Molecular Biology (13)

DNA mutations and repair mechanisms
Gene editing by CRISPR-Cas9

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Second year, First semester, 2024-2025

Mutations

What are mutations?

- A genetic mutation is a change in the genetic material.
  - Somatic mutations occur in somatic cells and are not transmitted.
  - Germline mutations occur in gametes and are heritable.

- The damaging effect of mutations has variable *sizes*.
  - Micromutations involve small regions of the DNA.
  - Macromutations involve chromosomes.

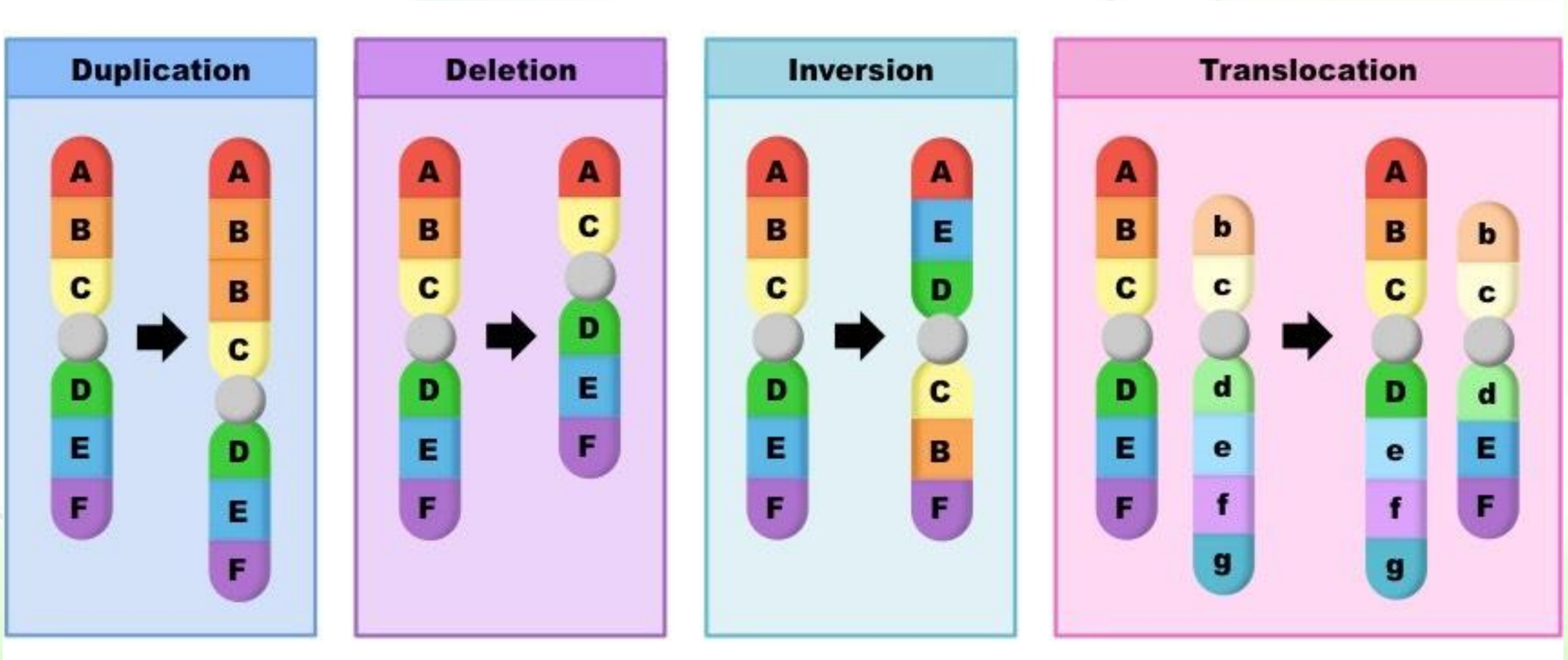
Causes of DNA mutations

- DNA mutations can arise spontaneously or can be induced.
- Spontaneous mutations are naturally occurring and arise in all cells.
  - They arise from a variety of sources, including errors in DNA replication and spontaneous lesions.
- Induced mutations are produced when an organism is exposed to a mutagenic agent (or mutagen).
  - Some mutagens are carcinogens (cancer-causing)
    - Ionizing radiation

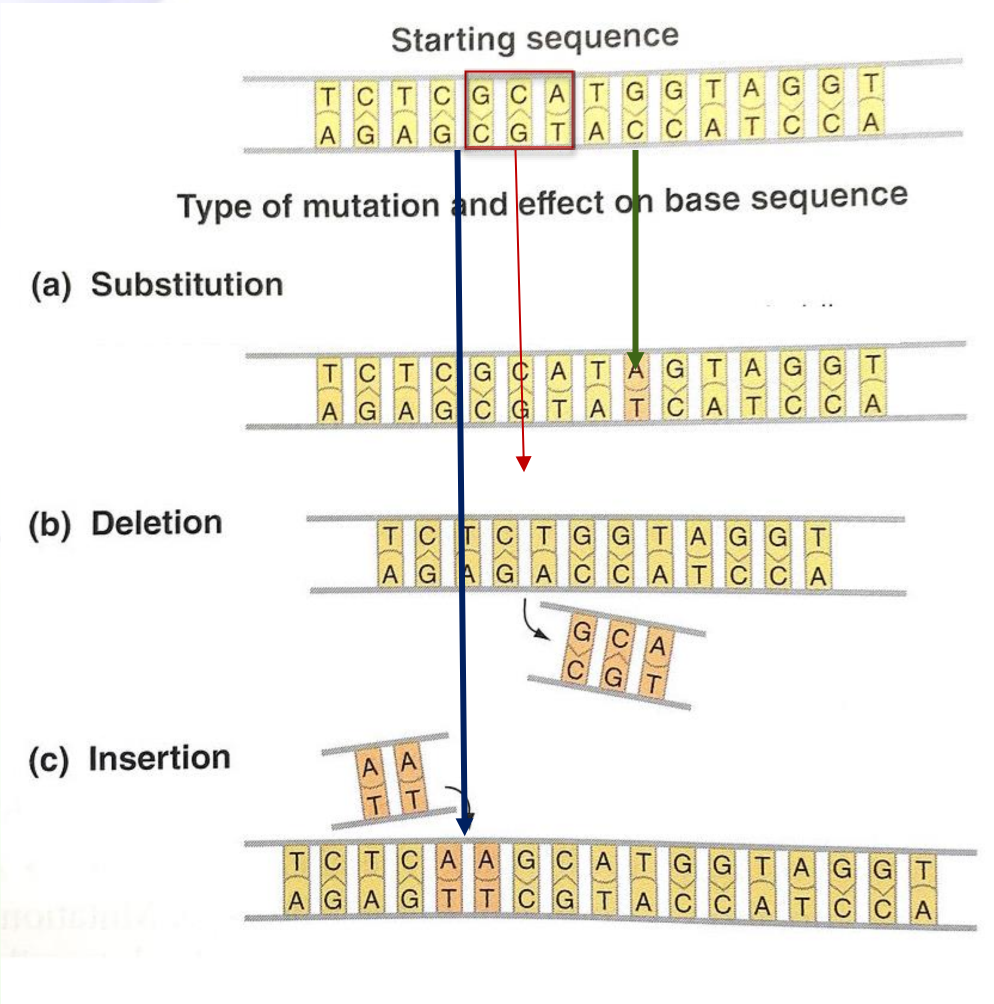
Macromutations

at the chromosomal level

- Translocations
- Inversion of DNA segments
- Duplications
- Deletions

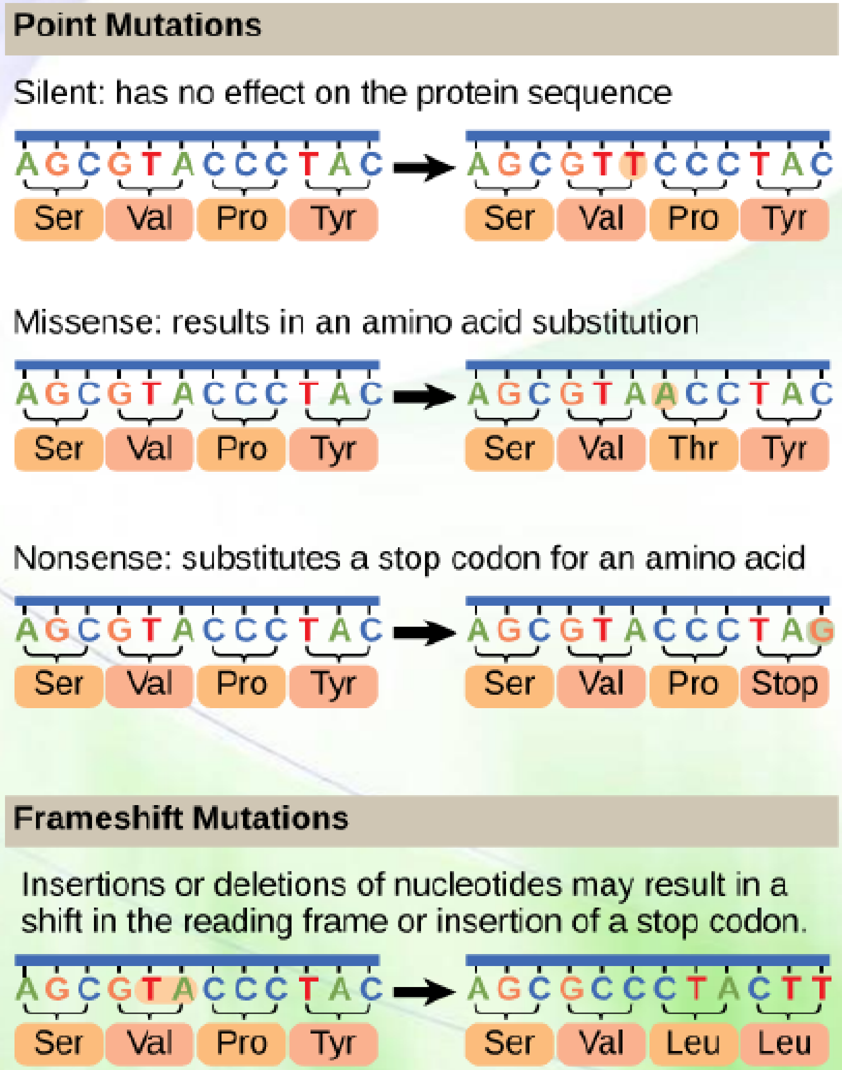


Types of micromutations



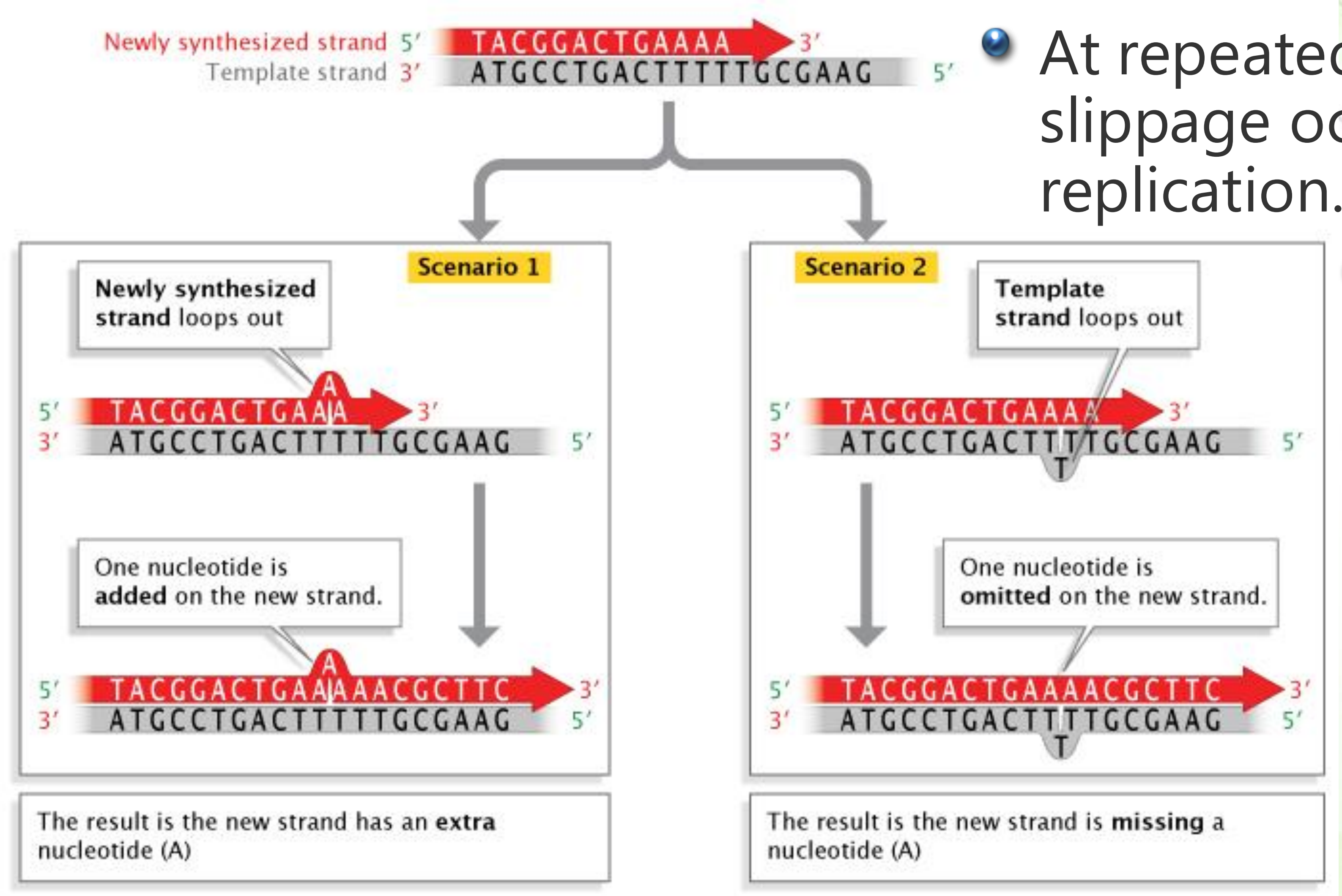
- Point mutations
  - The most common and include substitutions, insertion, and deletion
- Deletions or insertions of a few nucleotides to long stretches of DNA

Point mutations



- A point mutation occurs in a genome when a single base pair is added, deleted, or changed.
- *Trillions of mutations happen in our DNA daily*.

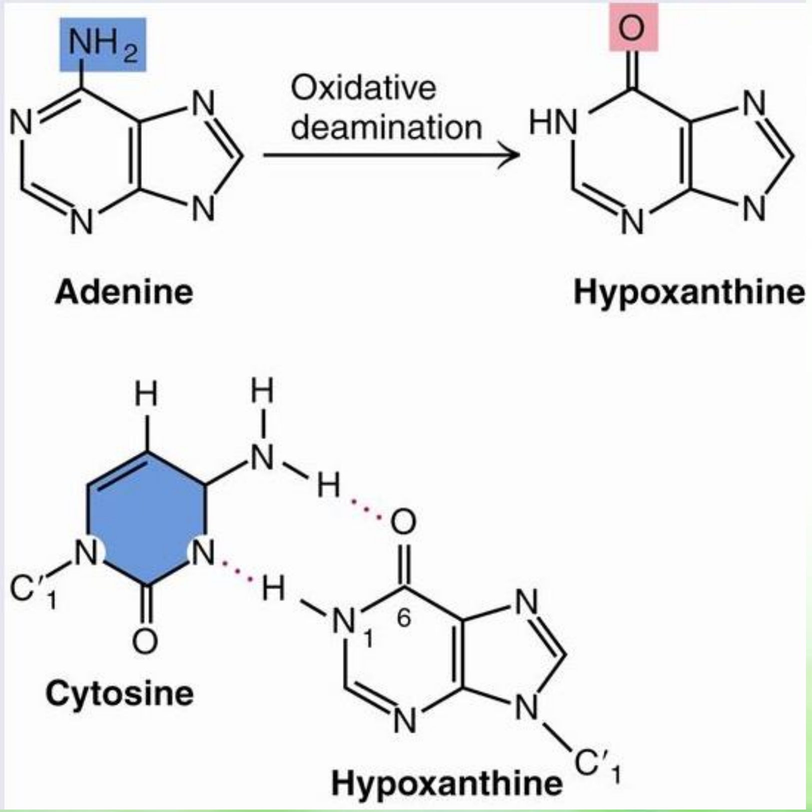
Repeated sequences, DNA replication, and strand slippage

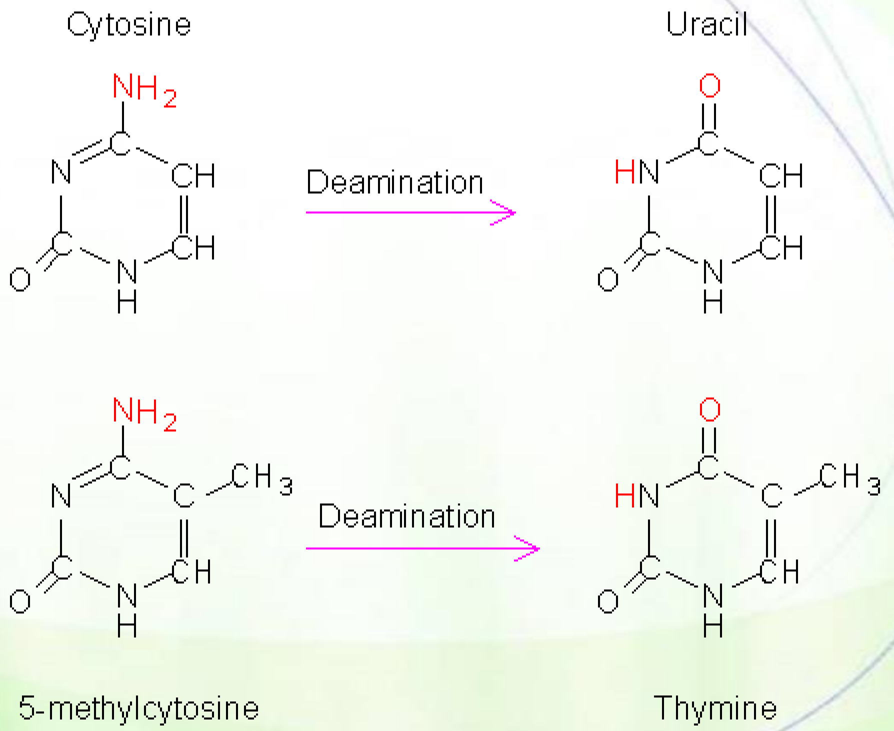


- At repeated sequences, strand slippage occurs during DNA replication.
- This results in adding or deleting a nucleotide on the newly synthesized strand.

Deamination (spontaneous)

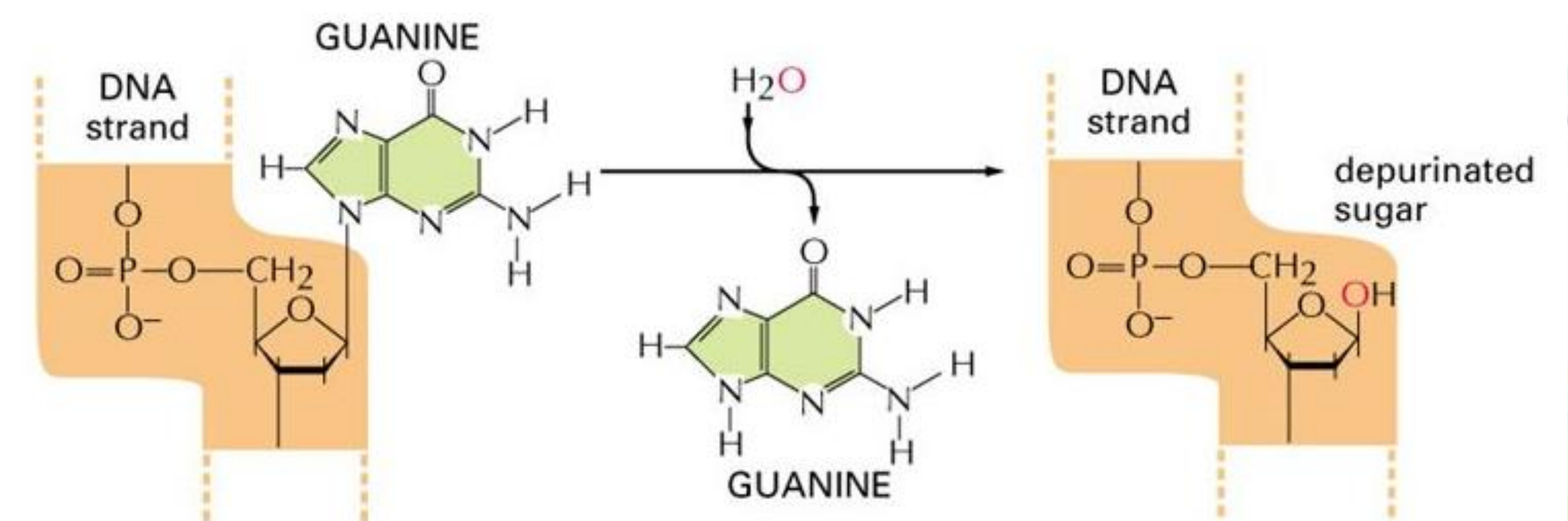
- The deamination of cytosine yields uracil.
- The deamination of methylated cytosine yields thymine.
- The deamination of adenine yields hypoxanthine.





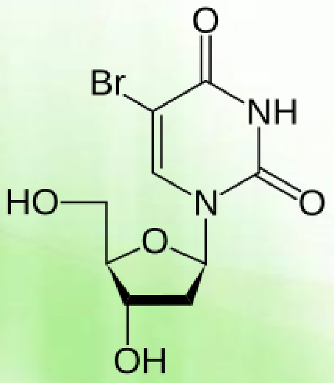
Depurination (spontaneous)

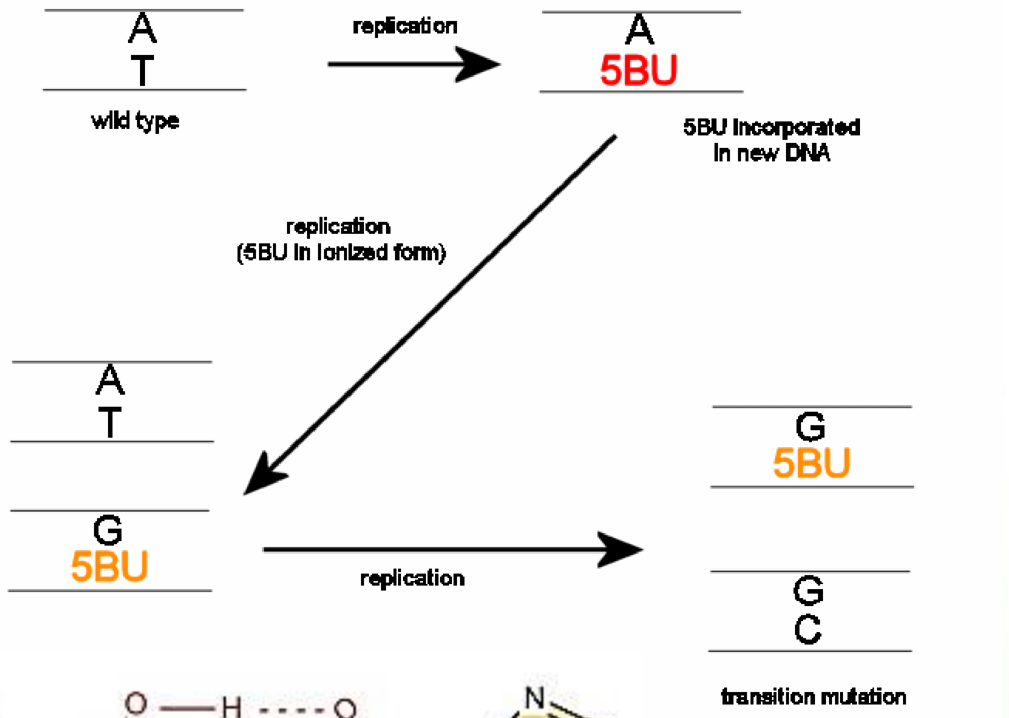
- Cleavage of the glycosidic bond between the base and deoxyribose creates an apyrimidinic or apurinic site (AP site).
- During replication, a random base can be inserted across from an AP site resulting in a mutation.

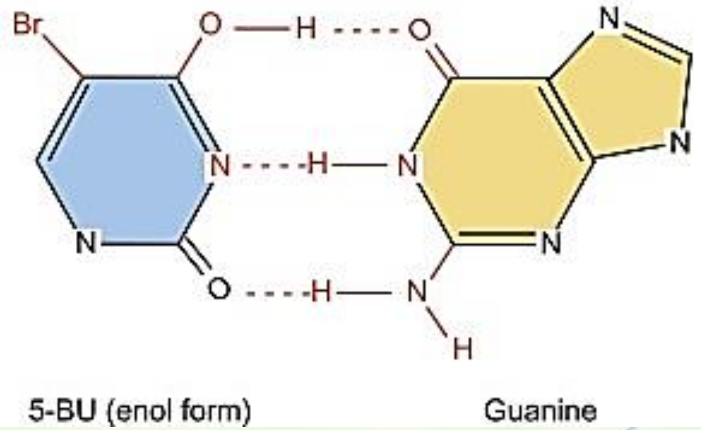


Incorporation of base analogs (induced)

- Base analogs have a similar structure to normal nucleotides and are incorporated into DNA during replication.
- 5-bromouracil (5-BU), an analog of thymine, pairs with adenine, but, when ionized, it pairs with guanine.
- Its deoxyriboside derivative (5-bromo-2-deoxy-uridine) is used to treat neoplasms.







Repair mechanisms

- Prevention of errors before they happen
- Direct reversal of damage
- Excision repair pathways
  - Base excision repair
  - Nucleotide excision repair
  - Transcription-coupled repair
- Mismatch repair and post-replication repair
- Translesion DNA synthesis
- Recombinational repair

Prevention of errors before they happen

Reactive oxygen species

- Enzymes neutralize potentially damaging compounds before they even react with DNA.
  - Example: detoxification of reactive oxygen species and oxygen radicals.

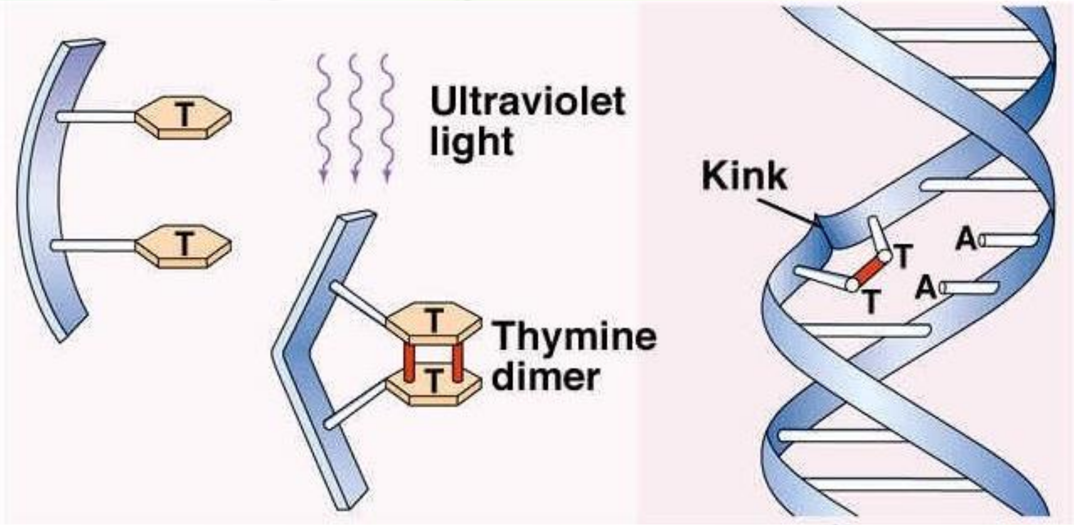
$$2\,O_2^{\cdot-} + 2\,H^+ \xrightleftharpoons{\text{Superoxide dismutase}} O_2 + H_2O_2$$

$$2\,H_2O_2 \xrightleftharpoons{\text{Catalase}} O_2 + 2\,H_2O$$

Direct reversal of damage

Pyrimidine dimers

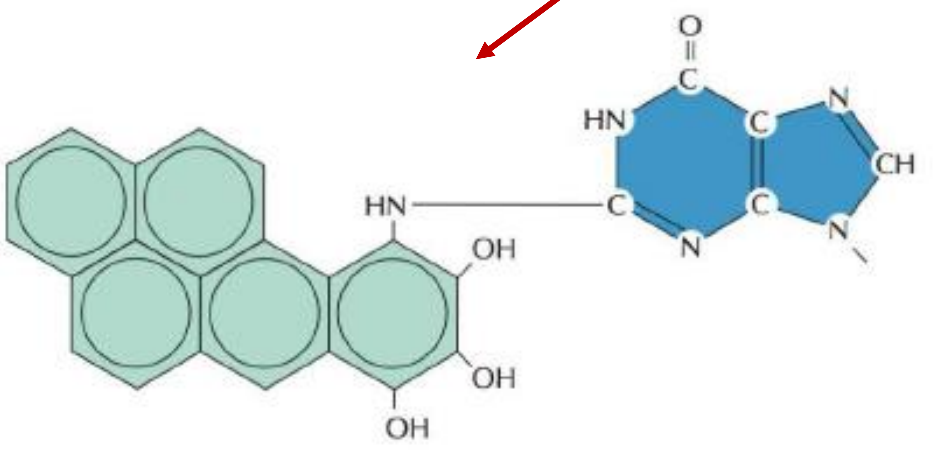
- The ultraviolet (UV) wavelength of sunlight causes the formation of covalent interactions (50–100 reactions per second) between two adjacent pyrimidine bases, commonly between two thymine, structures known as pyrimidine dimers.
- This product is mutagenic.
- Pyrimidine dimers are reversed in bacteria by enzymes known as photolyases, which do not exist in humans.

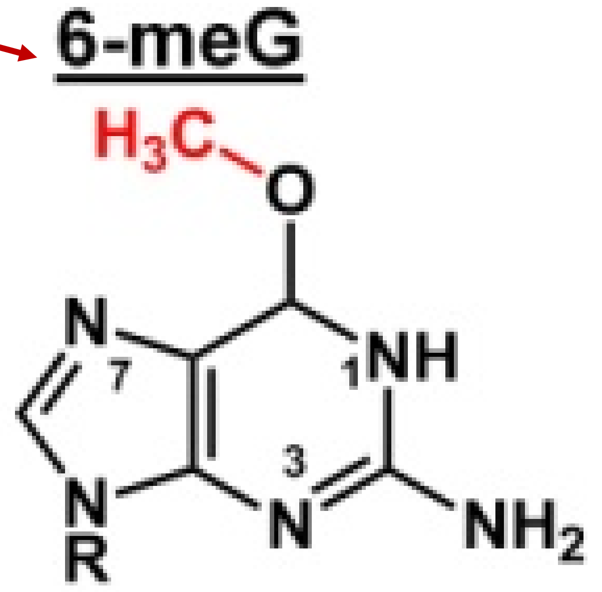


DNA structure is distorted and, thus, replication and transcription cannot proceed.

Specific mispairing

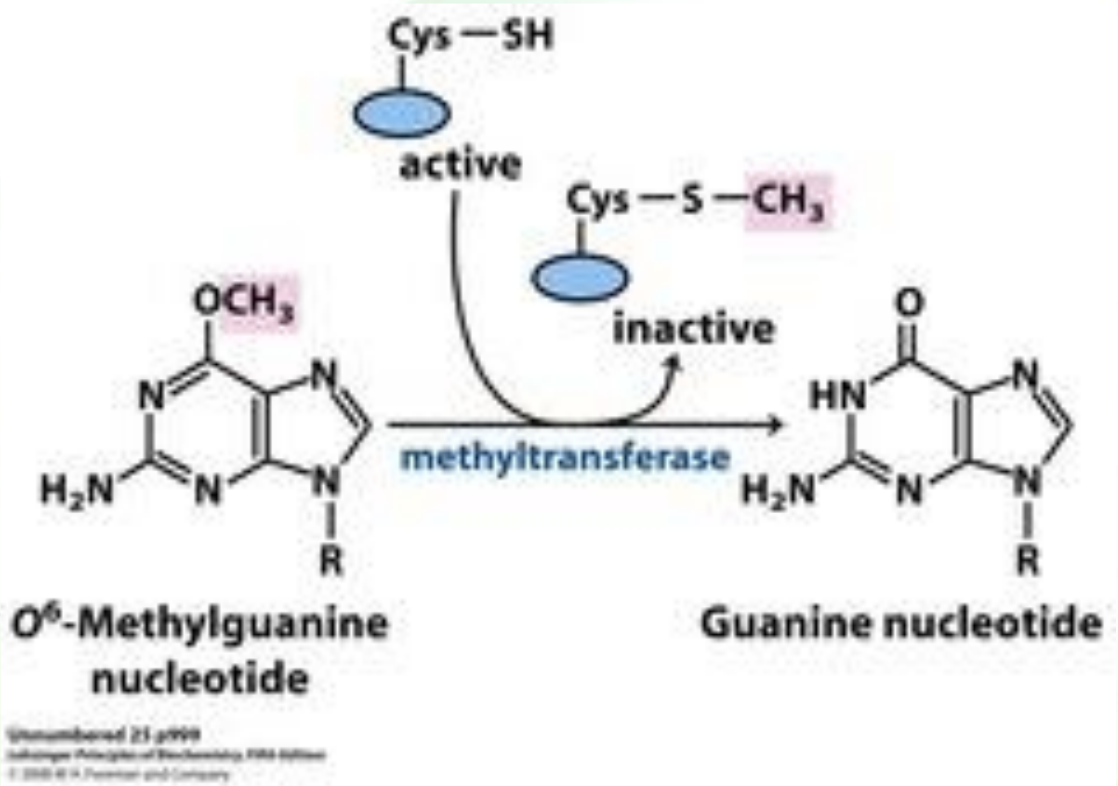
- Bases existing in DNA can be altered causing mispairing.
  - Alkylating agents can transfer methyl group to guanine forming 6-methylguanine, which pairs with thymine.
  - Addition of large chemical adducts by carcinogens.





Repair of O6-methylguanine

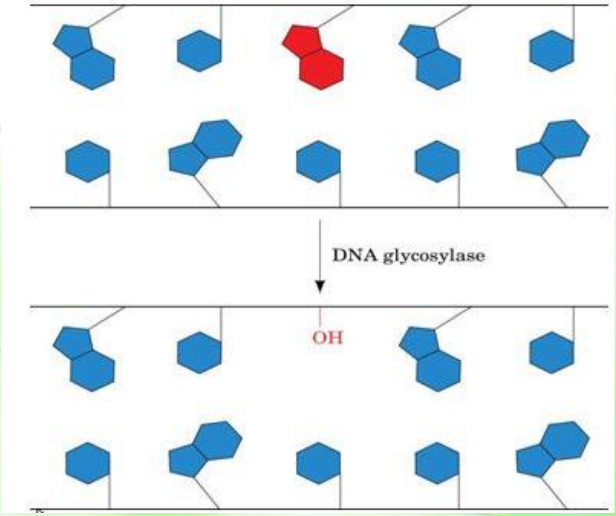
- This is done via O6-methylguanine methyltransferase.

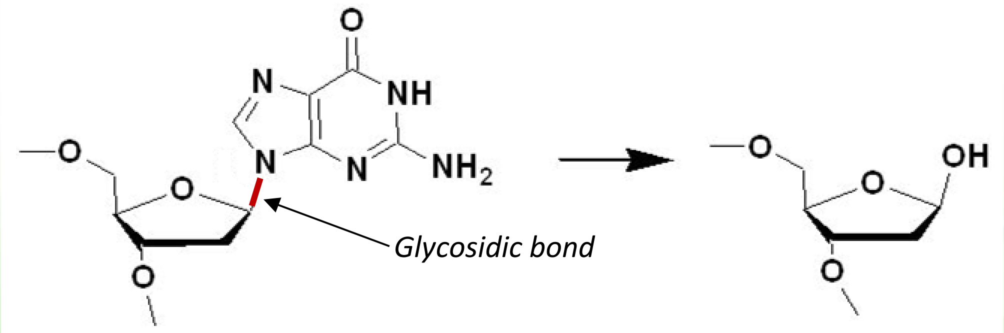


Excision repair pathways

Base excision repair pathway

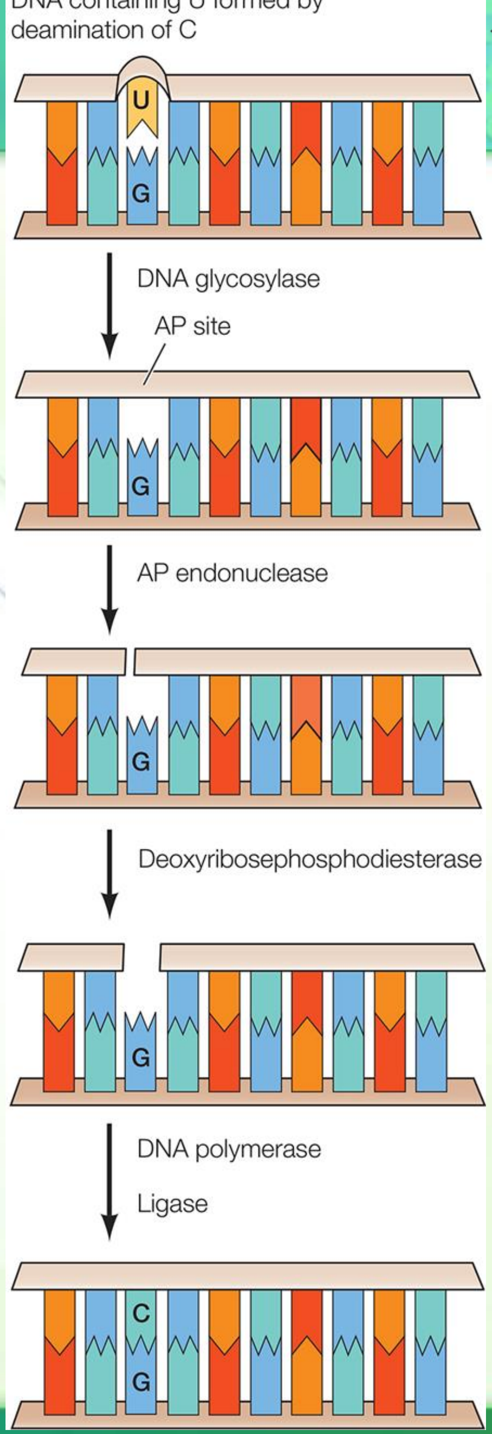
- Each cell in the human body can lose several thousand purine bases daily.
- DNA glycosylases do not cleave phosphodiester bonds, but instead cleave N-glycosidic (base-sugar) bonds of damaged bases, liberating the altered base and generating an apurinic or an apyrimidinic site, both are called AP sites.
- The AP site is repaired by an AP endonuclease repair pathway.





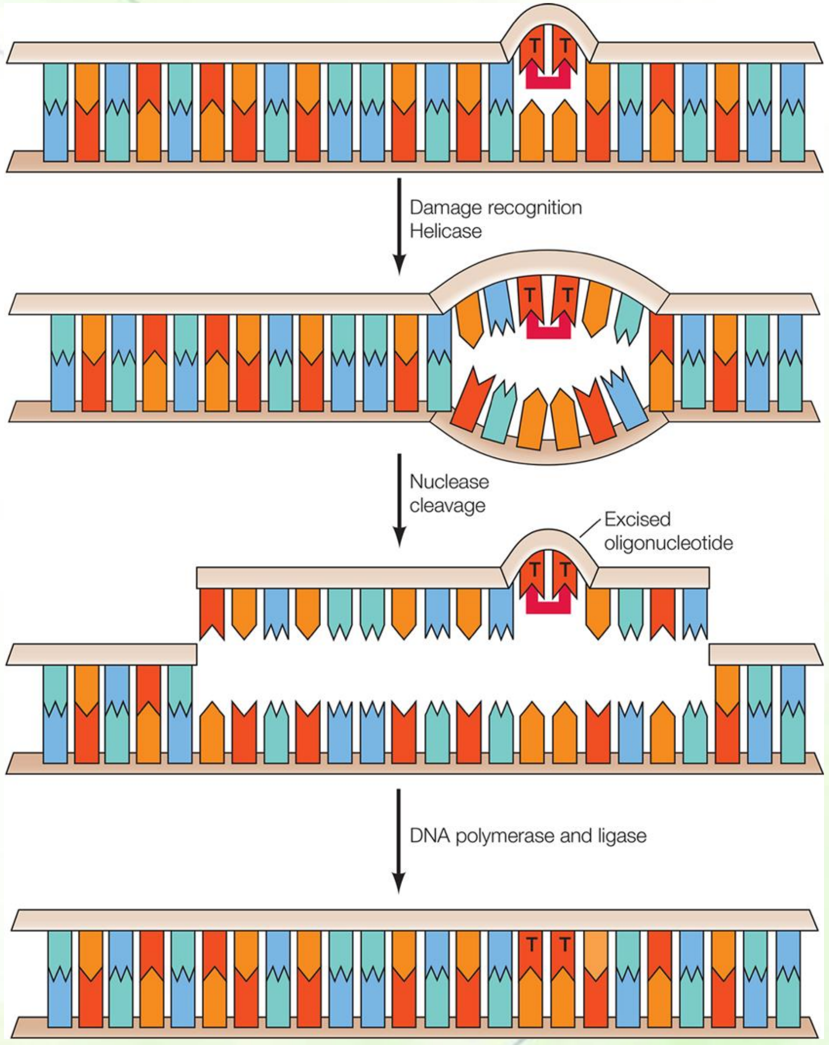
DNA glycosylases

- Numerous DNA glycosylases exist.
  - Example: uracil-DNA glycosylase, removes uracil from DNA.
    - Uracil residues, which result from the spontaneous deamination of cytosine or incorporation of dUTP can lead to a C→T transition, if unrepaired.
- AP endonucleases cleave the phosphodiester bonds at AP sites.
- The deoxyribose is removed.
- A DNA polymerase fills in the gap and DNA ligase and re-forms the bond.



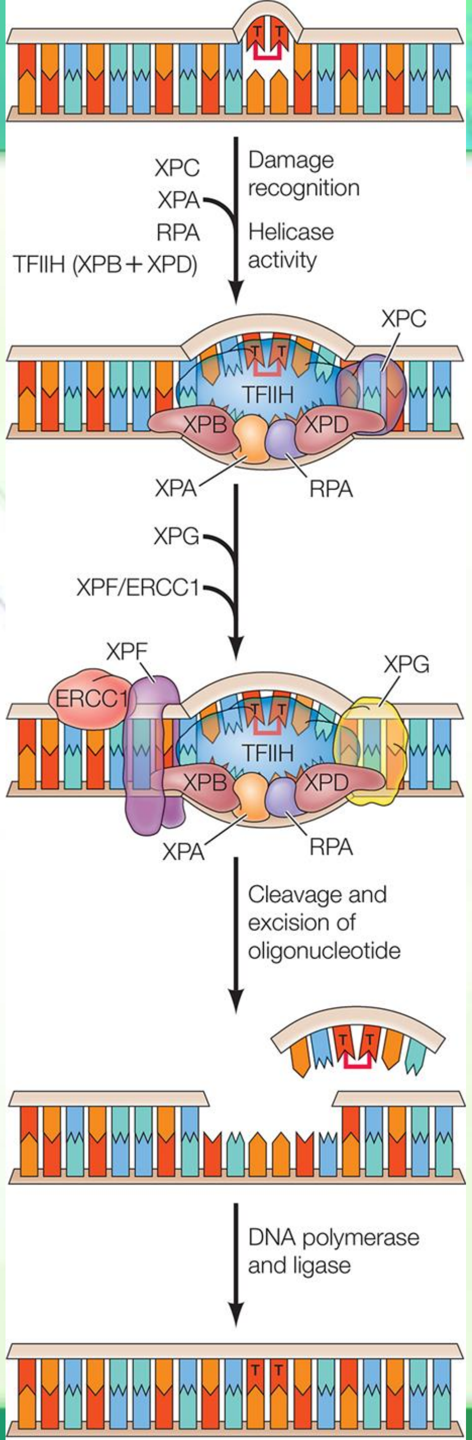
General excision repair
(nucleotide excision repair)

- Damaged DNA is recognized and then unwound around the site of damage by a helicase.
- The DNA is then cleaved on both sides of a thymine dimer, resulting in the excision of an oligonucleotide containing the damaged bases.
- The gap is then filled by DNA polymerase and sealed by ligase.



XP proteins

- DNA damage (e.g., a thymine dimer) is recognized by XPC protein.
- XPA, Replication protein A (RPA), which binds the single-stranded DNA during DNA replication, and TFIIH form a complex with XPC.
  - TFIIH contains the subunits, XPB and XPD helicases.
- DNA is unwound by TFIIH (XPB and XPD) and XPG.
- XPF/ERCC1 endonucleases are recruited and the DNA is cleaved, excising the damaged oligonucleotide.
- The resulting gap is filled by DNA polymerase and sealed by ligase.



In human…

- Defects in nucleotide excision repair cause a condition known as Xeroderma pigmentosum (XP) and Cockayne’s syndrome.
- Individuals with this disease are extremely sensitive to UV light and develop multiple skin cancers on the regions of their bodies that are exposed to sunlight.

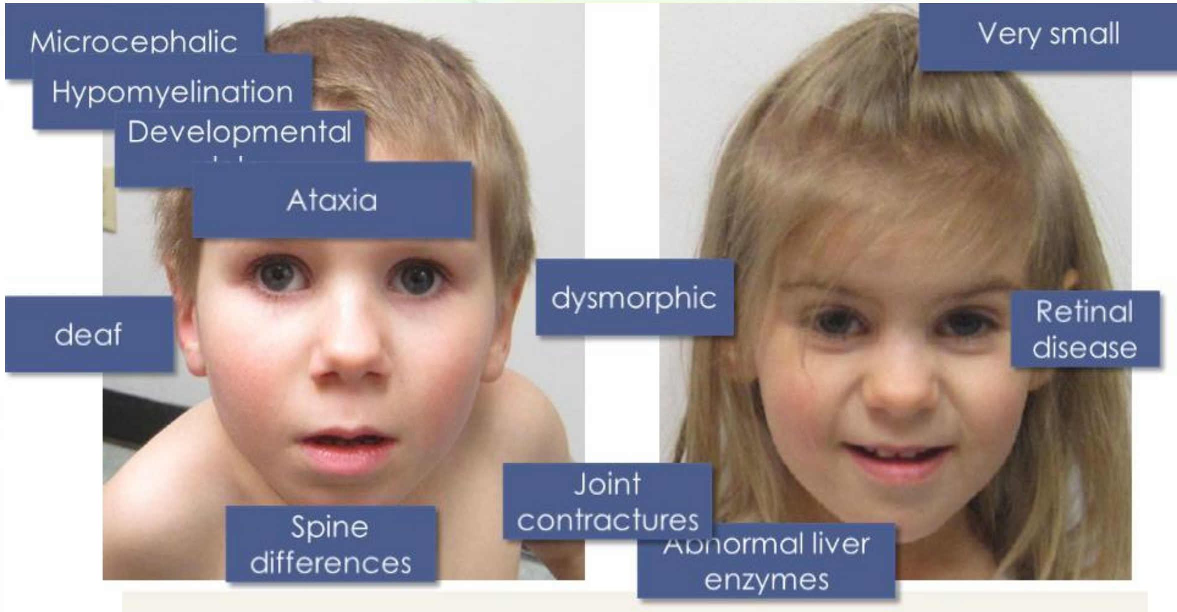
Transcription-coupled repair

Transcription-coupled repair

- There is a preferential repair of the transcribed strand of DNA for actively expressed genes.
- RNA polymerase pauses when encountering a lesion.
- The general transcription factor TFIIH and other factors carry out the incision, excision, and repair reactions.
- Then, transcription can continue normally.

Cockayne's syndrome

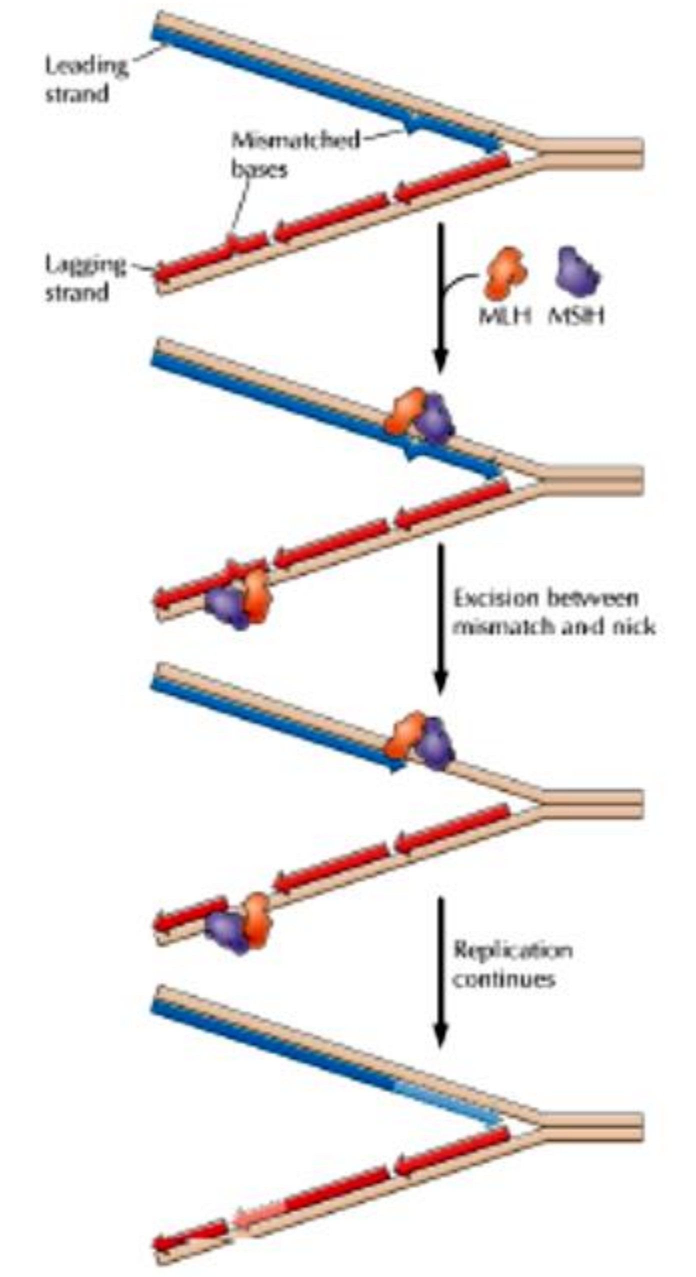
- Cockayne's syndrome: a condition caused by defects in XP proteins, but predominantly CSB.
- They recognize the RNA polymerase pausing at a site of mutation.
- It is caused by a defect in preferential DNA repair of transcriptionally active DNA.
- *Patients are characterized by short stature, an abnormally small head (microcephaly), and neurologic abnormalities that can lead to intellectual disability and may have skin that is sensitive to light (photosensitivity).*



Mismatch repair and replication-related repair

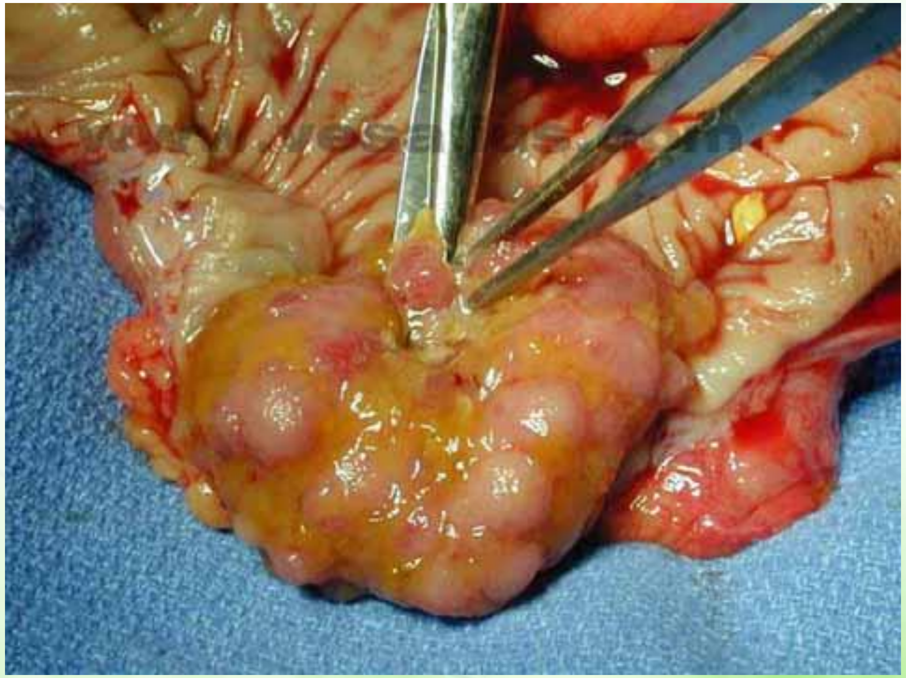
Mismatch repair in humans

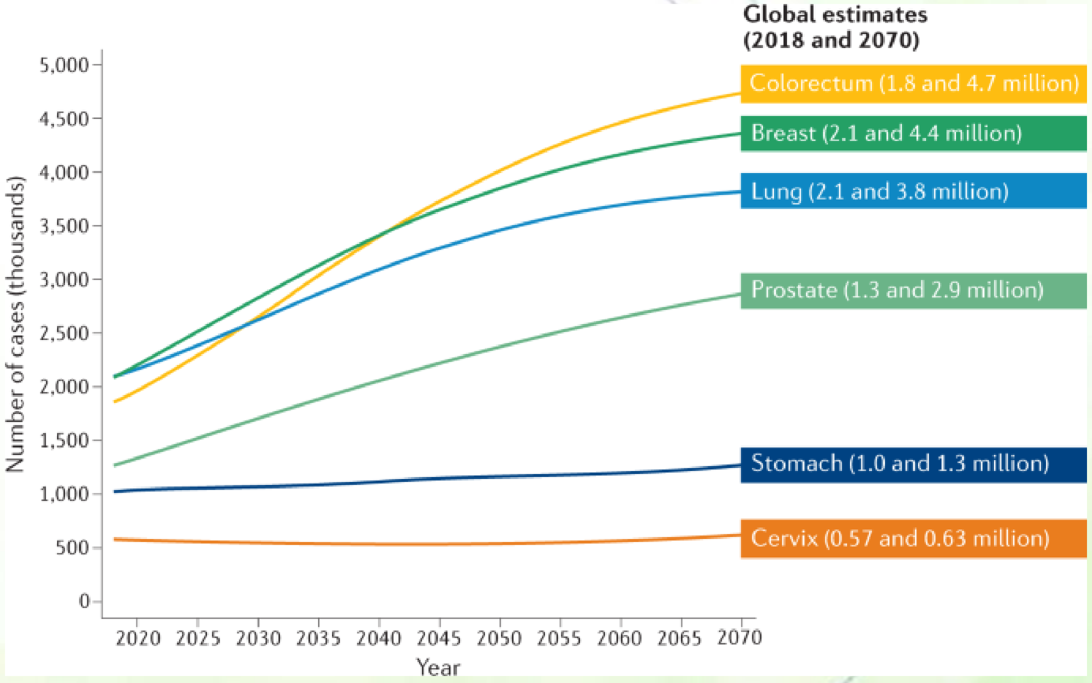
- During replication, MSH and MLH bind to mismatched bases within the lagging strand (Okazaki fragments) and the leading strand.
- DNA is excised and replication continues.
- Mismatch repair is 3-4 times more effective on the lagging strand than the leading strand, but DNA polymerase ε is more accurate than DNA polymerase δ.



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.



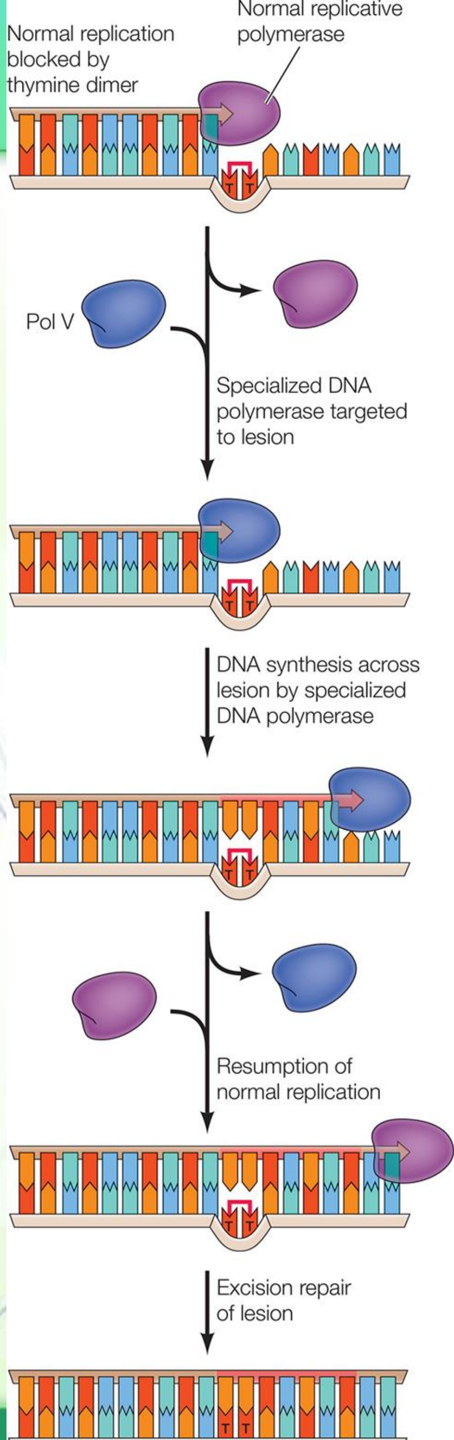


Colorectal
Breast
Lung

Translesion DNA synthesis

Translesion DNA synthesis

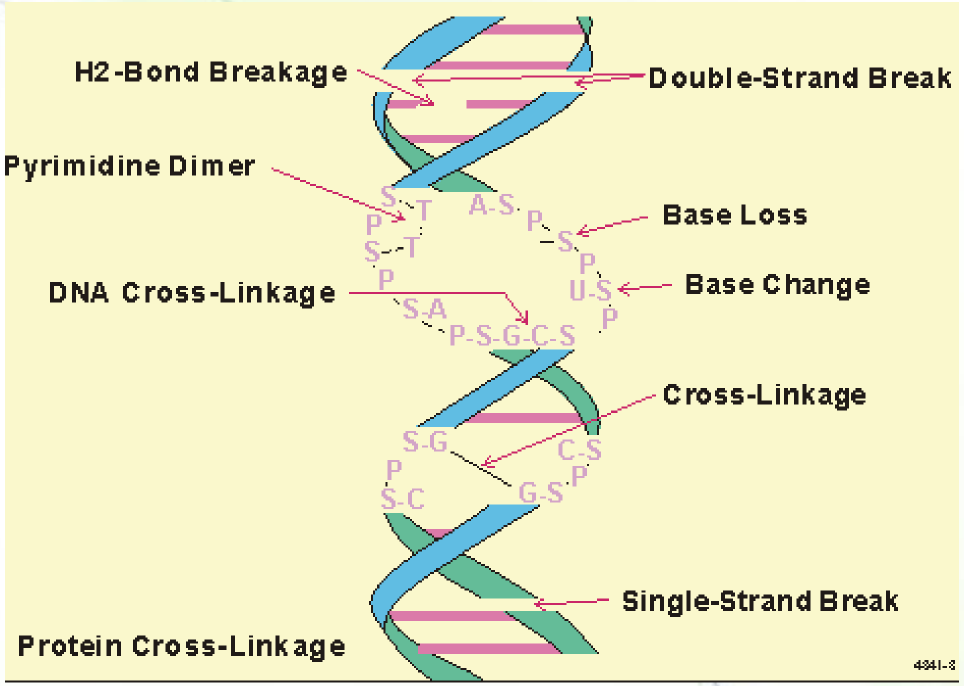
- Specialized DNA polymerases (not the typical replicative enzymes) can synthesize DNA over the lesions.
- But, they have low fidelity and lack proofreading mechanisms, and, hence, are error-prone.
- But, they are selective toward the introduction of A nucleotides, so that TT dimers are often replicated correctly.

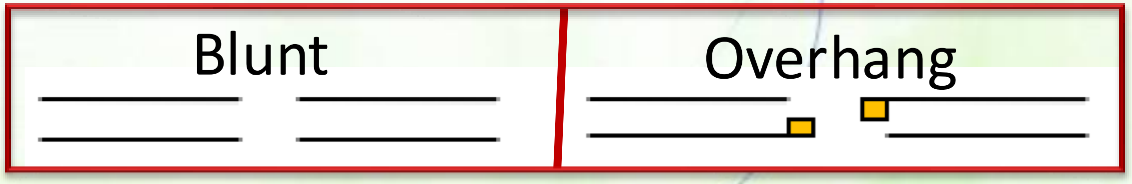


Recombinational repair

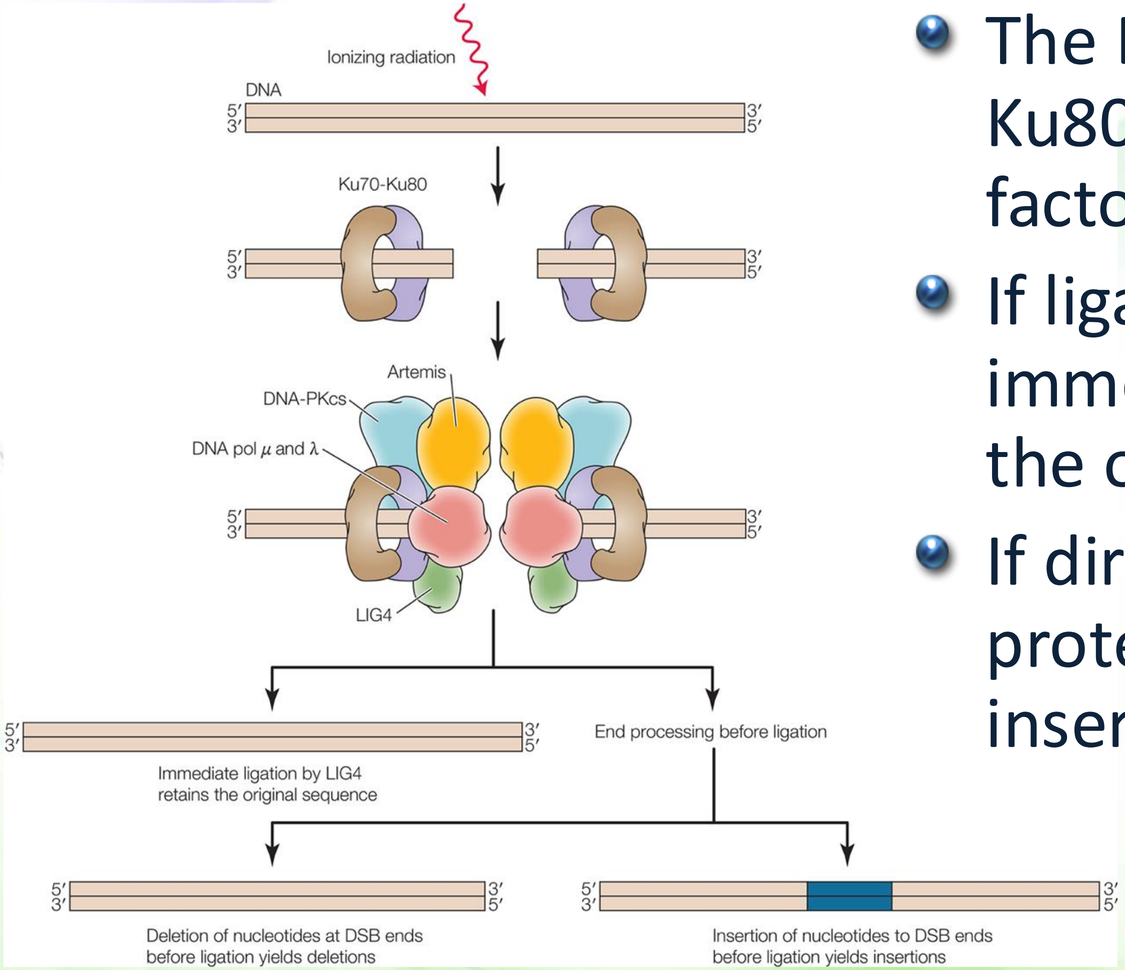
Ionizing radiation

- Ionizing radiation can cause different type of DNA damage:
  - Creation of AP sites
  - Base damage
  - Strand breaks, single (SSB) or double (DSB)
- 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





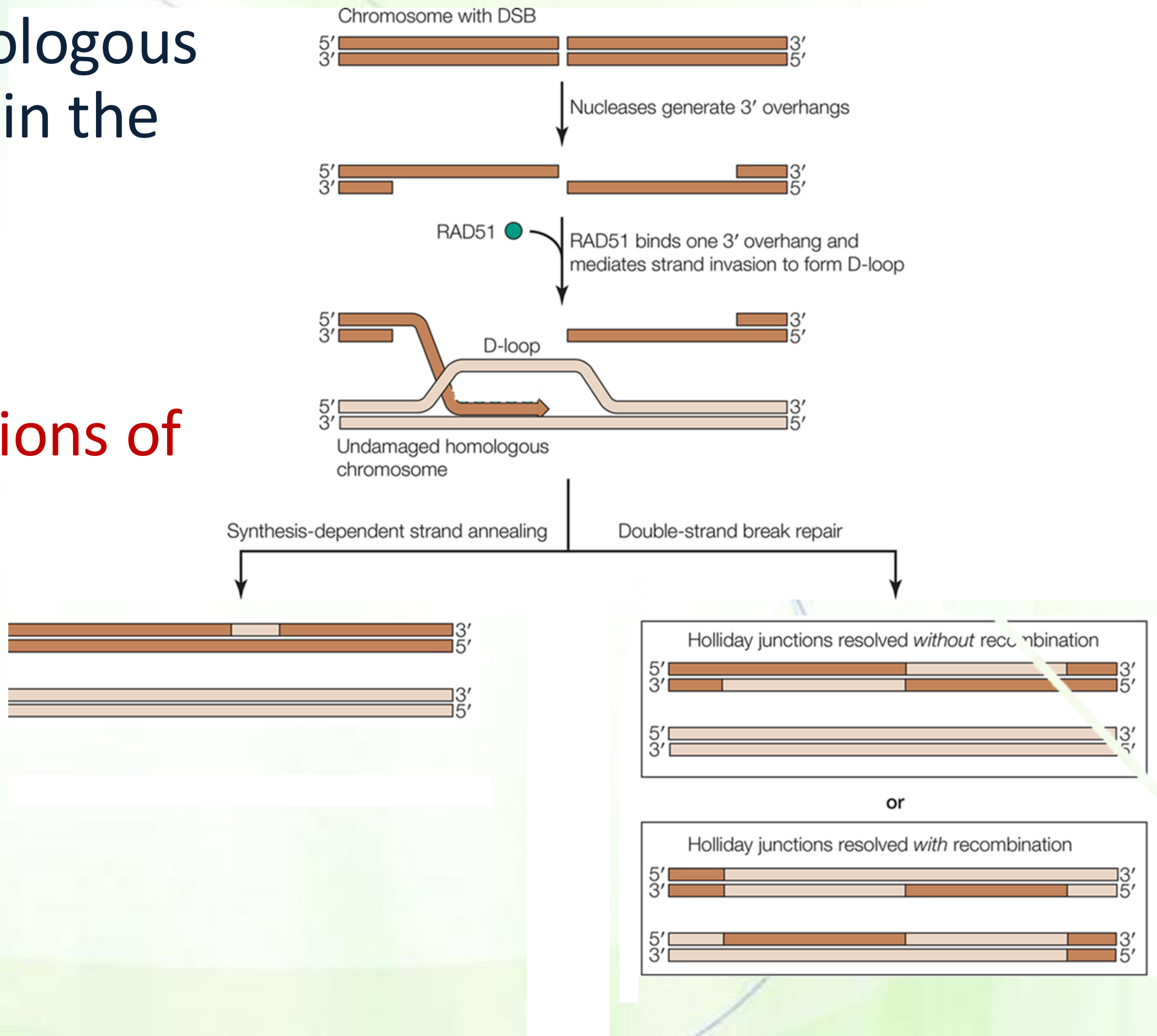
Nonhomologous end joining (NHEJ) repair



- 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 deletions or insertions (i.e., called INDELS) are introduced.

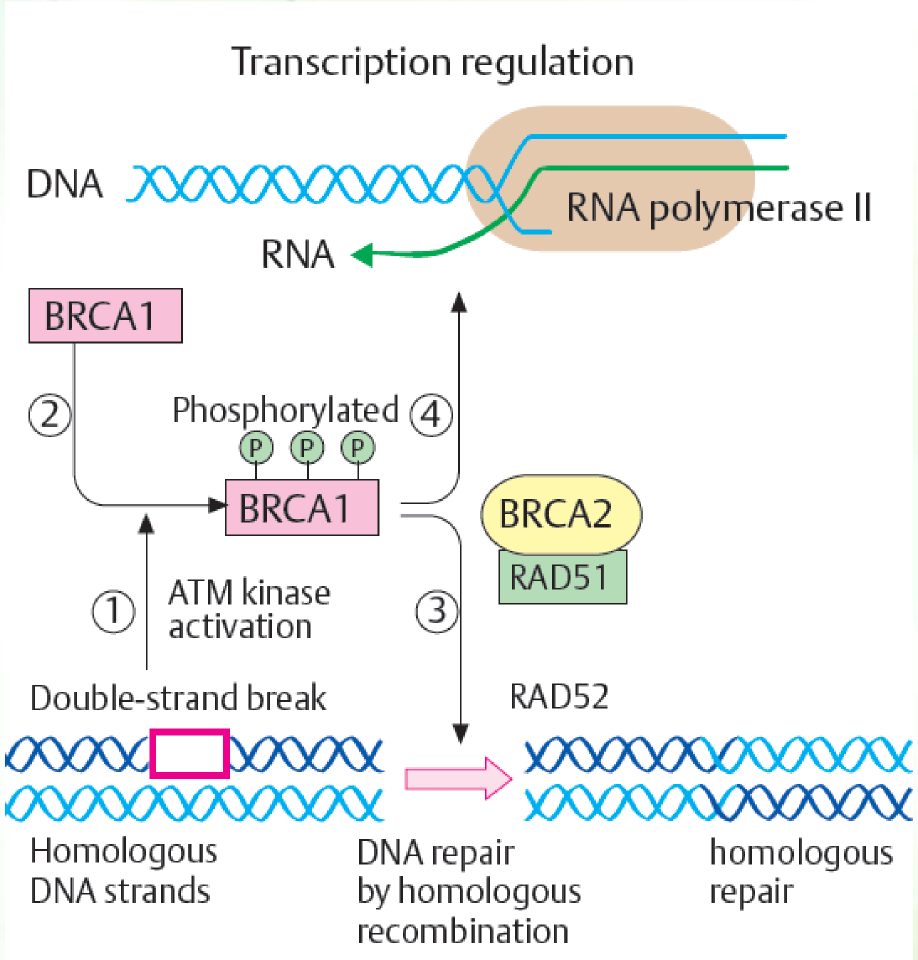
Homology-directed repair (HDR)

- Homology-directed repair (HDR), or homologous recombination, uses DNA sequences within the undamaged homologous chromosome.
  - More accurate than NHEJ.
- In germline cells, HDR generates genetic diversity by producing different combinations of gene alleles.



Breast cancer and BRCA genes

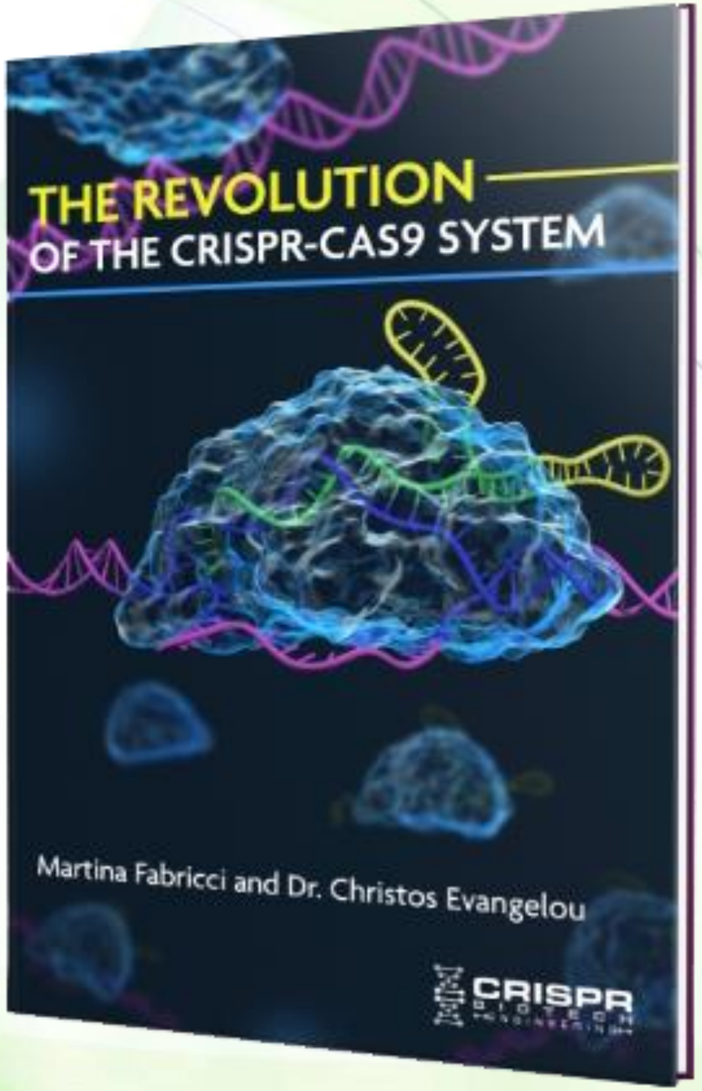
- Mutations in BRCA1 and BRCA2 genes are responsible for a portion of hereditary breast and ovarian cancers.
- BRCA1 activates homologous recombination repair of DNA double-stranded breaks.
- BRCA2 can recruit Rad51 to the ssDNA.
- BRCA1 is also involved in transcription and transcription-coupled DNA repair.



Wrap-up

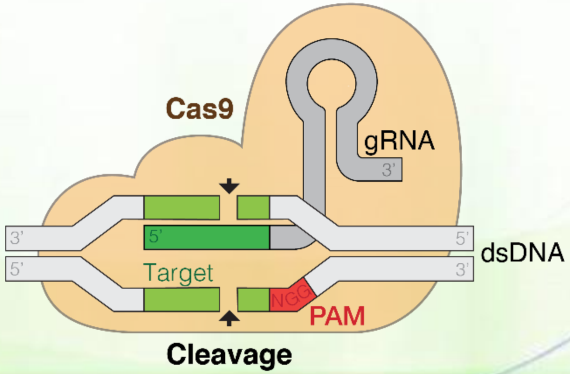
Type of DNA repair	Mechanism	Genes/proteins
Base excision repair	Removal of abnormal bases	DNA glycosylases
Nucleotide excision repair	Removal of thymine dimers and large chemical adducts	XP proteins, CSB
Mismatch repair	Correction of mismatched bases caused by DNA replication	MLH1, MSH2
Post-replication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

CRISPR-Cas9


THE REVOLUTION
OF THE CRISPR-CAS9 SYSTEM
Martina Fabricci and Dr. Christos Evangelou
CRISPR

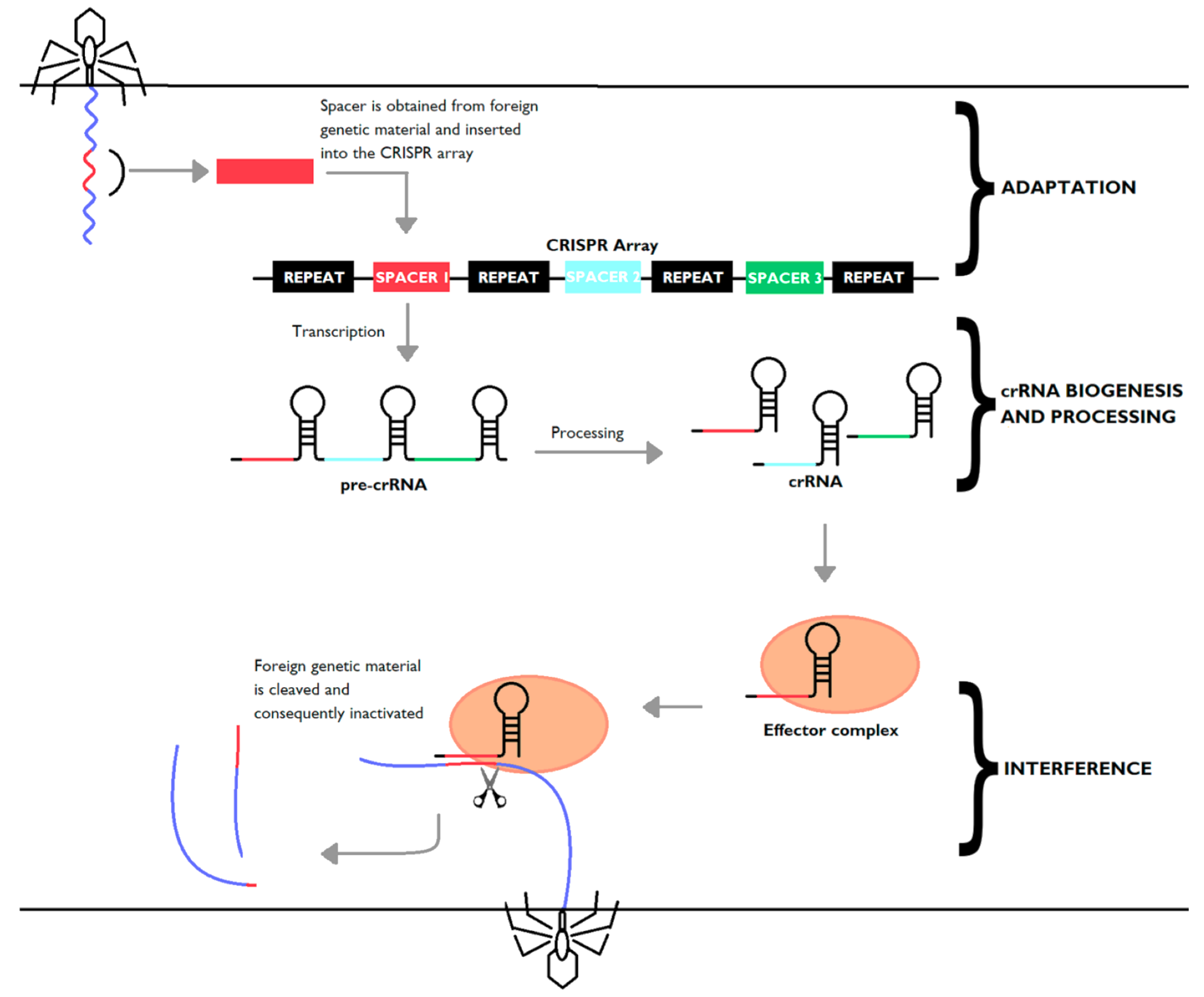
What is CRISPR/Cas9?

- 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 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.

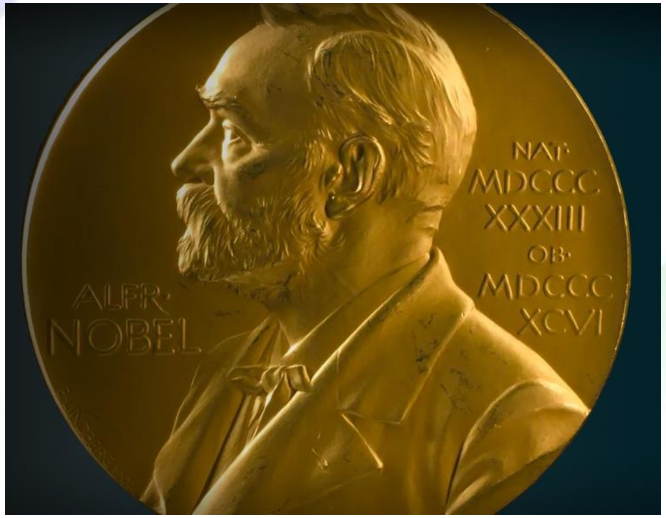


The biological function

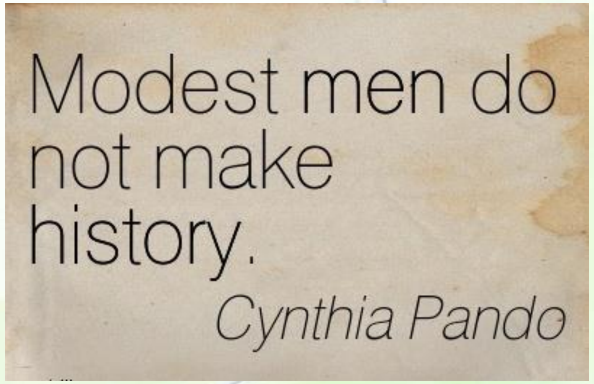
- When a phage infects a bacterial cell, the cell degrades the phage DNA into smaller pieces and integrates one of these fragments into the CRISPR cluster.
- When the phage infects the cell again, the cell transcribes the DNA into RNA (guide RNA or gRNA), which is integrated into the Cas9 nuclease and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



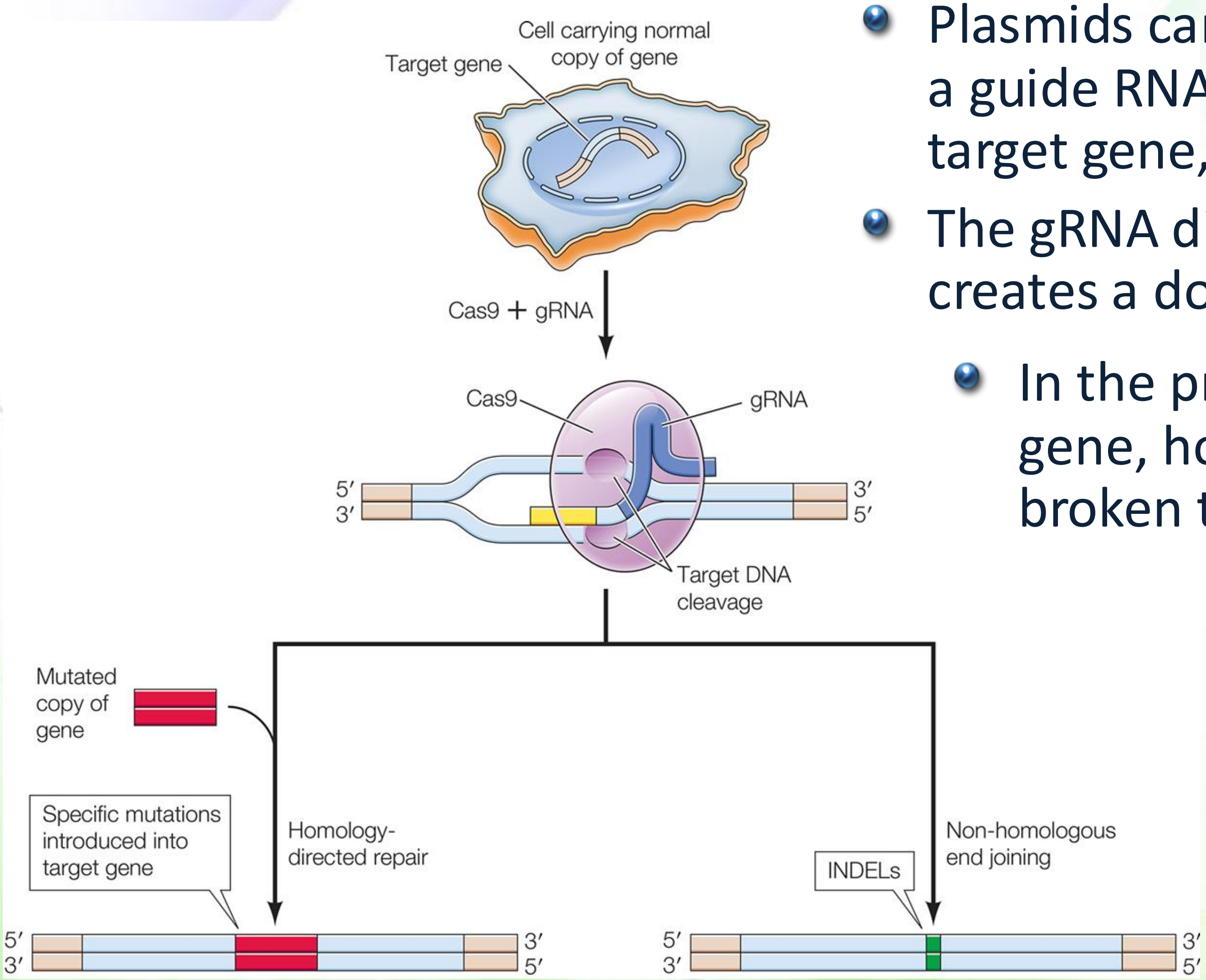
In 2020...



Emmanuelle Charpentier and Jennifer Doudna

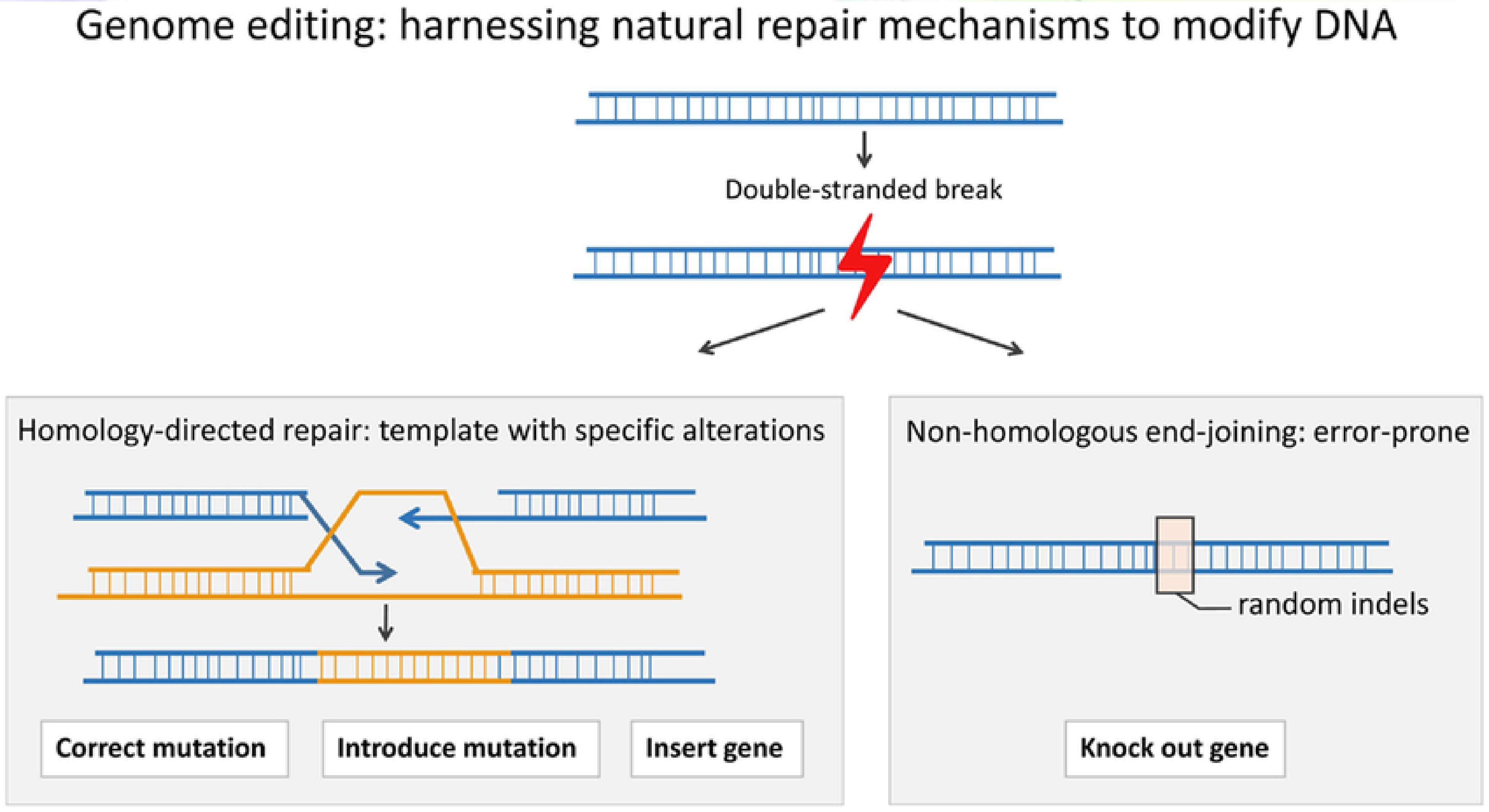


The experiment

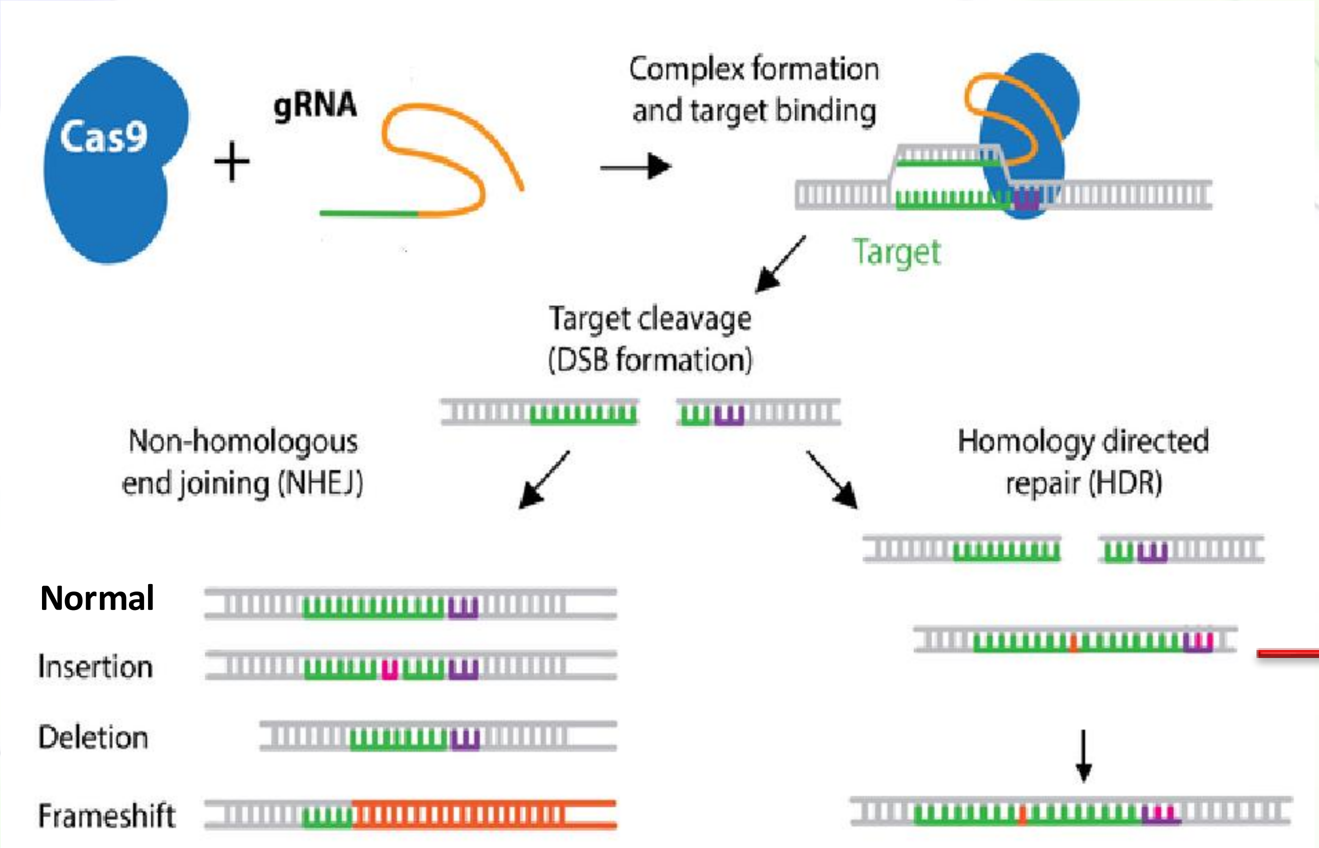


- Start with cells.
- Plasmids carrying genes for Cas9 and a gene expressing a guide RNA (gRNA), i.e. sequences homologous to the target gene, are introduced into the cells.
- The gRNA directs Cas9 to the target gene and Cas9 creates a double-stranded break.
  - In the presence of a homologous copy of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



Gene editing



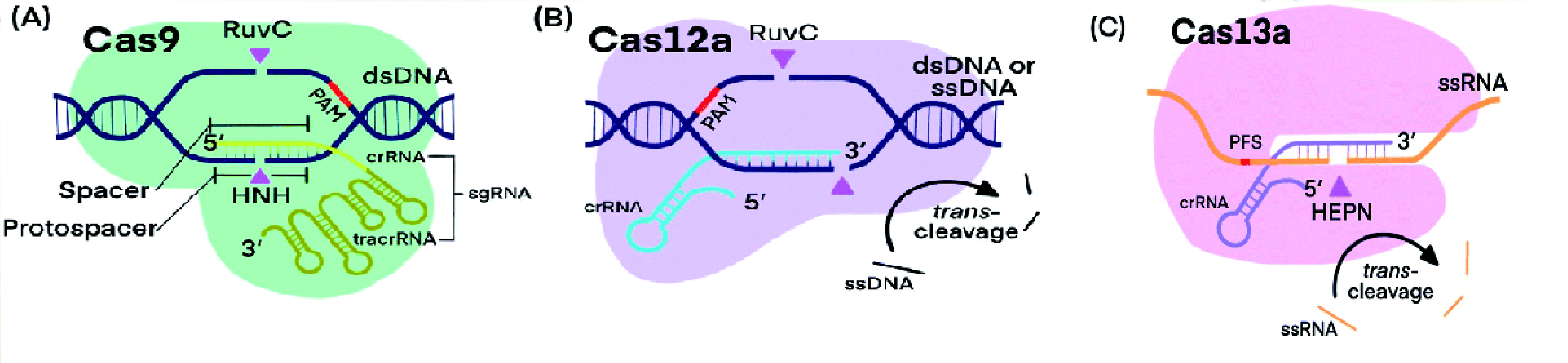
This DNA is introduced into cells so the DNA repair mechanism uses it for recombination.

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).

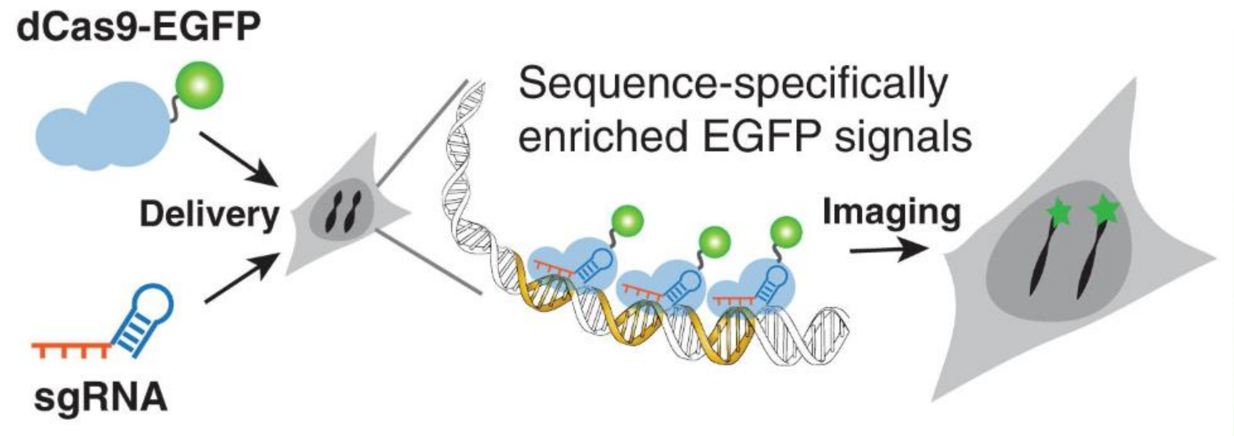
Other Cas enzymes

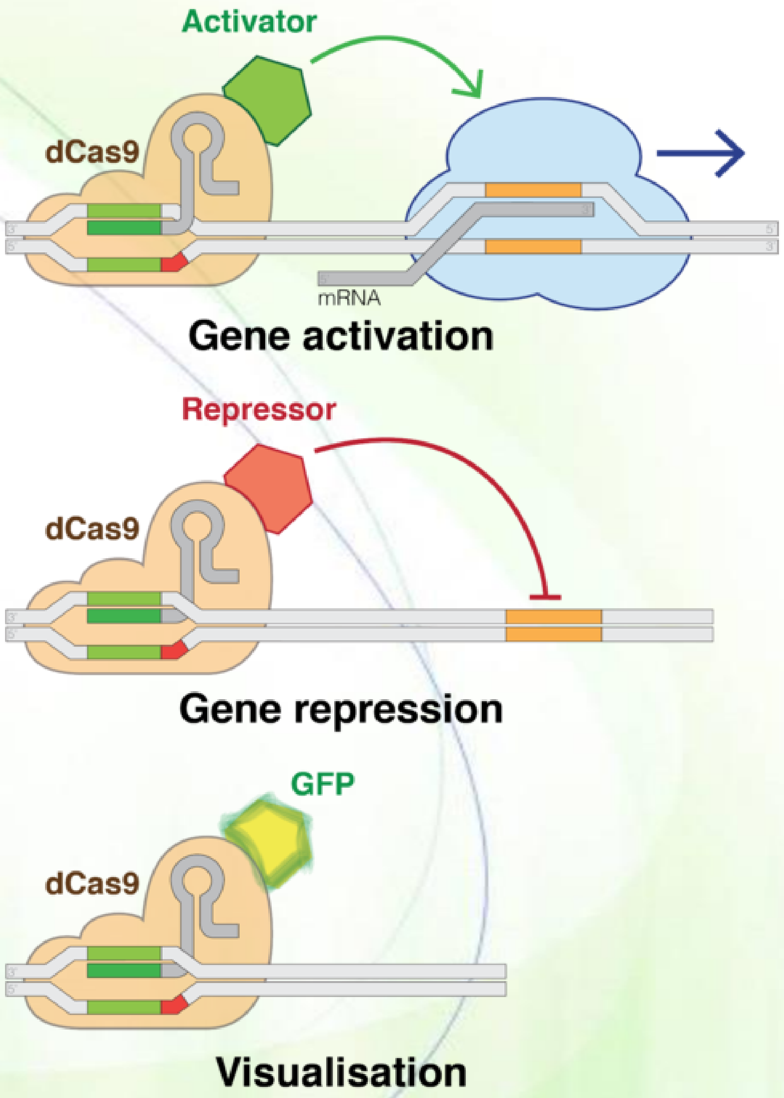
- Engineered Cas9: can make single-stranded DNA cuts.
- Cas12a: can make staggered cuts.
- Cas13a: A RNA endonuclease



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.





FDA NEWS RELEASE

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

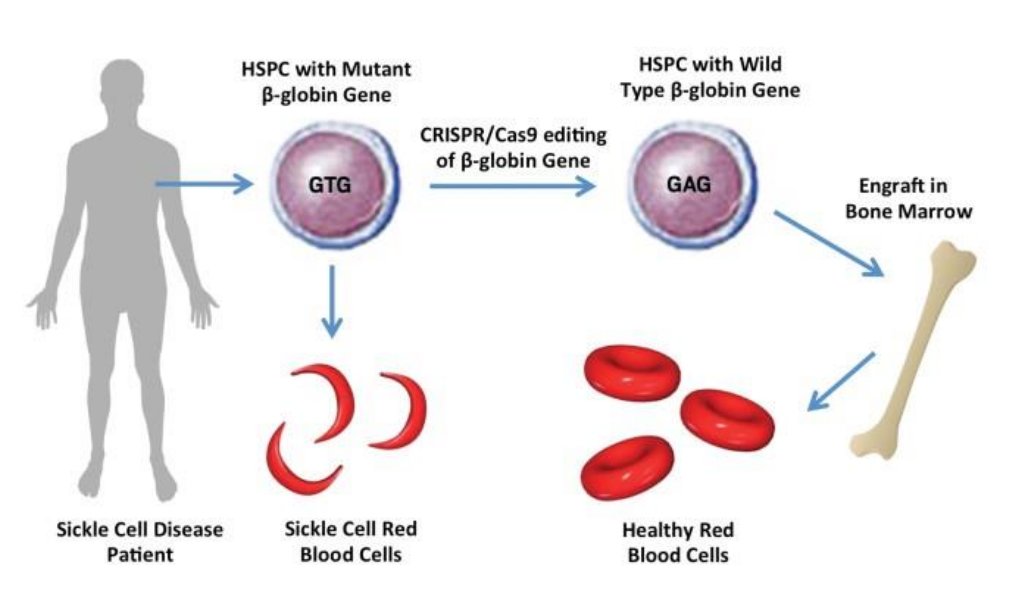
For Immediate Release: December 08, 2023

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

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CRISPR Study Is First to Change DNA in Participants

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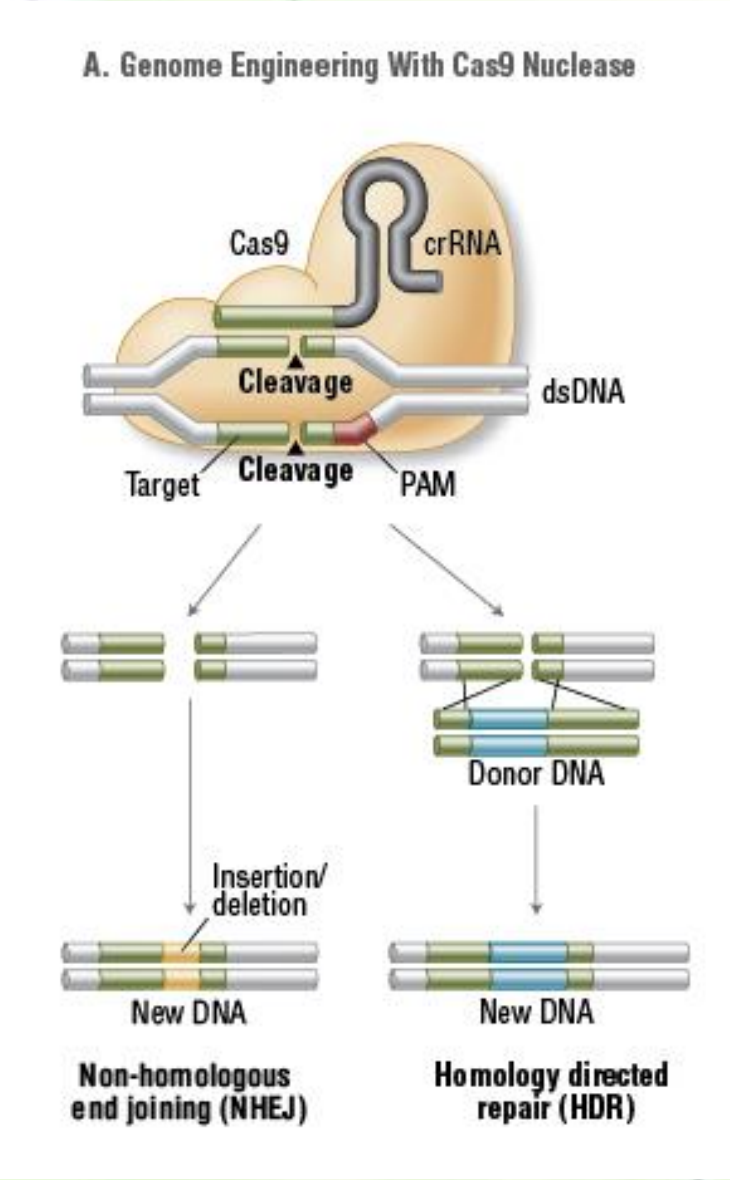
Controversial issue

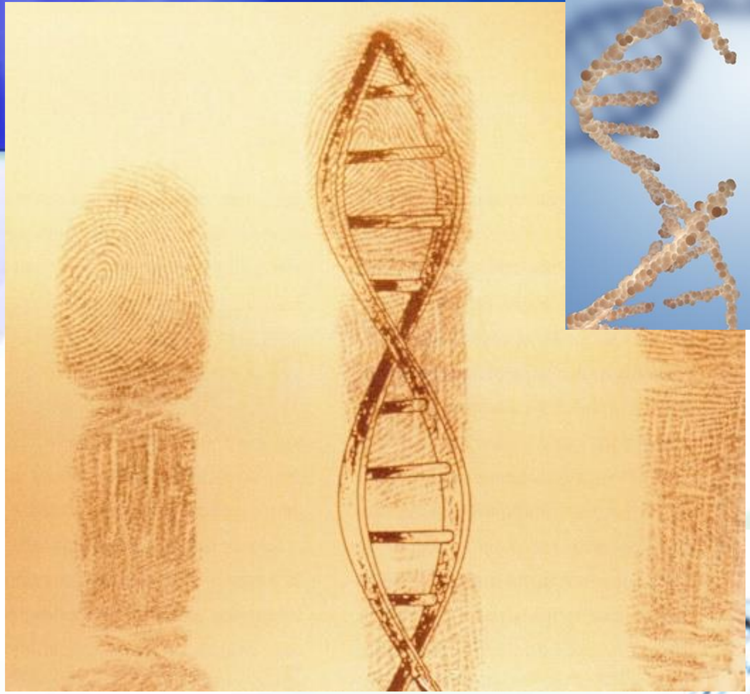
Gene repair

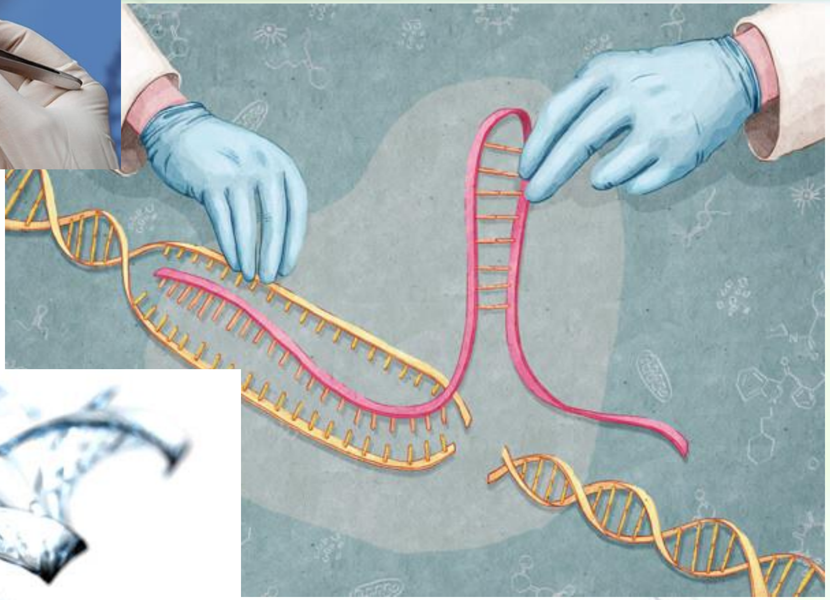
UK scientists ready to genetically modify human embryos

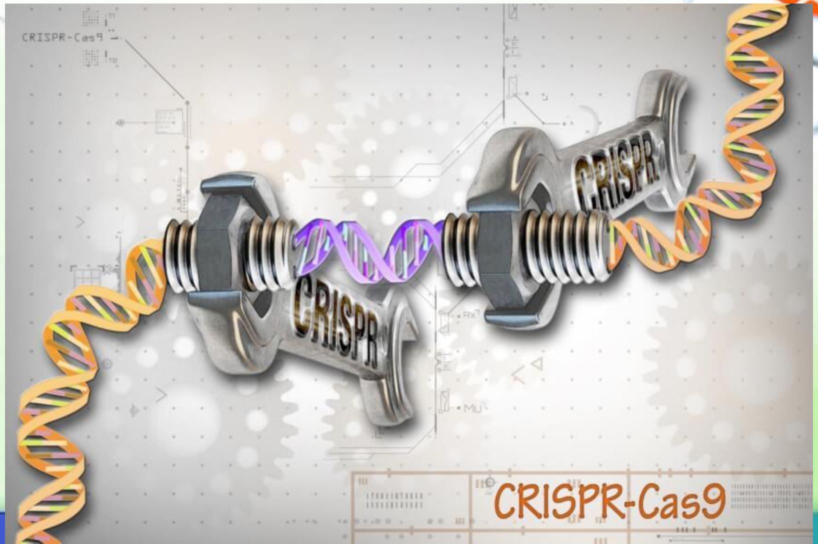
Researchers awaiting approval to use gene editing in embryos, which they hope will help them understand early stage life and improve fertility treatment

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





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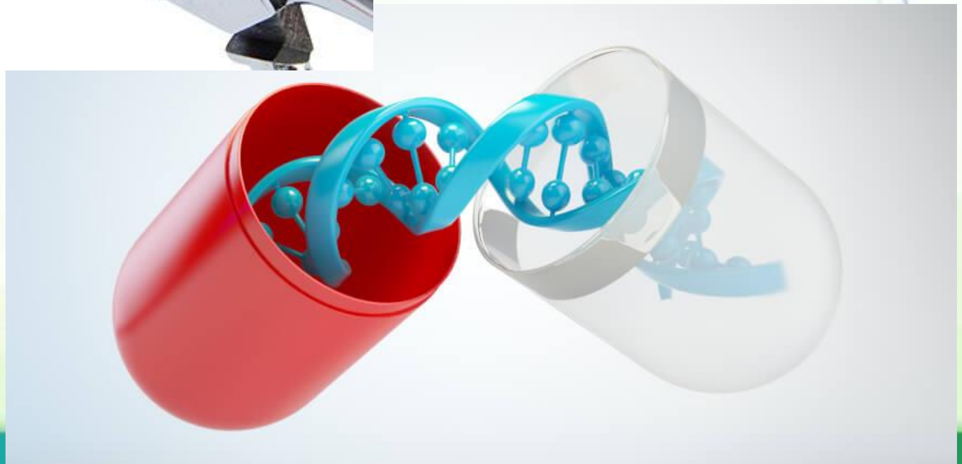
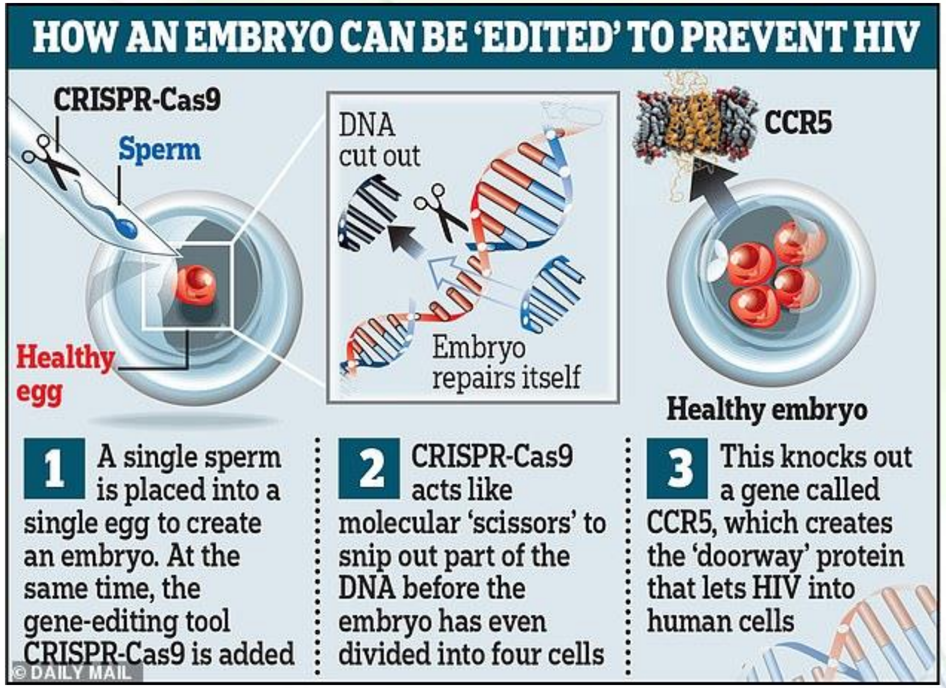


The dark side of science

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

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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

Ian Sample *Science editor*
@iansample
Tue 31 Dec 2019 00.23 GMT

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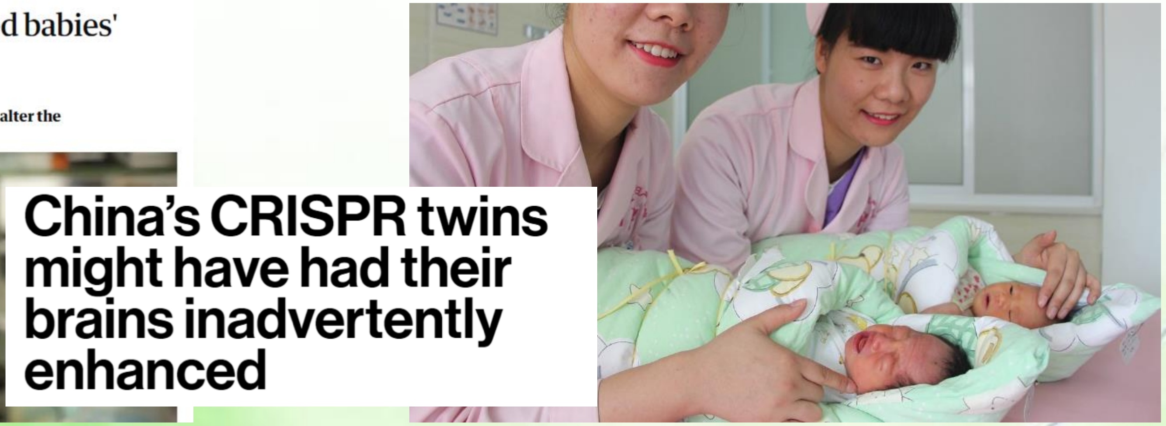

This is molecular biology in a nutshell