Further Explanation, u can skip

1. Gene Knockout for Studying Functions:

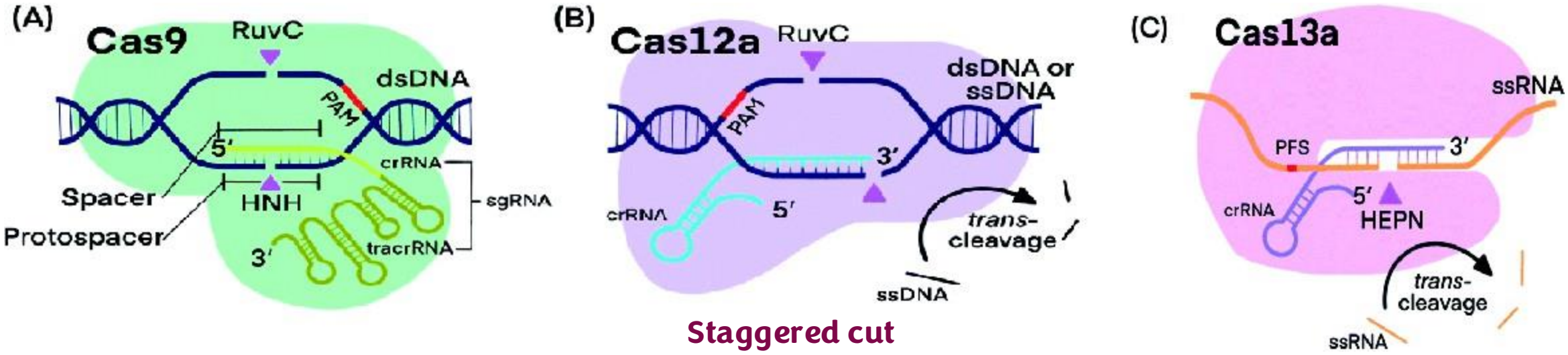
- CRISPR-Cas9 can disrupt a gene to study its function. Cas9, guided by gRNA, cuts the DNA, and the repair via non-homologous end joining (NHEJ) introduces mutations.
  - **Application:** In diseases caused by a specific gene, disrupting it can stop the production of harmful proteins. For example, in oncogenic cells, disabling the mutant gene ensures only the normal gene remains functional.

2. Gene Replacement Using Homology-Directed Repair (HDR):

- CRISPR-Cas9 can also replace defective genes by activating the HDR pathway with a provided homologous DNA template.
- **Mechanism:** Cas9 makes a targeted cut in the DNA, and the cell uses the homologous template to insert a functional gene, replacing the damaged or defective gene.
- **Additional Capability:** HDR can also be used to introduce specific mutations or edits, in addition to replacing or adding new genes.

Other Cas enzymes

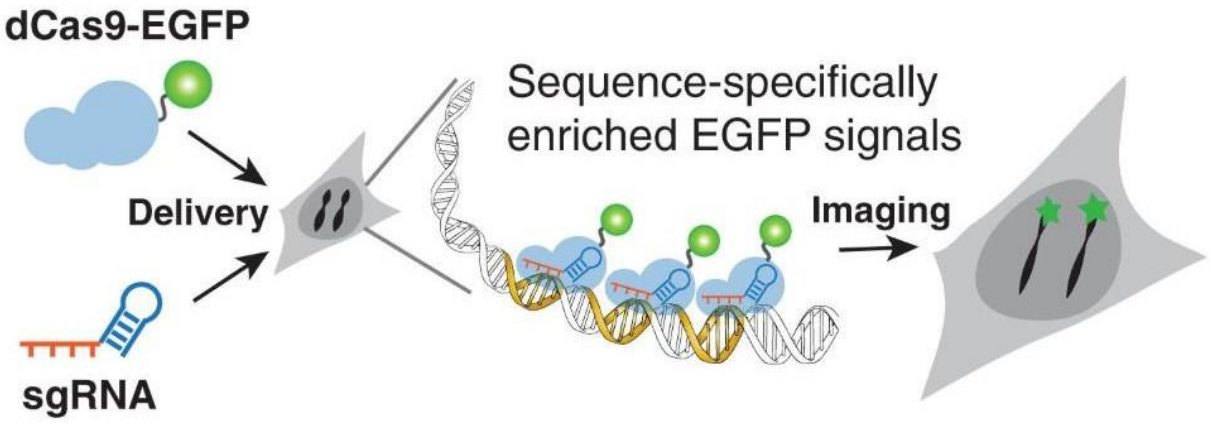
- Cas9: introduces cuts on both DNA strands, resulting in blunt ends.
- Engineered Cas9: can make single-stranded DNA cuts.
- Cas12a: can make staggered cuts, resulting in overhangs in the DNA strands.
- Cas13a: an RNA endonuclease, targets specific RNA molecules.

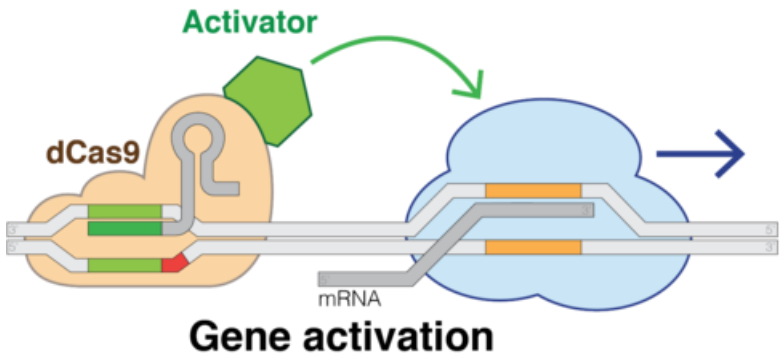


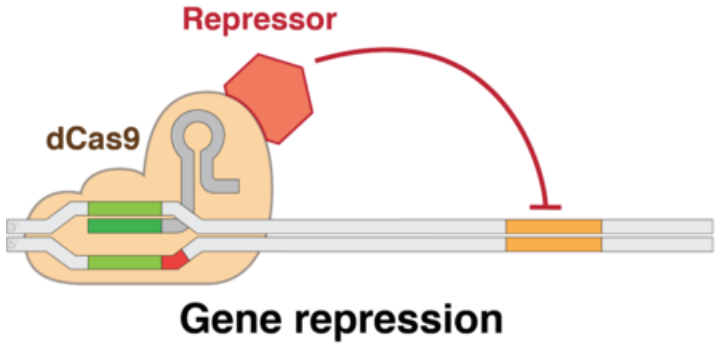
Staggered cut

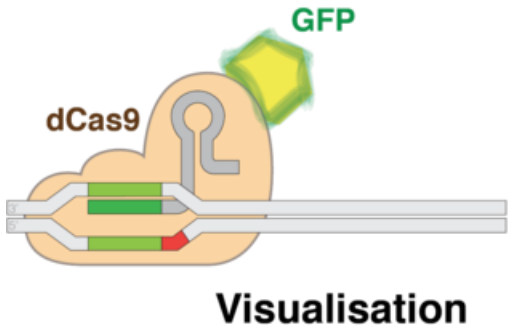
Other creative uses

- Transcriptional regulatory factors can be added to an enzymatically inactive or “dead” Cas9 (dCas9), enabling these factors to turn genes on or off.
- GFP can be added to dCas9 to find a particular stretch of DNA in the cell or even visualize the three-dimensional architecture of a chromosome.



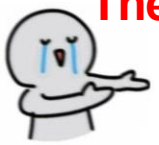






Applications of Modified Cas6 Enzymes:

1. **Dead Cas9 (dCas9):**
   - dCas9 is an enzymatically inactive version of Cas9 that can still bind to DNA but does not cut it. By guiding dCas9 to a specific region with gRNA, various functional proteins can be attached to dCas9 to manipulate gene expression or study chromatin structure.
2. **Gene Activation:**
   - Transcription activators can be attached to dCas9 to activate a specific gene. This approach can be used to study gene expression or reprogram cells by activating desired genes.
   - **Example:** In oncogenic cells, the same technique can be applied, but with transcriptional inhibitors attached to dCas9, effectively repressing oncogenic gene expression.
3. **Chromosome and Chromatin Study:**
   - By attaching GFP (Green Fluorescent Protein) to dCas9, specific regions of the genome can be visualized. This allows researchers to study chromatin structure (e.g., condensed vs. relaxed states) in real-time, as only targeted regions become visible.



The rest of the slides, starting from this one, illustrate some examples on uses for techniques previously discussed; understand the concept of gene therapy. Specific details about each study are not part of the lecture.

FDA NEWS RELEASE

FDA Approves First Gene Therapies to Treat Patients with Sickle Cell Disease

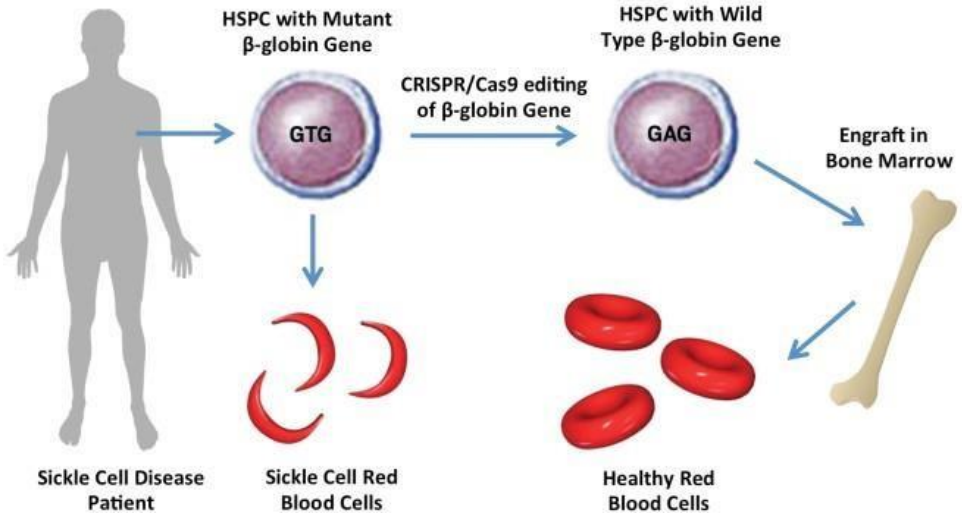
For Immediate Release: December 08, 2023

https://www.fda.gov/news-events/press-announcements/fda-approves-first-gene-therapies-treat-patients-sickle-cell-disease

Selection-free genome editing of the sickle mutation in human adult hematopoietic stem/progenitor cells

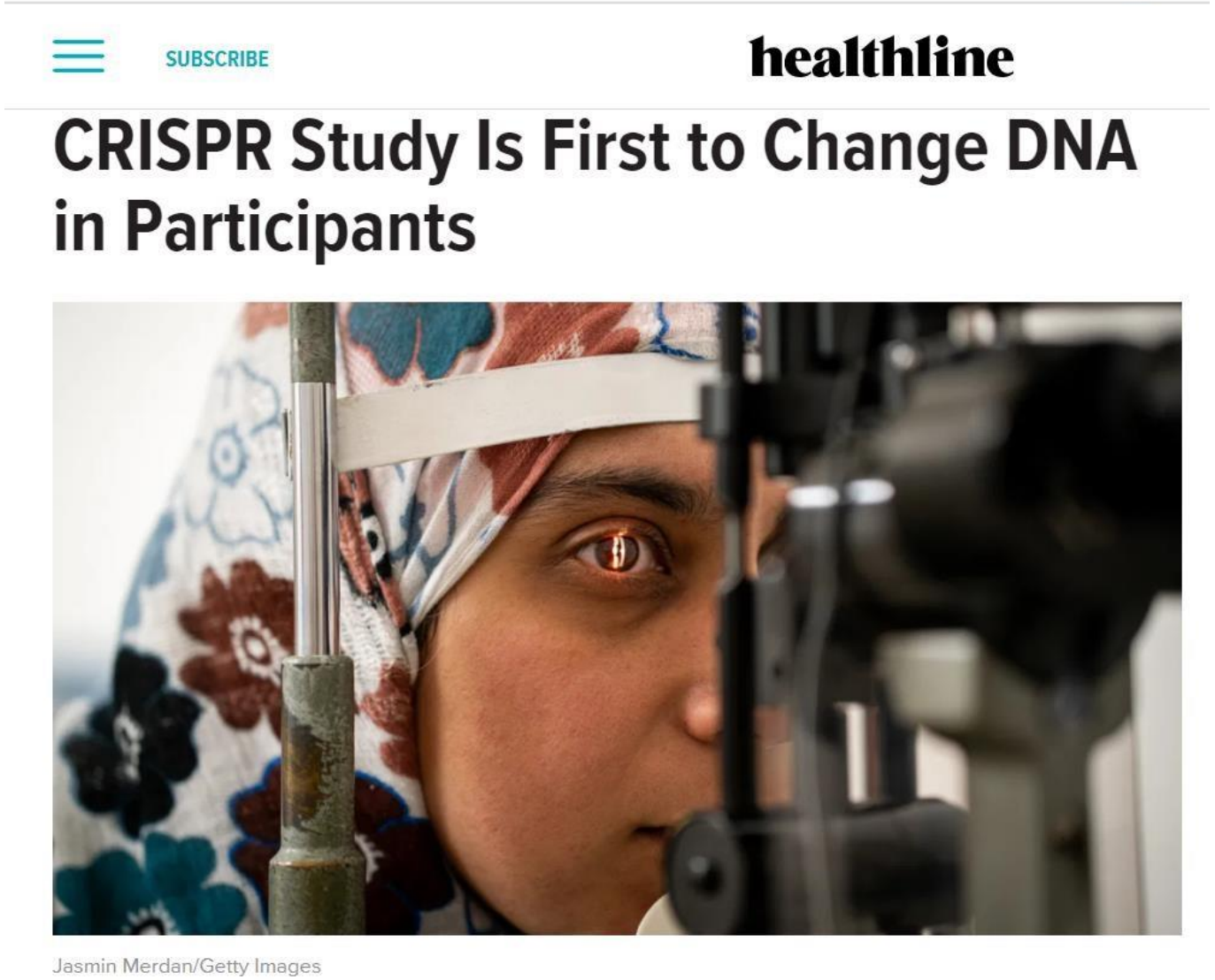
MARK A. DEWITT, WENDY MAGIS, NICOLAS L. BRAY, TIANJIAO WANG, JENNIFER R. BERMAN, FABRIZIA URBINATI, SEOK-JIN HEO, THERESE MITROS, DENISE P. MUÑOZ, [...], AND JACOB E. CORN +5 authors Authors Info & Affiliations

SCIENCE TRANSLATIONAL MEDICINE • 12 Oct 2016 • Vol 8, Issue 360 • p. 360ra134 • DOI: 10.1126/scitranslmed.aaf9336



The bright side of science

https://www.healthline.com/health-news/crispr-study-is-first-to-change-dna-in-participants



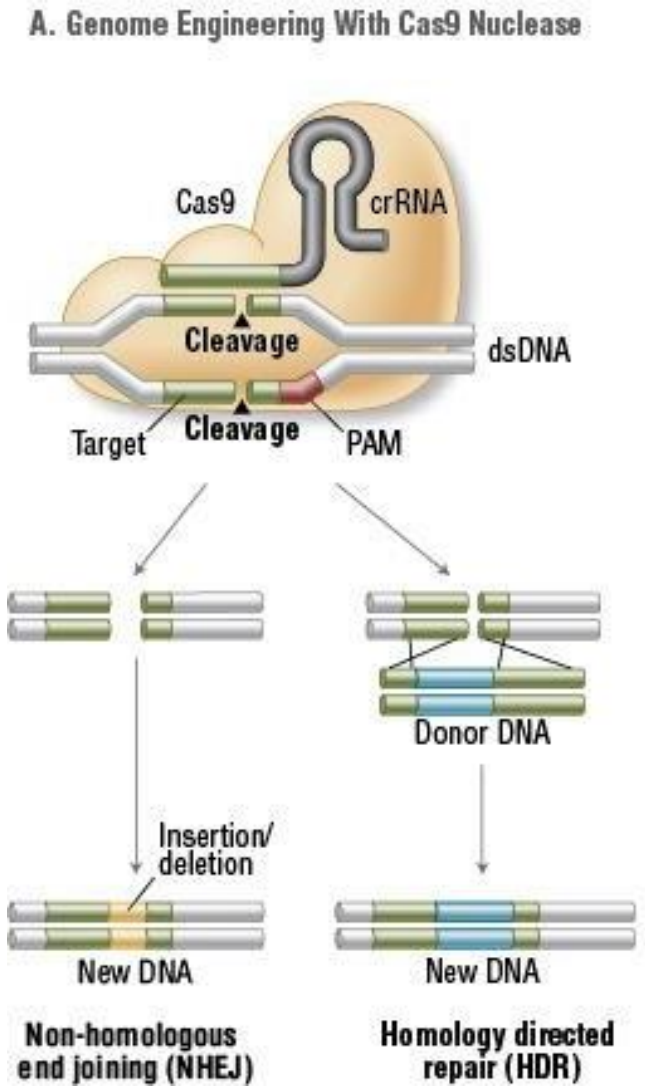
Controversial issue

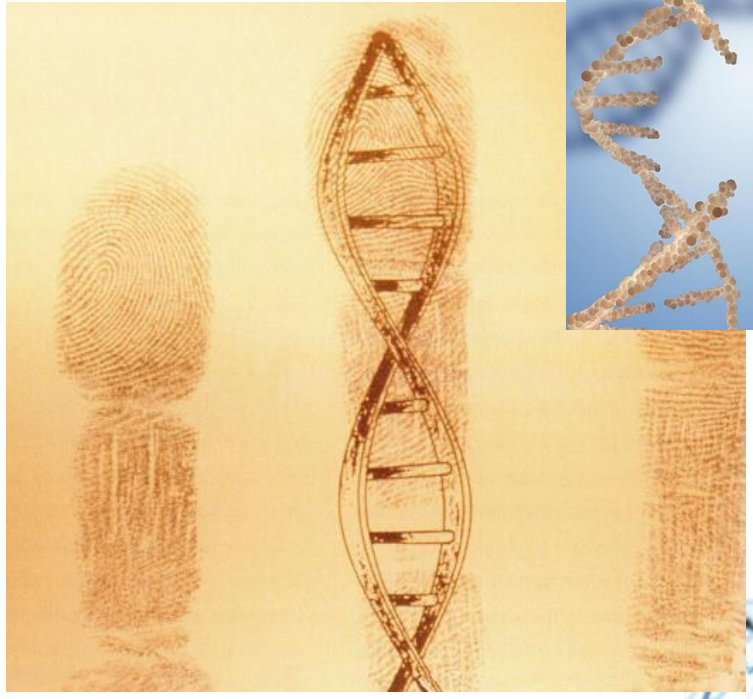
Gene repair

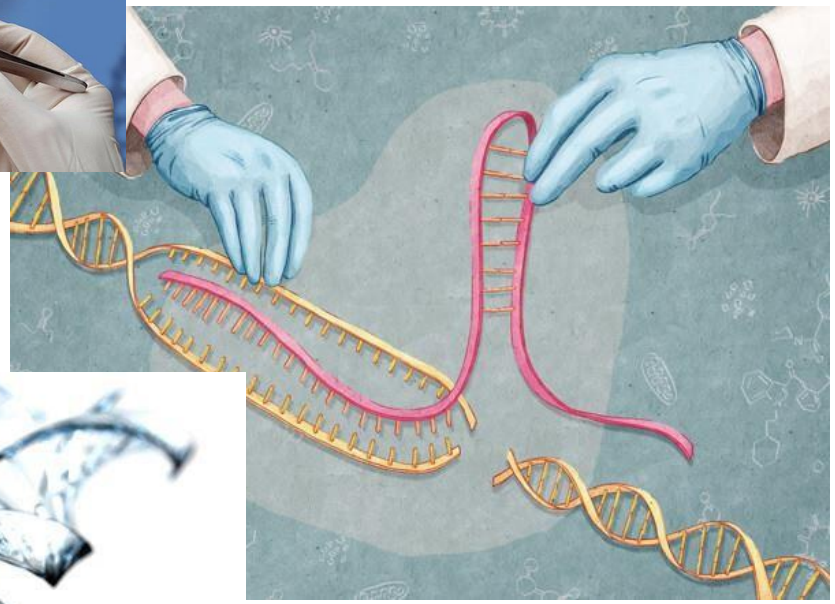
UK scientists ready to genetically modify human embryos

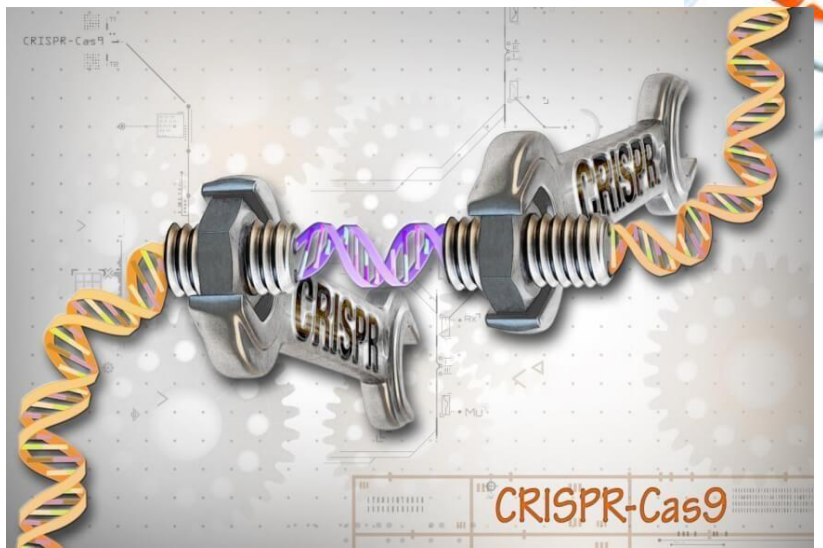
Researchers awaiting approval to use gene editing in embryos, which they hope will help them understand early stage life and improve fertility treatment

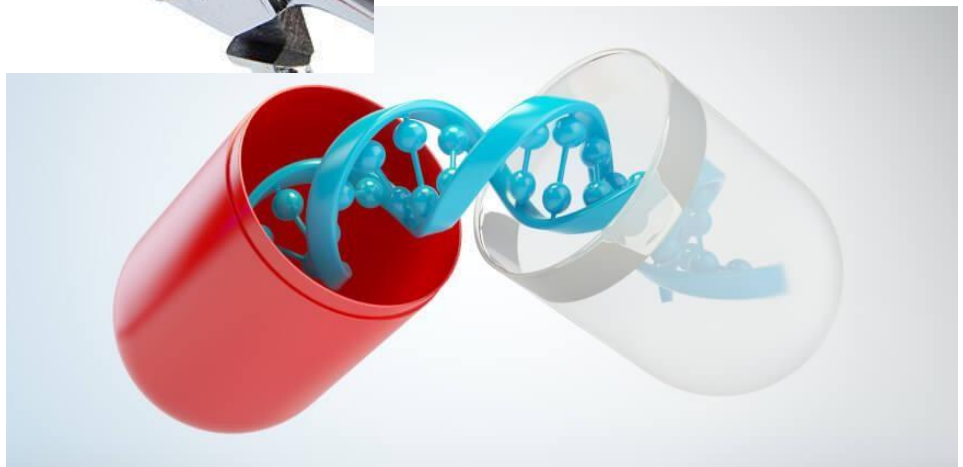
https://www.theguardian.com/science/2016/jan/13/uk-scientists-ready-to-genetically-modify-human-embryos





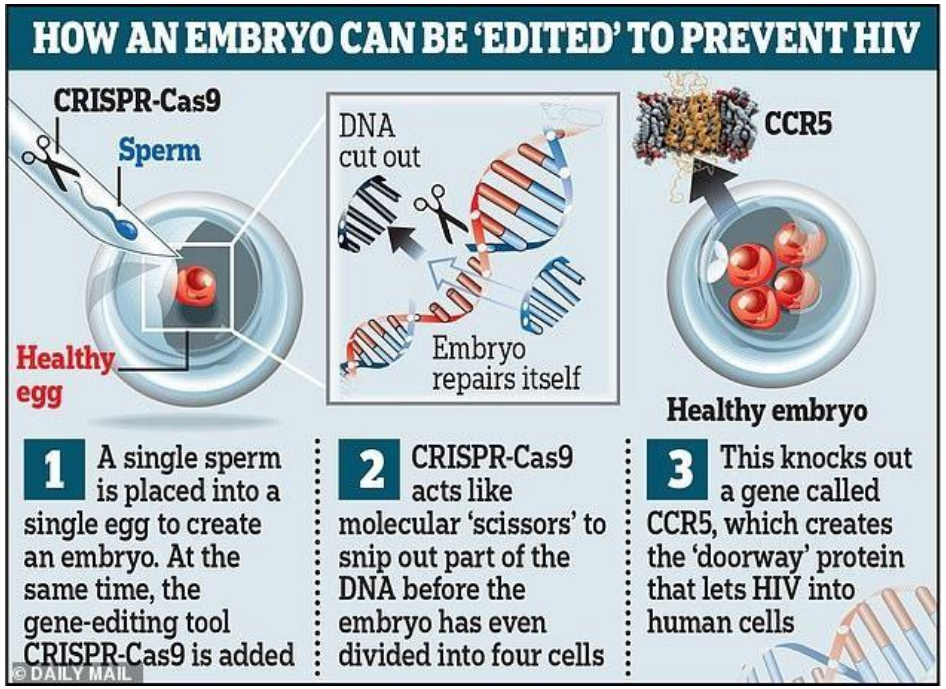



CRISPR-Cas9
CRISPR
CRISPR
CRISPR-Cas9



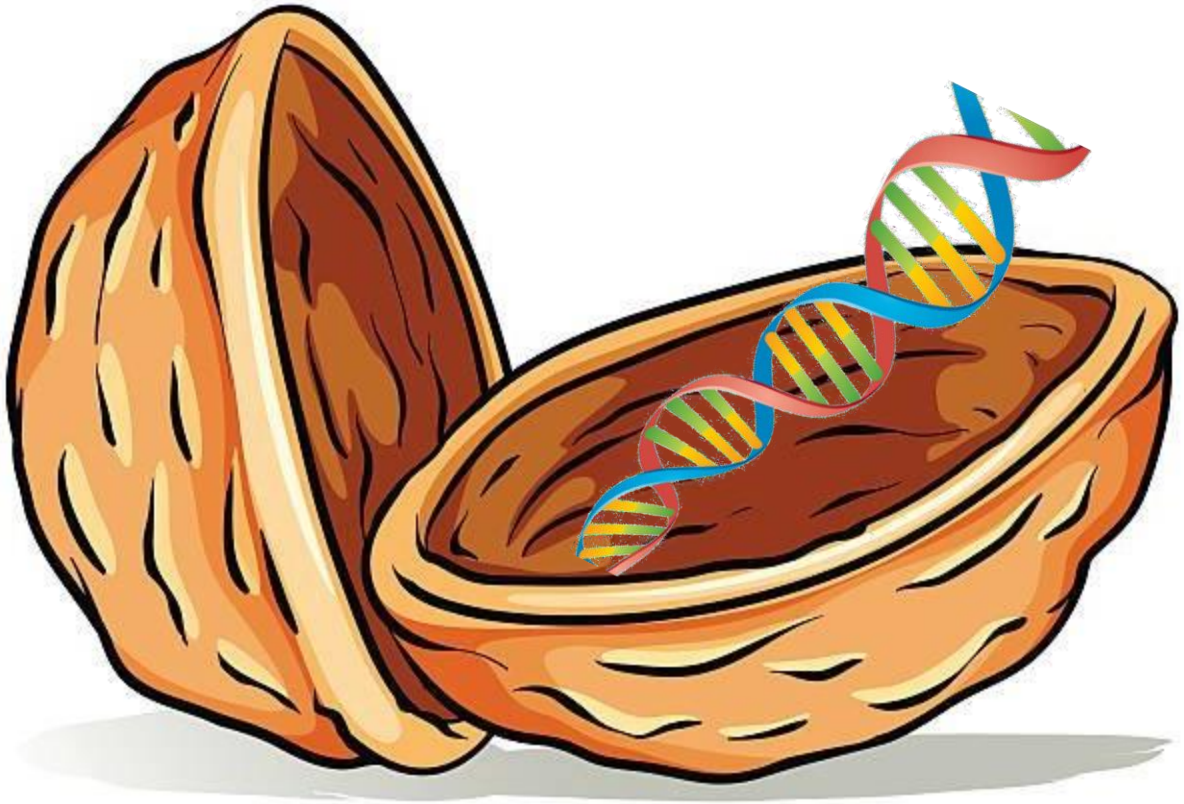
The dark side of science

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

This is molecular biology in a nutshell



رسالة من الفريق العلمي:

﴿فَلِلَّهِ الْحَمْدُ﴾

وبهذا نُنهي موديفايدات المولي بحمد الله تعالى

أكرِموا الفريق العلميّ بدعواتِكم، لعلّ الله أن يكتب بها التوفيق لكم ولهم

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	Slide 8	the more the MSI (more repeats) the more aggressive the tumor is	the more the MSI (more repeats) the less aggressive the tumor is
V1 → V2			