DNA mutations

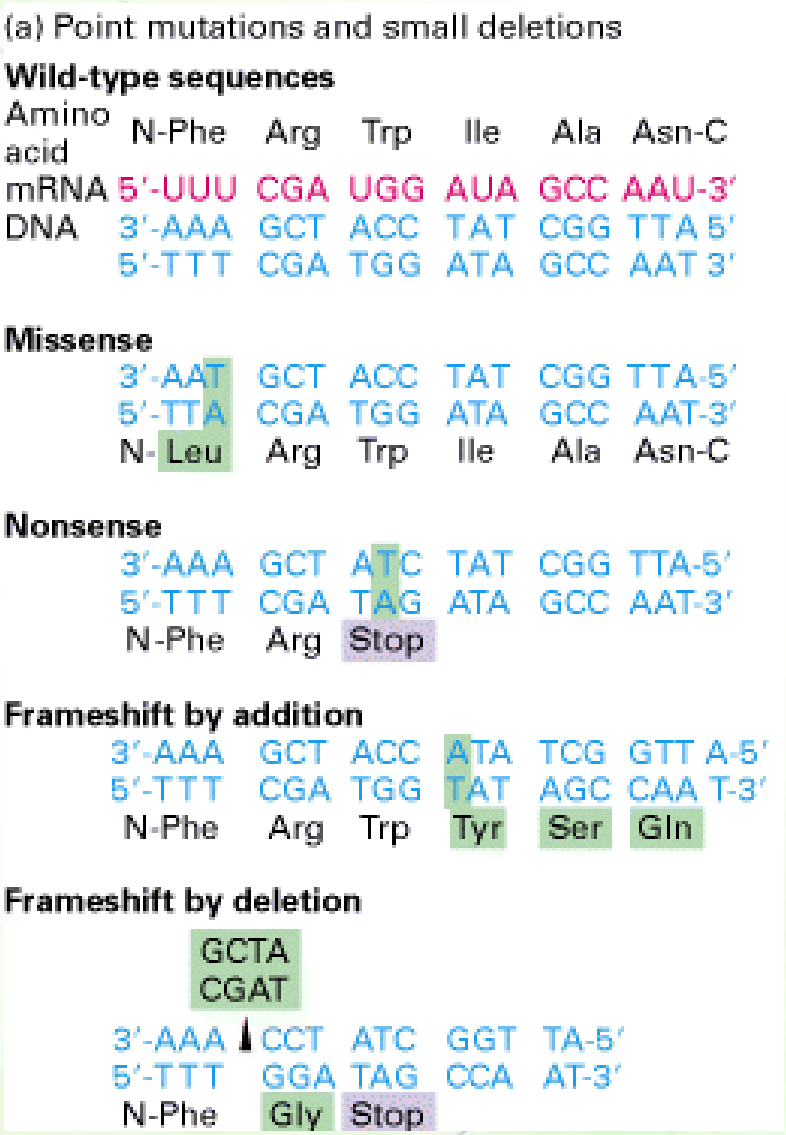
http://www.ncbi.nlm.nih.gov/books/NBK21897/
https://www.ncbi.nlm.nih.gov/books/NBK21936/

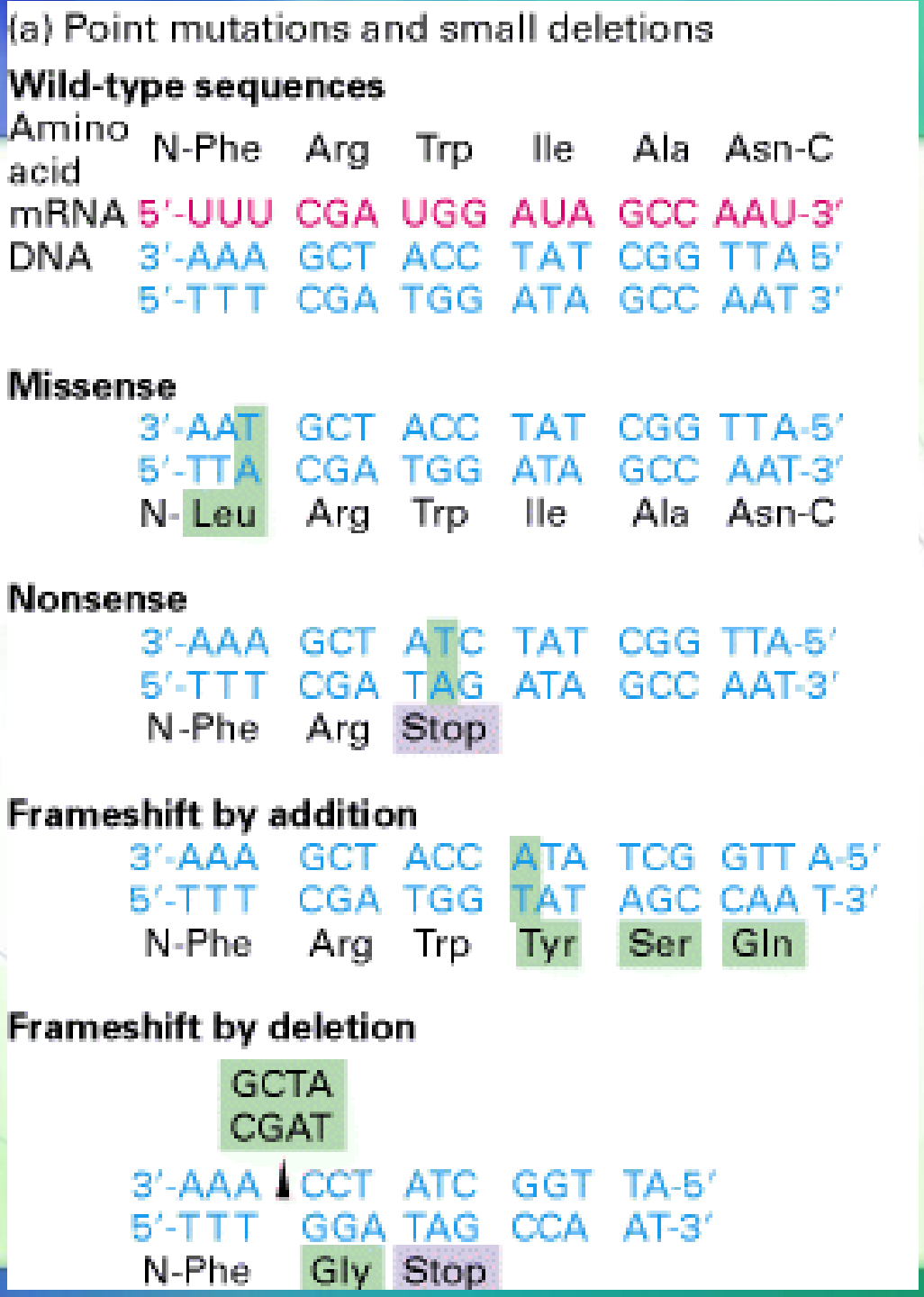
Types of mutations

- Micromutation that involve small regions of the DNA
- Macromutations that involve the chromosomes as a whole

DNA micromutations

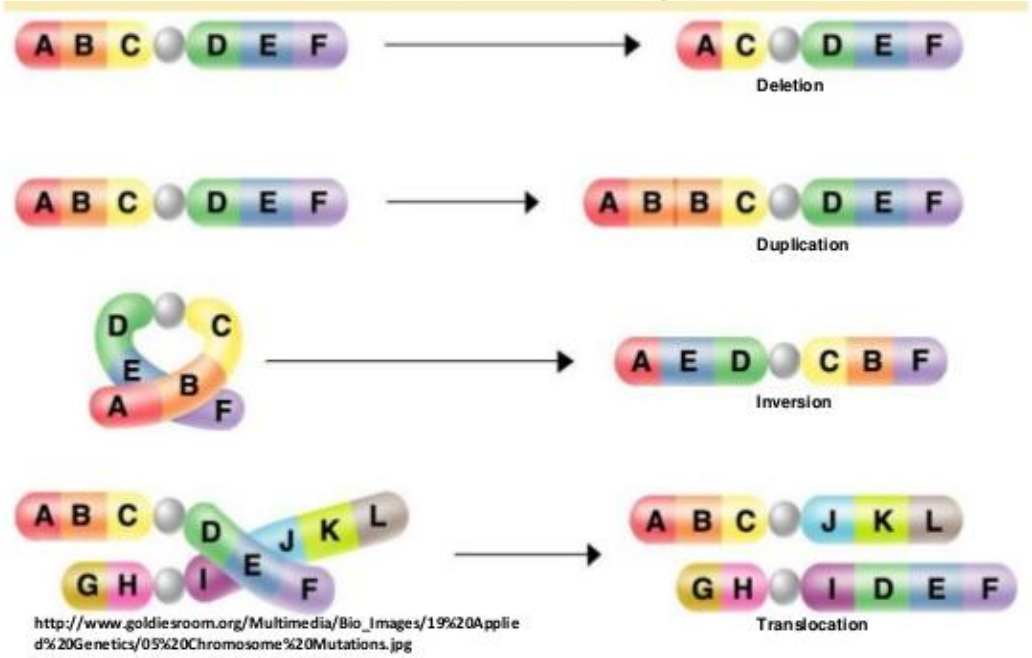
- Single-base mutations can result in different types of mutations such as:
  - missense: a change of an amino acid
  - nonsense: premature termination of protein synthesis
  - frameshift: altering the reading of amino acid sequence
  - silent: does not lead to any change at the protein level, but contributes to genetic variability among individuals




(a) Point mutations and small deletions
Wild-type sequences
Amino acid N-Phe Arg Trp Ile Ala Asn-C
mRNA 5′-UUU CGA UGG AUA GCC AAU-3′
DNA 3′-AAA GCT ACC TAT CGG TTA 5′
5′-TTT CGA TGG ATA GCC AAT 3′
Missense
3′-AAT GCT ACC TAT CGG TTA-5′
5′-TTA CGA TGG ATA GCC AAT-3′
N- Leu Arg Trp Ile Ala Asn-C
Nonsense
3′-AAA GCT ATC TAT CGG TTA-5′
5′-TTT CGA TAG ATA GCC AAT-3′
N-Phe Arg Stop
Frameshift by addition
3′-AAA GCT ACC ATA TCG GTT A-5′
5′-TTT CGA TGG TAT AGC CAA T-3′
N-Phe Arg Trp Tyr Ser Gln
Frameshift by deletion
GCTA
CGAT
3′-AAA CCT ATC GGT TA-5′
5′-TTT GGA TAG CCA AT-3′
N-Phe Gly Stop

DNA micromutations (cont.)

- Translocations, that bring different regions of gene segments together
- Deletions of a few nucleotides to long stretches of DNA,
- Insertions and duplications of nucleotides or long stretches of DNA
- Inversion of DNA segments



http://www.goldiesroom.org/Multimedia/Bio_Images/19%20Applied%20Genetics/05%20Chromosome%20Mutations.jpg

Causes of DNA mutation

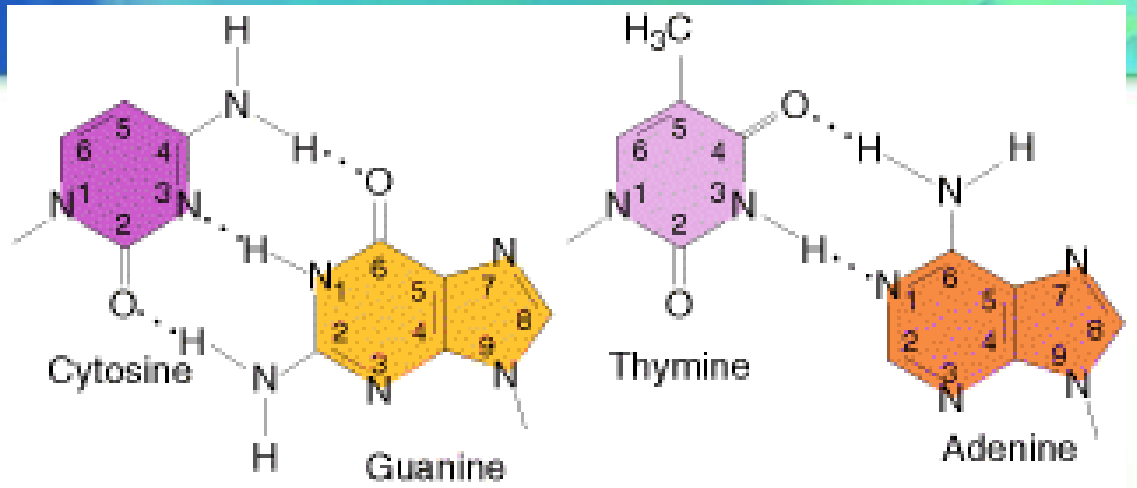
- DNA mutations can arise spontaneously or induced.

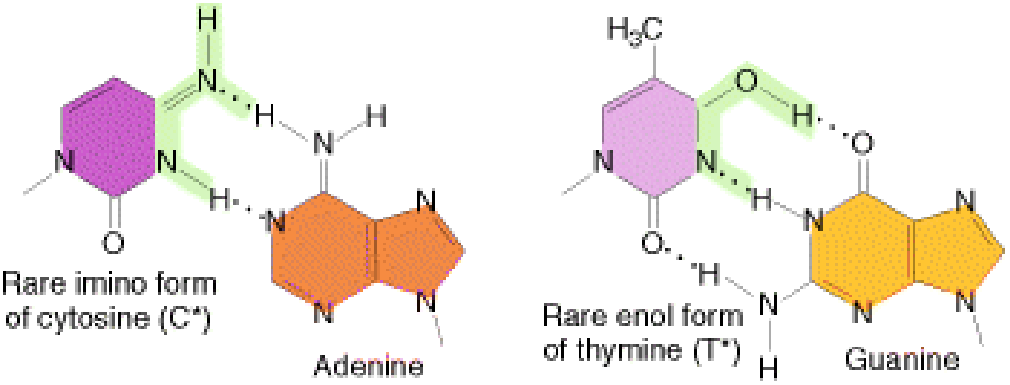
- Spontaneous mutations are naturally occurring mutations and arise in all cells.
  - They arise from a variety of sources, including errors in DNA replication and spontaneous lesions
- Induced mutations are produced when an organism is exposed to a mutagenic agent, or mutagen.

Sources of errors in DNA replication

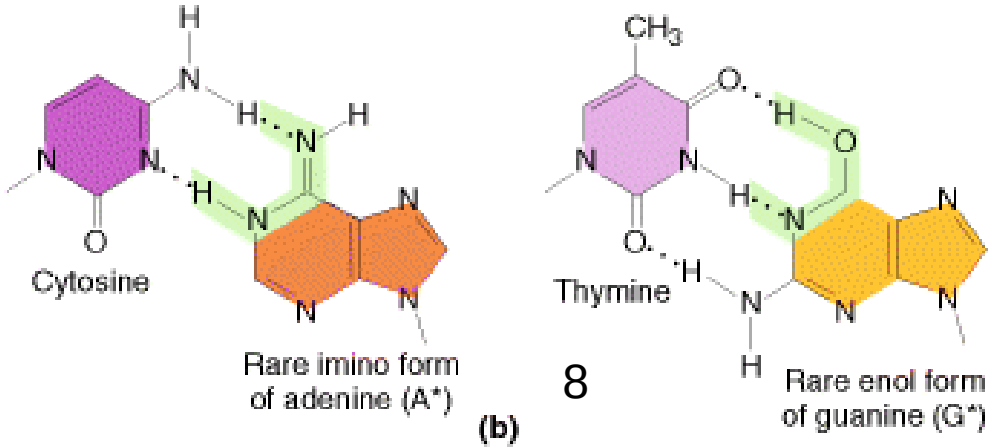
- Formation of inaccurate nucleotide pairs (A-C or G-T) leading to base substitution.
  - Mispairing happens since each base can appear in several forms, called tautomers (interconverting constitutional isomers of organic compounds)

Following DNA replication, tautomers lead to either transition or transversion mutations.





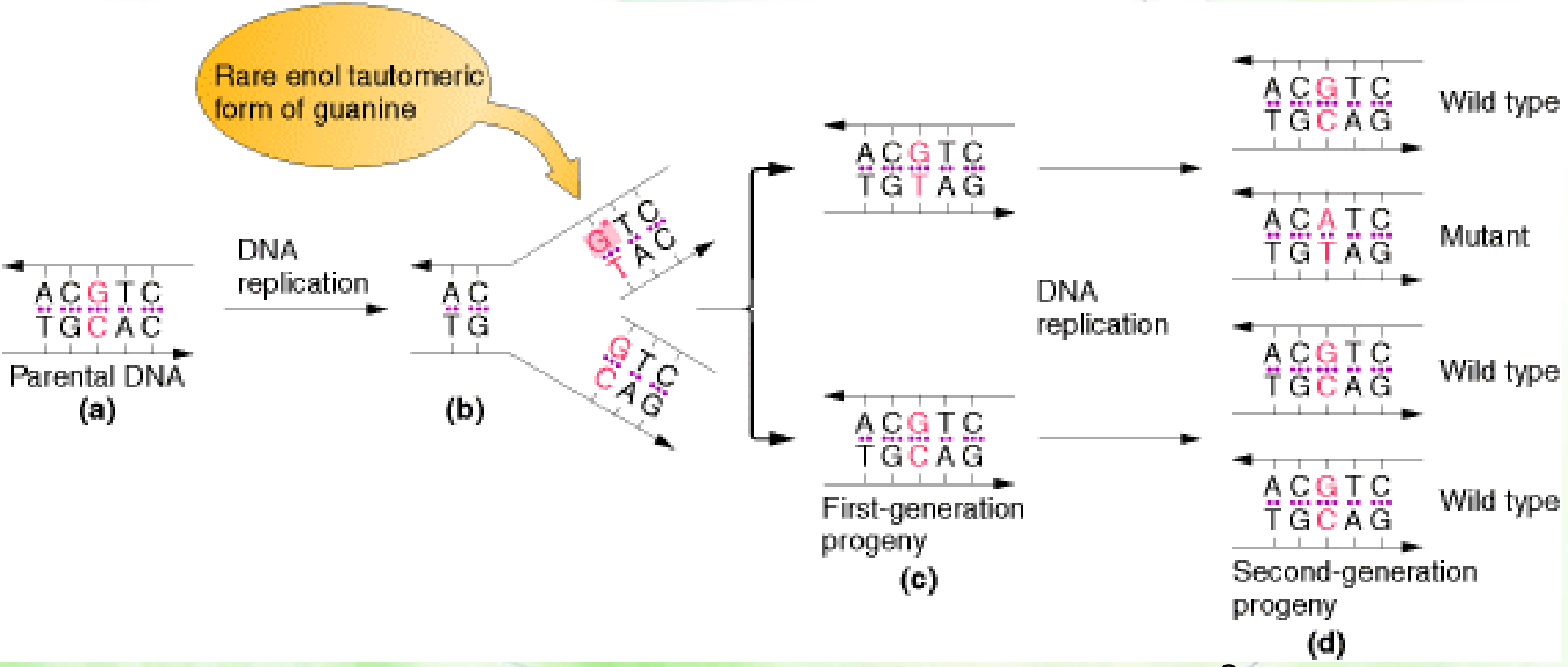
(a)



(b)

Transitions

- A purine substitutes for a purine or a pyrimidine for a pyrimidine

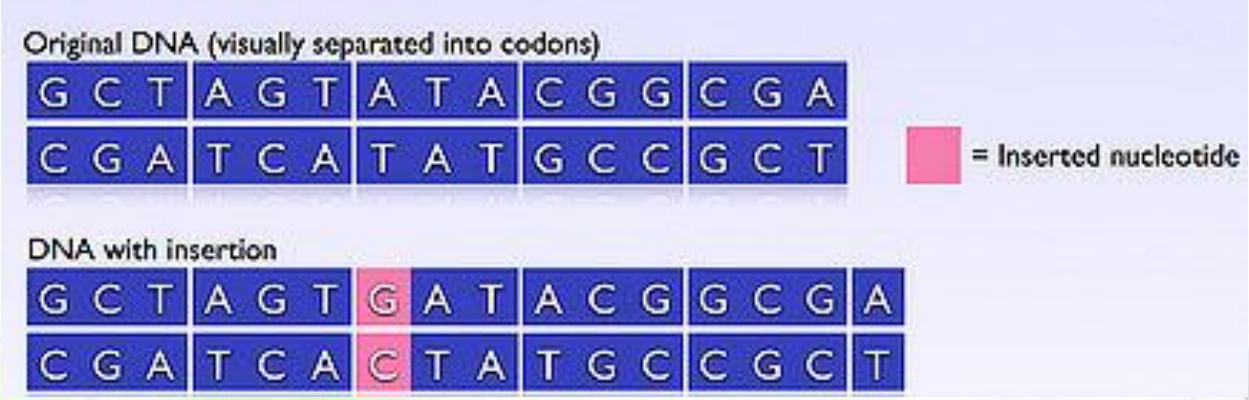


Transversions

- In transversion mutations, a pyrimidine substitutes for a purine or vice versa
- In this case, a replication error would require mispairing of a purine with a purine or a pyrimidine with a pyrimidine

Other errors of DNA replication

- Frameshift mutations
  - Insertion and deletion of one or a few bases can change the reading "frame" of codons and can lead to changes in the amino acid sequence of the produced protein.
  - These mutations often occur at repeated sequences.

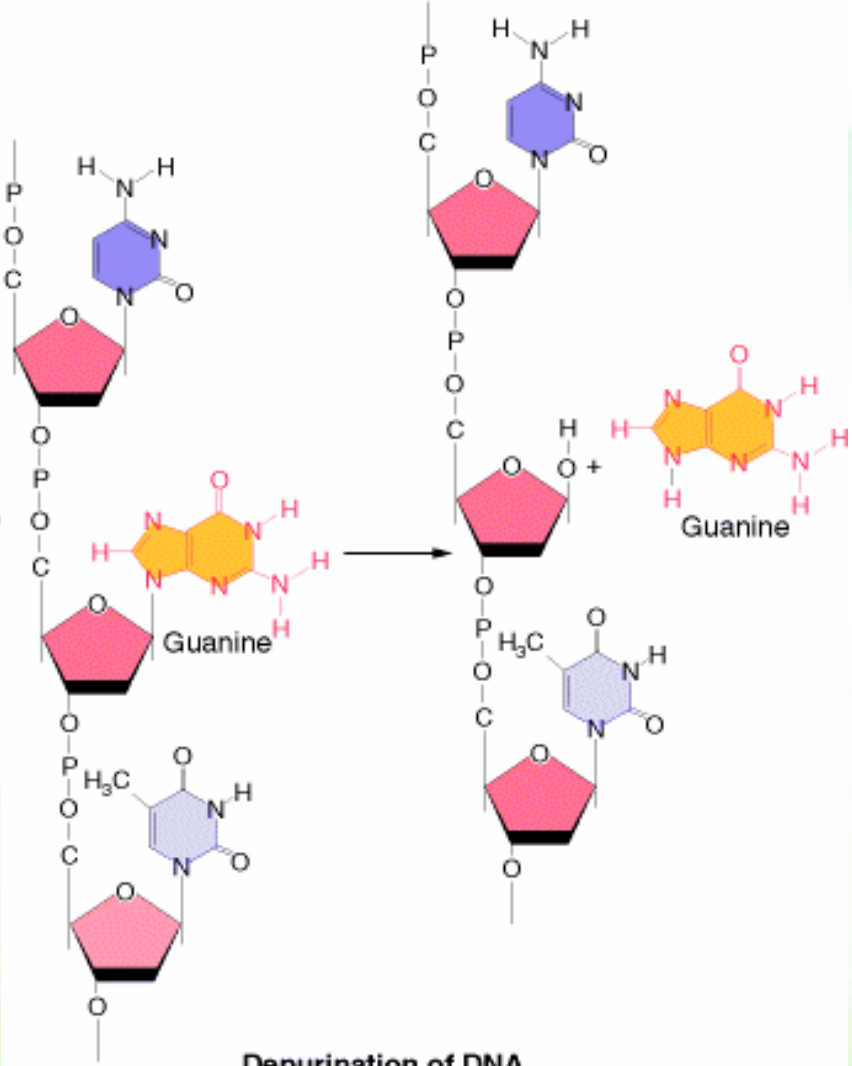


- Large deletions and duplications
  - They also often occur at sequence repeats

Spontaneous lesions

- Spontaneous lesions are naturally occurring type of DNA damage that can generate mutations
  - Depurination
  - Deamination
  - Oxidatively damaged bases

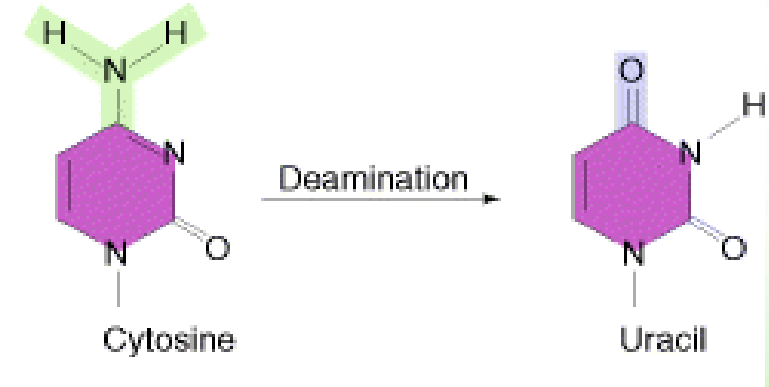
Depurination

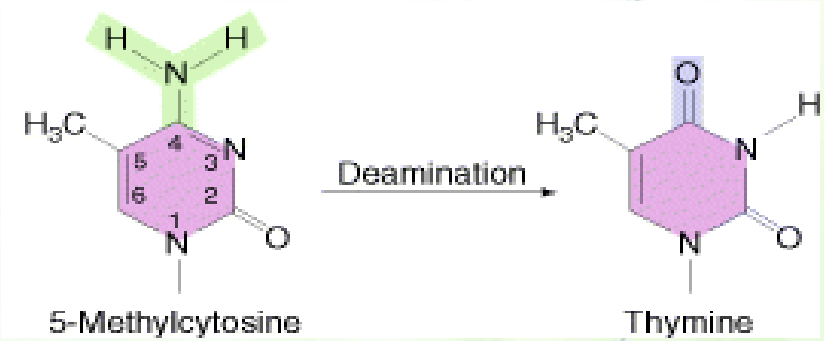


- Cleavage of the glycosidic bond between the base and deoxyribose.
- During replication, a random base can be inserted across from an apurinic site resulting in a mutation.

Deamination

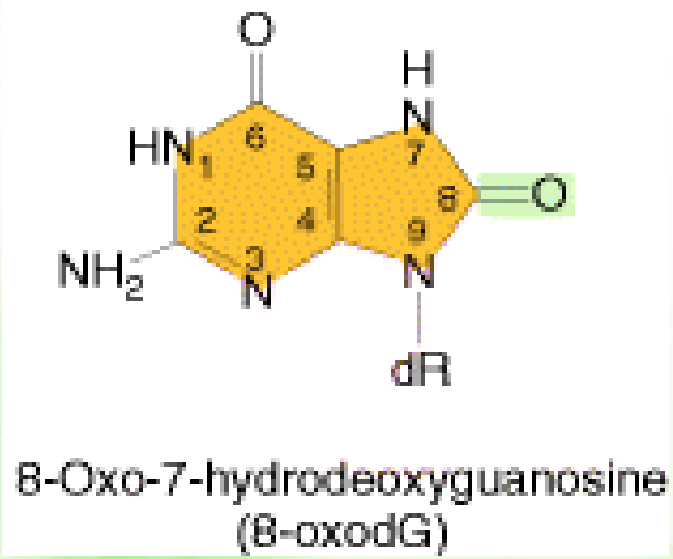
- The deamination of cytosine yields uracil
  - Uracil residues will pair with adenine during replication, resulting in the conversion of a G-C pair into an A-T pair (a GC→AT transition)
- Methylated cytosine can also be deaminated resulting in its conversion to thymine and leading to a transition mutation





Oxidatively damaged bases

- Active oxygen species such as hydrogen peroxide (H2O2) are normally produced during metabolism
  - Example: 8-oxo-7-hydrodeoxyguanosine (8-oxodG, or GO) product mispairs with A, resulting in G→T transversions.



8-Oxo-7-hydrodeoxyguanosine
(8-oxodG)

Spontaneous mutations and human diseases

- **Expansion of a three-base-pair repeat**
  - Kearns-Sayre syndrome: mitochondrial encephalomyopathies.
- **Expansion of a three-base-pair repeat**
  - Fragile X syndrome (CGG repeats in the FMR-1 gene)
  - Kennedy disease (X-linked spinal and bulbar muscular atrophy (CAG repeats in the androgen receptor)
  - Myotonic dystrophy (CTG repeat in the non-coding region of a kinase gene)
  - Huntington disease (CAG repeats in HTT gene)

Induced mutations

Mechanisms of mutagenesis

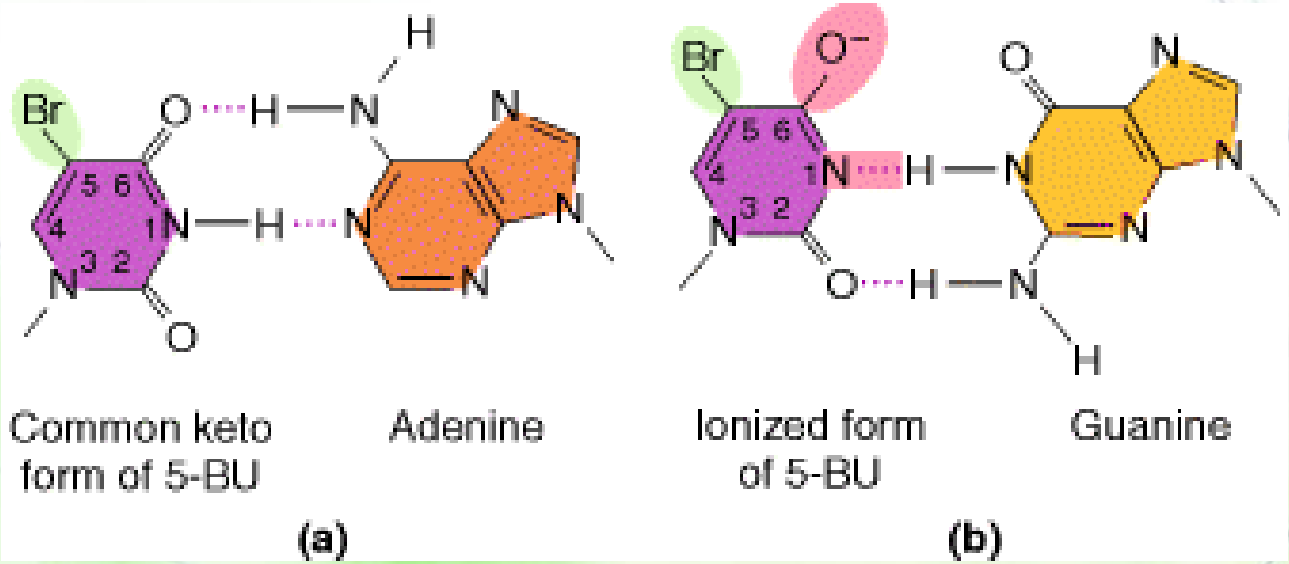
- Mutagens induce mutations by at least three different mechanisms:
  - replace a base in the DNA (base analog)
  - alter an existing base so that it specifically mispairs with another base (alkylation)
  - damage a base so that it can no longer pair with any base under normal conditions

Incorporation of base analogs

- Some chemical compounds are similar to the normal bases of DNA that they are incorporated into DNA in place of normal bases; such compounds are called base analogs.
- These analogs cause incorrect nucleotides to be inserted opposite to them during replication.

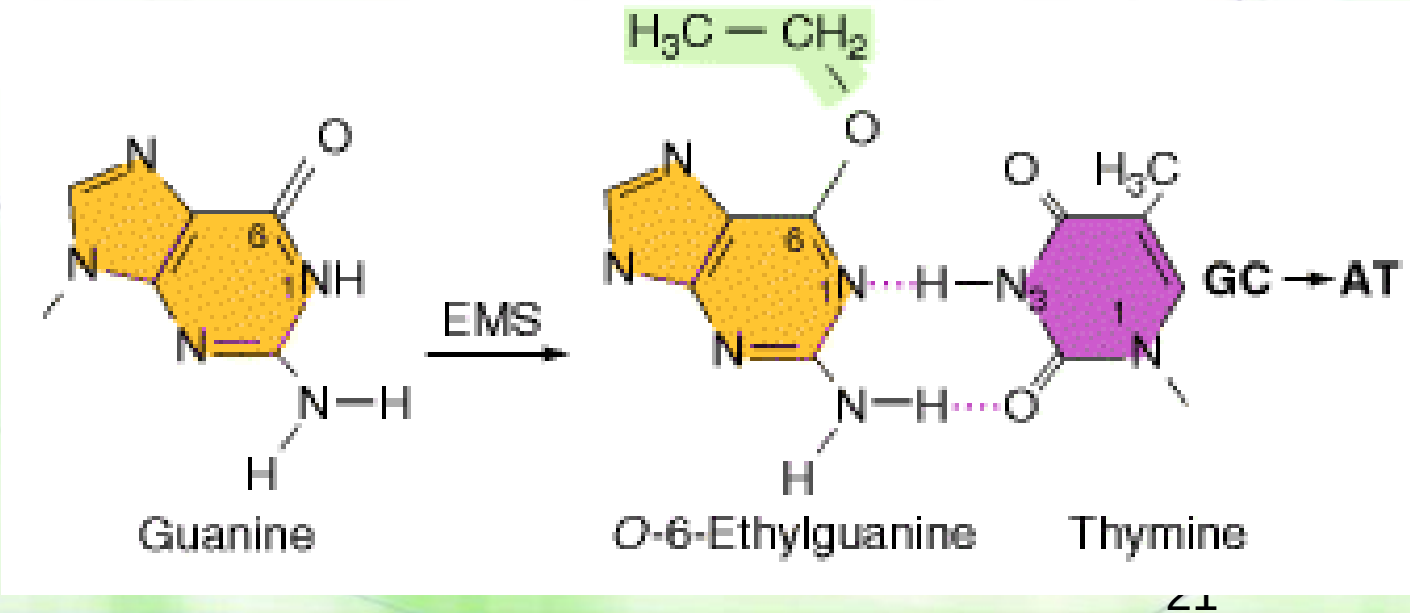
5-bromouracil

- 5-bromouracil (5-BU) is an analog of thymine
- The normal structure of 5-BU pairs with adenine
- When ionized, 5-BU pairs with guanine
- 5-BU causes transition mutations



Specific mispairing

- Some mutagens are not incorporated into the DNA but are altered and cause specific mispairing.
  - Example, the addition of an alkyl group to guanine leads to the formation of O-6-alkylguanine and results in direct mispairing with thymine and a GC → AT transition.

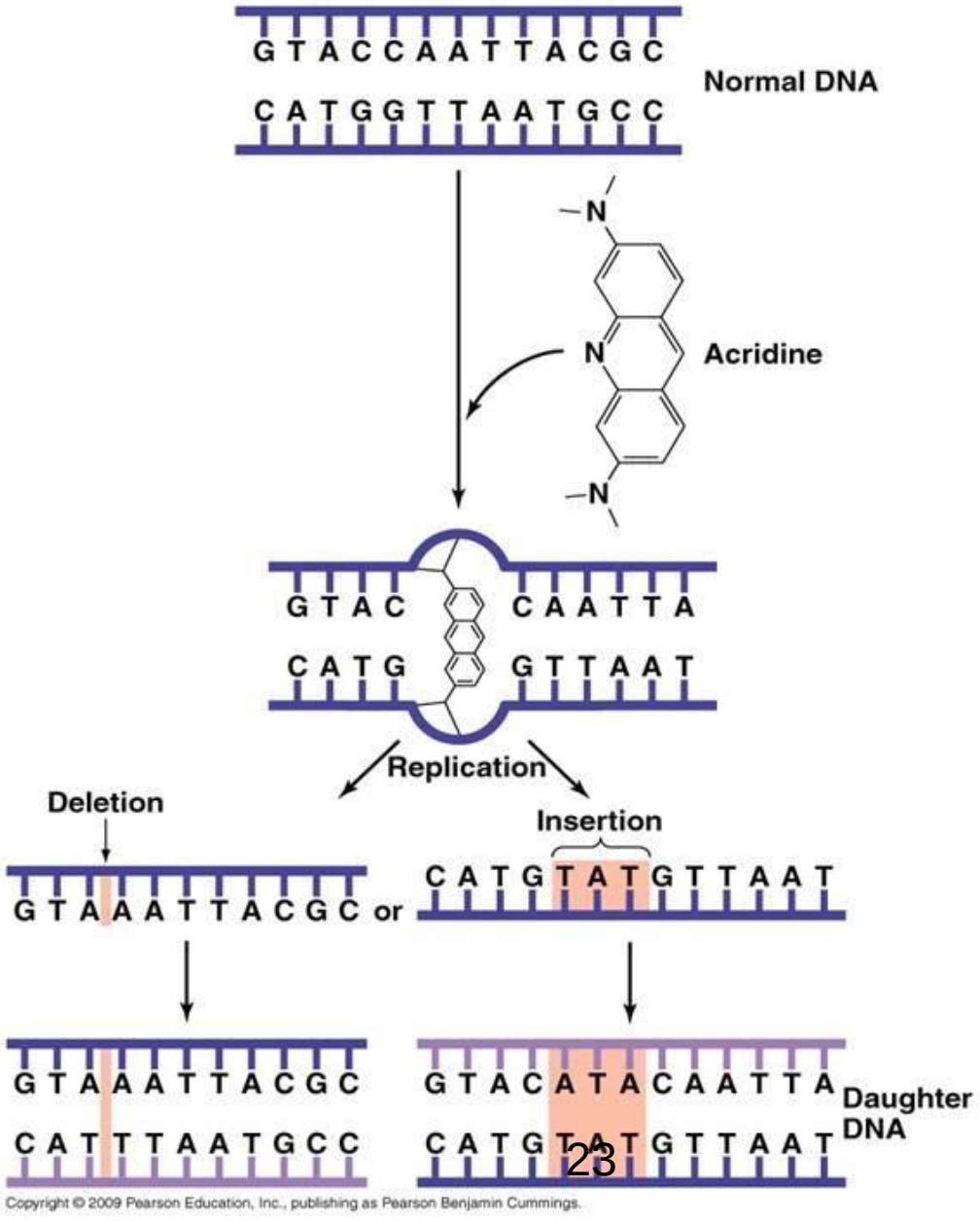


Base damage

- A large number of mutagens damage one or more bases, so no specific base pairing is possible.
- Ionizing radiation results in the formation of ionized and excited molecules that can cause damage to cellular components including DNA.
- Many different types of reactive oxygen species are produced that can
  - damage bases,
  - cause breakage of the N-glycosidic bond (AP sites), or
  - cause strand breaks

Intercalating agents

- The intercalating agents such as proflavin and ethidium bromide are planar molecules that can insert themselves (intercalate) between the stacked nitrogen bases imitating base pairs.
- The intercalated agent can cause single-nucleotide-pair insertions or deletions.



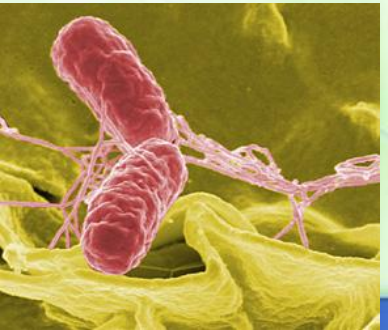
Mutagens and carcinogens

- Mutagenicity and carcinogenicity are clearly correlated when most mutagens are also carcinogens (approximately 90 percent).
- Rapid tests make use of microbes to test for mutagenicity
- The most widely used test was developed in the 1970s by Bruce Ames, who worked with Salmonella typhimurium.
- Chemicals detected by this test can be regarded as potential carcinogens.

http://www.ncbi.nlm.nih.gov/books/NBK21788/

Features of Salmonella typhimurium

- The Ames test uses a mutant strain of S. typhimurium
  - cannot grow in the absence of the amino acid histidine because a mutation has occurred in a gene that encodes one of the enzymes necessary for histidine biosynthesis
  - They also carry a mutation that inactivates a DNA repair system
  - They also carry a mutation that eliminates the protective lipopolysaccharide coating of wild-type Salmonella to enable the entry of many different chemicals into the cell



Reversion mutation

- In order for these cells to survive in the absence of histidine, they must have a mutation that corrects the original mutation that prevented the production of the missing enzyme
- This type of mutation is known as reversion, because this second mutation returns the mutant to the wild-type genotype
- This reversion can happen spontaneously or as the result of a mutagen

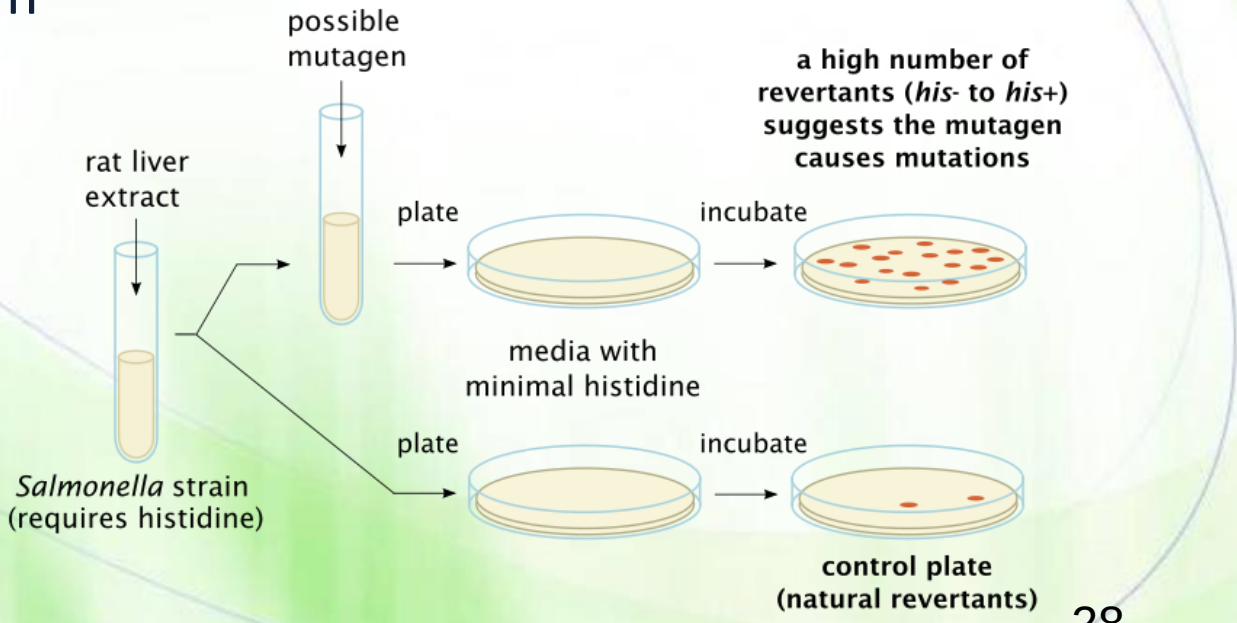
What is considered a mutagen?

- A compound must increase the number of colonies grown in the absence of histidine by double compared to cells grown in the absence of the compound

Water	Motor oil	Alcohol	Drug X	شيبس أبو 5 قروش
10	50	43	9	200

Role of liver enzymes

- In mammals, chemicals are normally detoxified or broken down by liver enzymes
- In some cases, the action of liver enzymes can create a toxic or mutagenic compound from a substance that was originally safe
- Ames incorporated mammalian liver enzymes in his bacterial test system

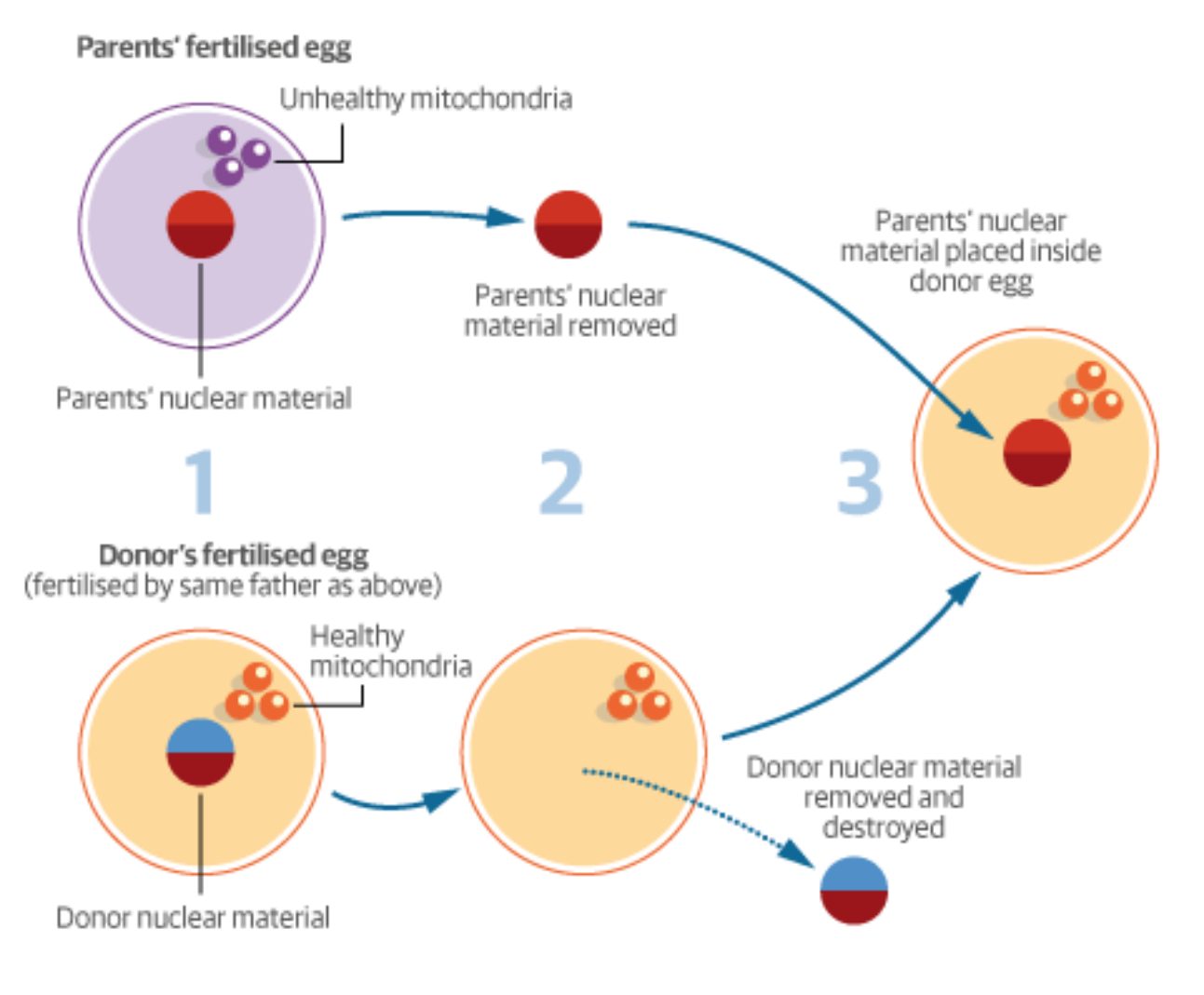


Example

Condition	Water	Motor oil	Alcohol	Drug X	شيبس أبو 5 قروش
-liver enzymes	10	50	43	9	200
+liver enzymes	12	22	50	35	500

Controversial issue

Three-parent babies



https://www.theguardian.com/science/2015/feb/02/three-parent-babies-explained

DNA repair mechanisms

Resources

- This lecture
- Cooper, pp 207-219
- An Introduction to Genetic Analysis. 7th edition. Griffiths AJF, Miller JH, Suzuki DT, et al., New York: W. H. Freeman; 2000. (http://www.ncbi.nlm.nih.gov/books/NBK22004/)

DNA repair

- Maintaining genetic stability requires not only an accurate mechanism of DNA replication, but also mechanisms for repairing DNA damage.

- These mechanisms are collectively called DNA repair.

Repair mechanisms

- Prevention of errors before they happen
- Direct reversal of damage
- Excision-repair pathways
- Postreplication repair

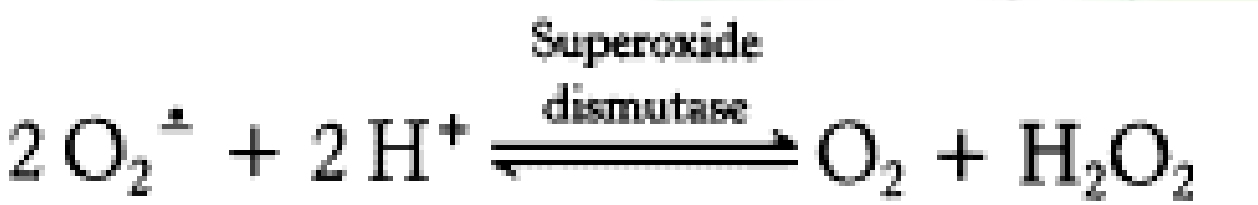
Prevention of errors before they happen

Reactive oxygen species

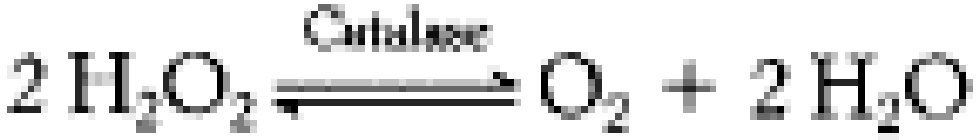
- Some enzymatic systems neutralize potentially damaging compounds before they even react with DNA

- One example of such a system is the detoxification of reactive oxygen species and oxygen radicals, which are produced during oxidative damage to DNA

- Superoxide dismutase

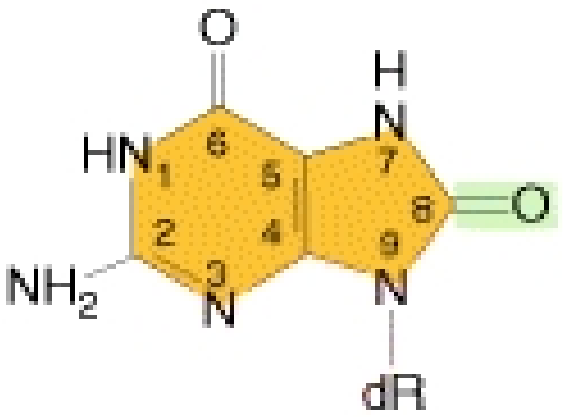


- Catalase



8-oxodG

- The enzyme product of the mutT gene prevents the incorporation of 8-oxydeoxyguanosine (8-oxodG) into DNA.
- 8-oxodG is formed from free radical attack of DNA and pairs with A rather than C.

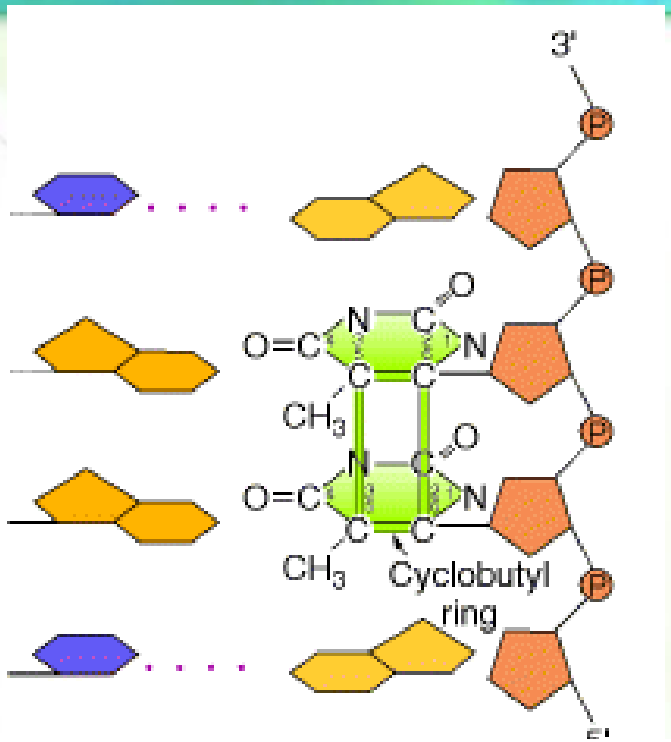


8-Oxo-7-hydrodeoxyguanosine
(8-oxodG)

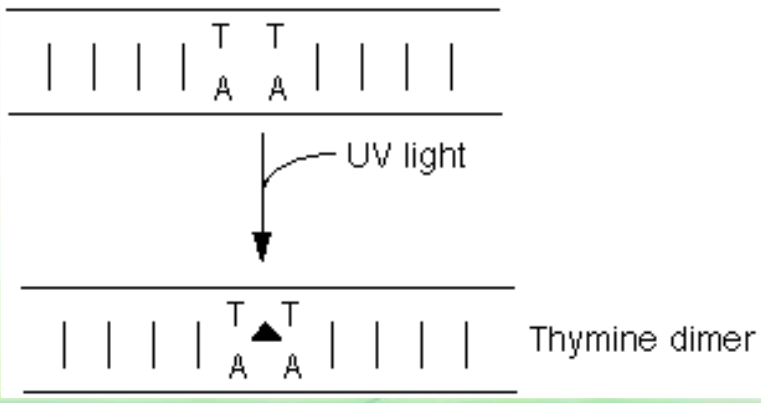
Direct reversal of damage

Cyclobutane pyrimidine

- Some lesions can be repaired by reversal of DNA damage.
- UV light that hits DNA results in the formation of a covalent interaction between two adjacent pyrimidine bases forming structures known as cyclobutane pyrimidine dimers, most frequently between two thymines.
- This product is a mutagenic photodimer.



Photoreactivation Repair



Exision-repair pathways

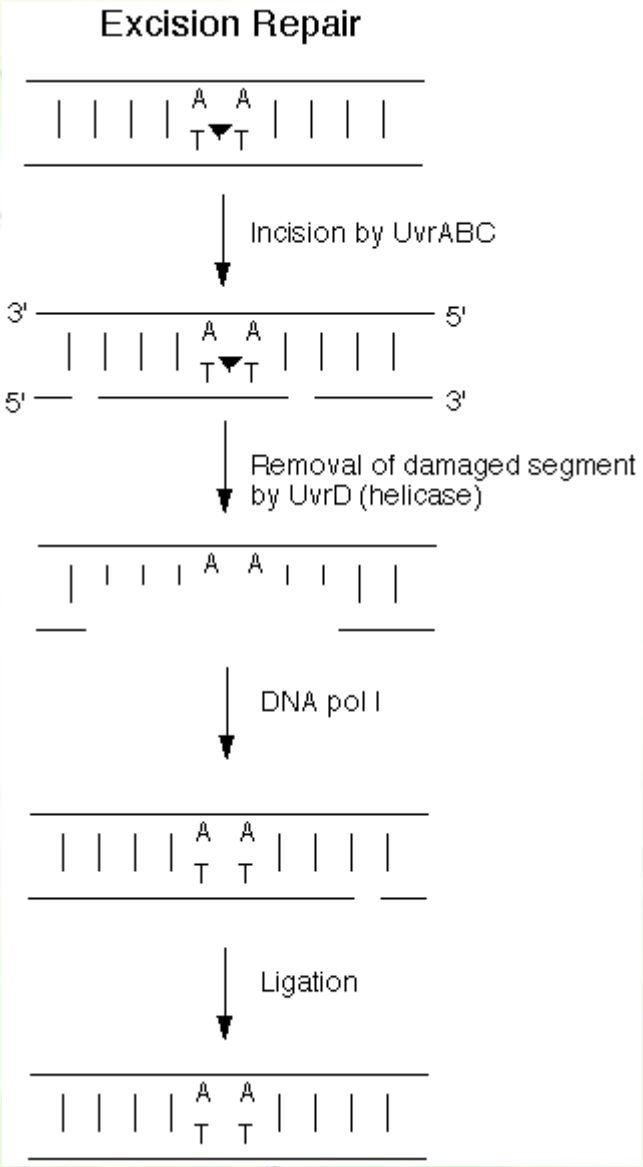
Types

- General excision repair
- Coupling of transcription and repair
- Specific excision pathways

General excision repair

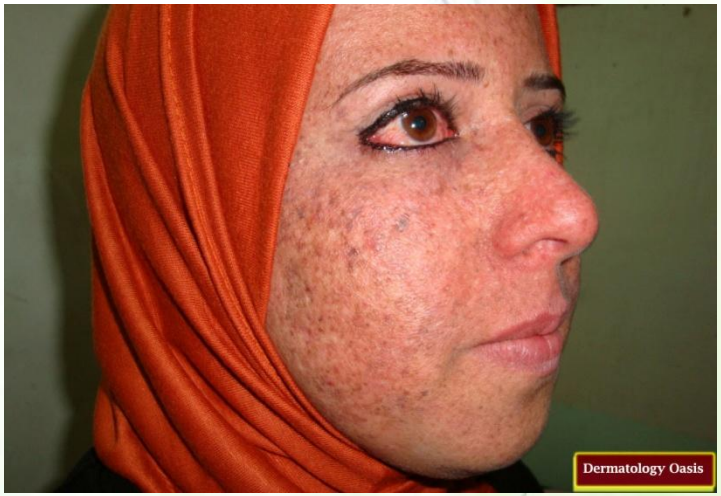
AKA: nucleotide excision repair

- This system includes the breaking of a phosphodiester bond on either side of the lesion, on the same strand, resulting in the excision of an oligonucleotide.
  - In bacteria, the UvrABC protein complex does this work.
- A helicase removes the strand.
- The gap is filled by DNA polymerase (in bacteria, it is DNA pol I), and a ligase seals the breaks.



In human…

- In human cells, the process is more complex than its bacterial counterpart. However, the basic steps are the same as those in E. coli
- Defect in this mechanism causes a condition known as Xeroderma pigmentosum.



Transcription and repair

- In both eukaryotes and prokaryotes, 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.

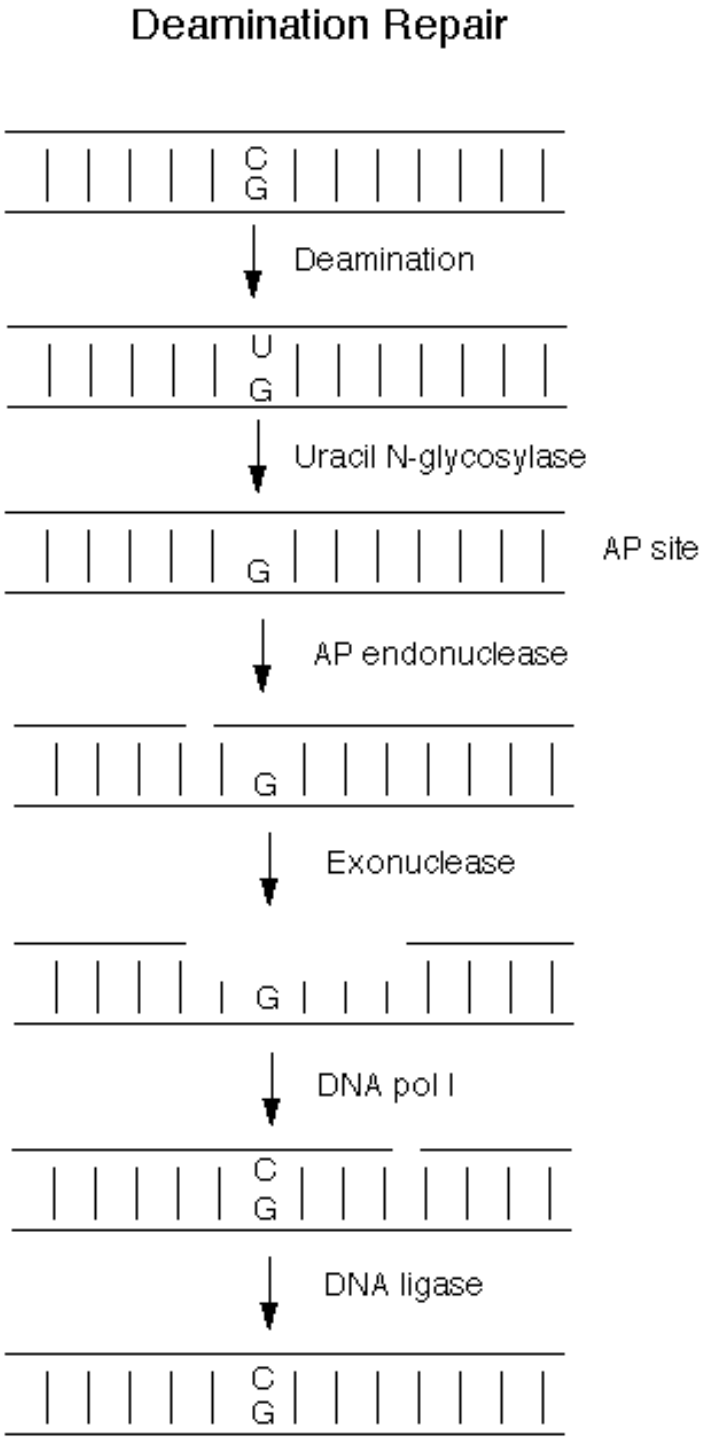
Speaific exaision pathways

DNA glycosylase repair pathway

- 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 resulting AP site is then repaired by an AP endonuclease repair pathway.

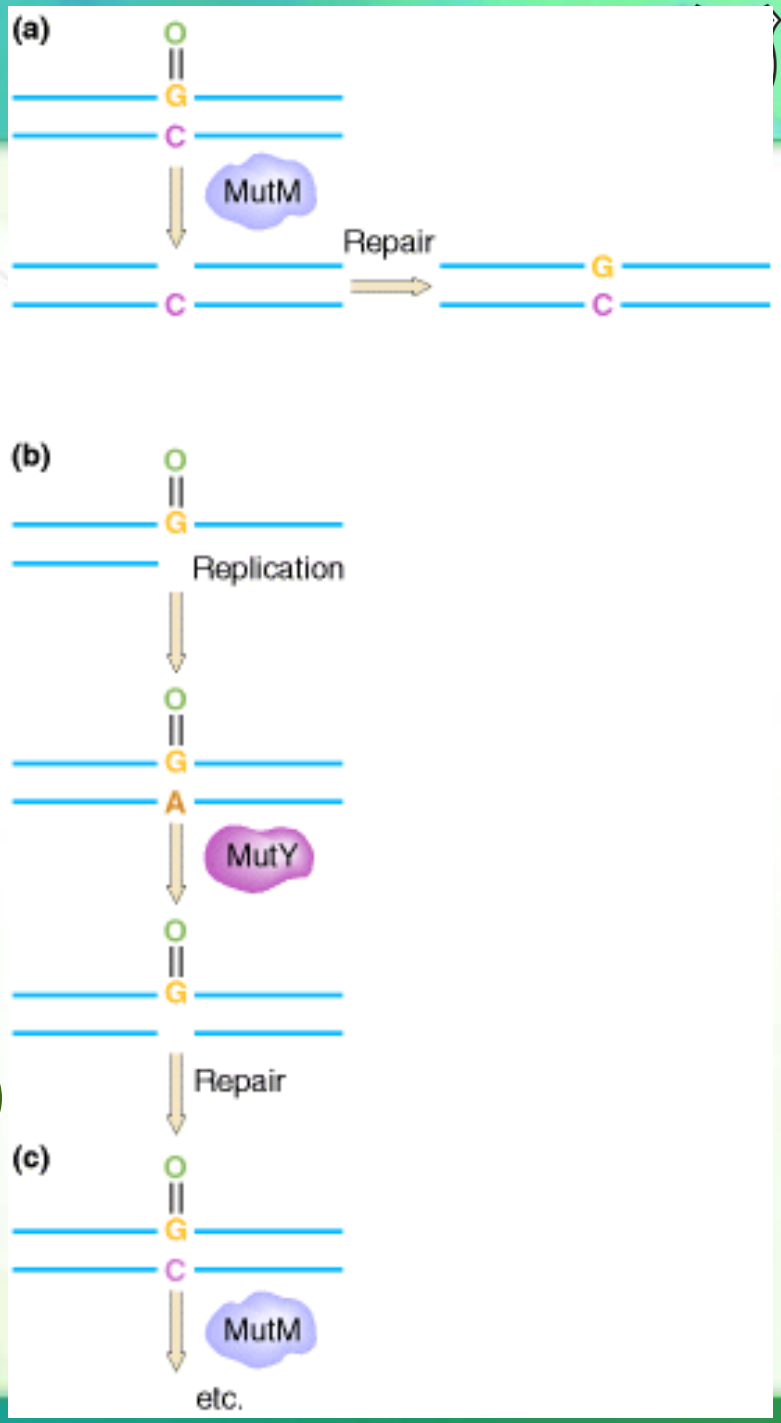
DNA glycosylases

- Numerous DNA glycosylases exist.
  - Example: uracil-DNA glycosylase, removes uracil from DNA.
  - Uracil residues, which result from the spontaneous deamination of cytosine can lead to a C→T transition if unrepaired.
- The AP endonucleases cleaves the phosphodiester bonds at AP sites.
- An exonuclease removes the strand, DNA polymerase I fills in the gap, and DNA ligase and re-forms the bond.



GO system

- mutM removes 8-oxodG, or GO, lesion from DNA.
- If DNA is replicated,
  - mutM first removes the GO lesion
  - Then, mutY removes the mispaired adenine from the opposite strand, leading to restoration of the correct cytosine by repair synthesis (mediated by DNA polymerase I) and allowing subsequent removal of the GO lesion by the mutM product



Postreplication repair

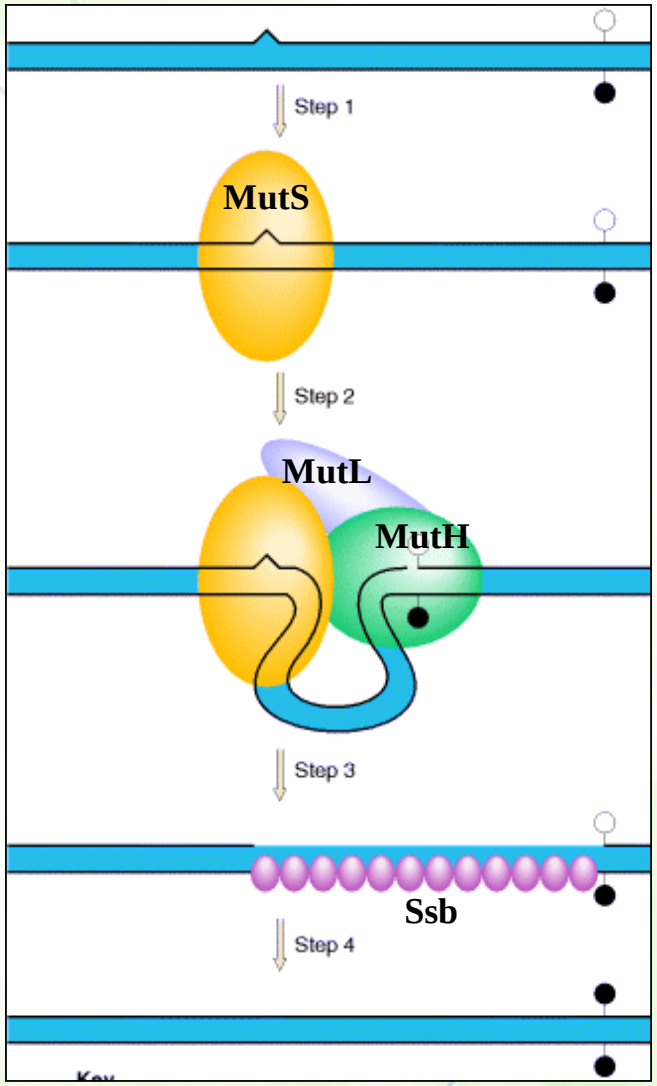
Mismatch repair

- Some repair pathways are capable of recognizing errors even after DNA replication has already occurred.
- One such system, termed the mismatch repair system, can detect mismatches that occur in DNA replication.

The mechanism

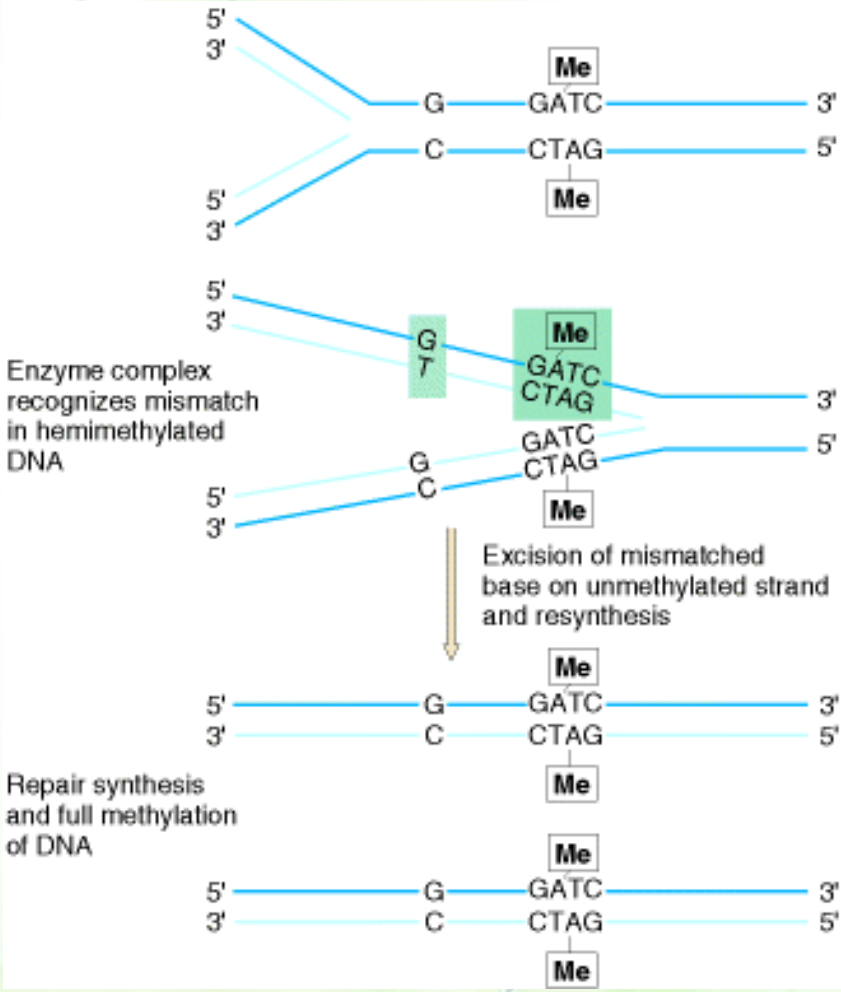
- Recognize mismatched base pairs.
- Determine which base in the mismatch is the incorrect one.
- Excise the incorrect base and carry out repair synthesis
- This is mediated by the mut protein system.

- BUT…How can the mismatch repair system determine whether G or T is incorrect?



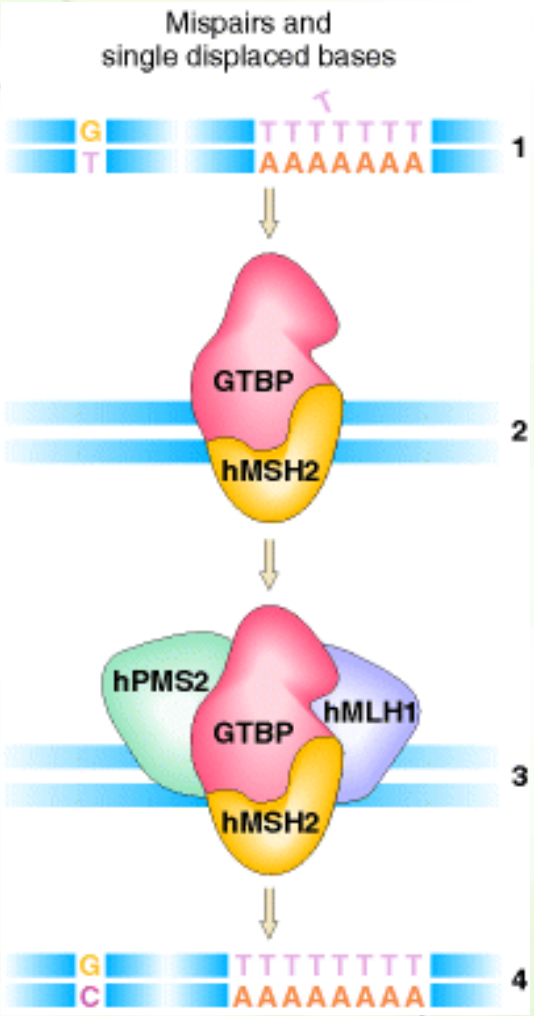
DNA methylation

- DNA is methylated following replication by the enzyme, adenine methylase.
- However, it takes the adenine methylase several minutes to methylate the newly synthesized DNA.
- The mismatch repair system in bacteria takes advantage of this delay to repair mismatches in the newly synthesized strand.



In humans

- The mismatch repair system has also been characterized in humans.
- Two of the proteins, hMSH2 and hMLH1, are very similar to their bacterial counterparts, MutS and MutL, respectively.



Hereditary nonpolyposis colon cancer (HNPCC)

- Several genes responsible a type of colon cancer have been identified, including MSH2 and MLH1.
- The protein products of the MSH2 and MLH1 genes appear to have critical roles in the recognition and repair of DNA mismatches.
- They are homologous to the mutL and mutS genes in bacteria and yeast.

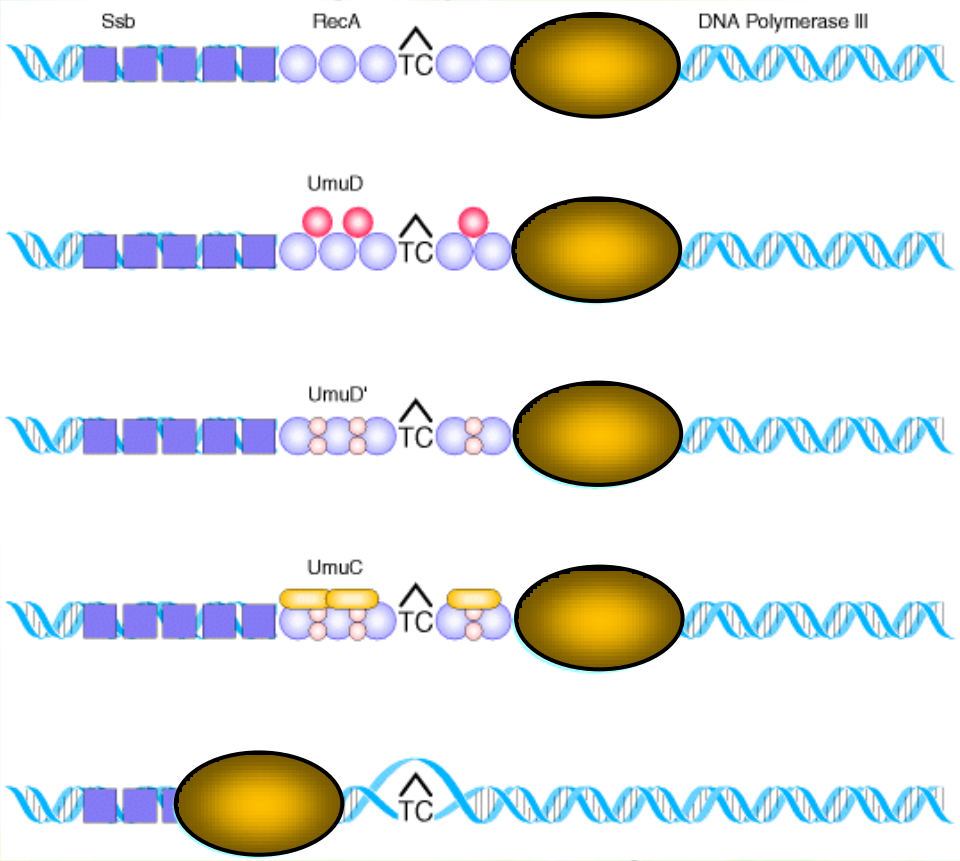
SOS system

- A large number of mutagens such as ultraviolet light damage one or more bases, resulting in a replication block, because DNA synthesis will not proceed past a base that cannot specify its complementary partner by hydrogen bonding

- In bacterial cells, such replication blocks can be bypassed by inserting nonspecific bases. The process requires the activation of a special system, the SOS system

The SOS mechanism

- DNA polymerase III pauses at a type of damage called a TC photodimer.
- This region attracts single-strand-binding protein (Ssb), as well as the RecA protein, which signals the cell to synthesize the UmuC and UmuD proteins forming the SOS system.
- The SOS system allows DNA polymerization to continue past the dimer.



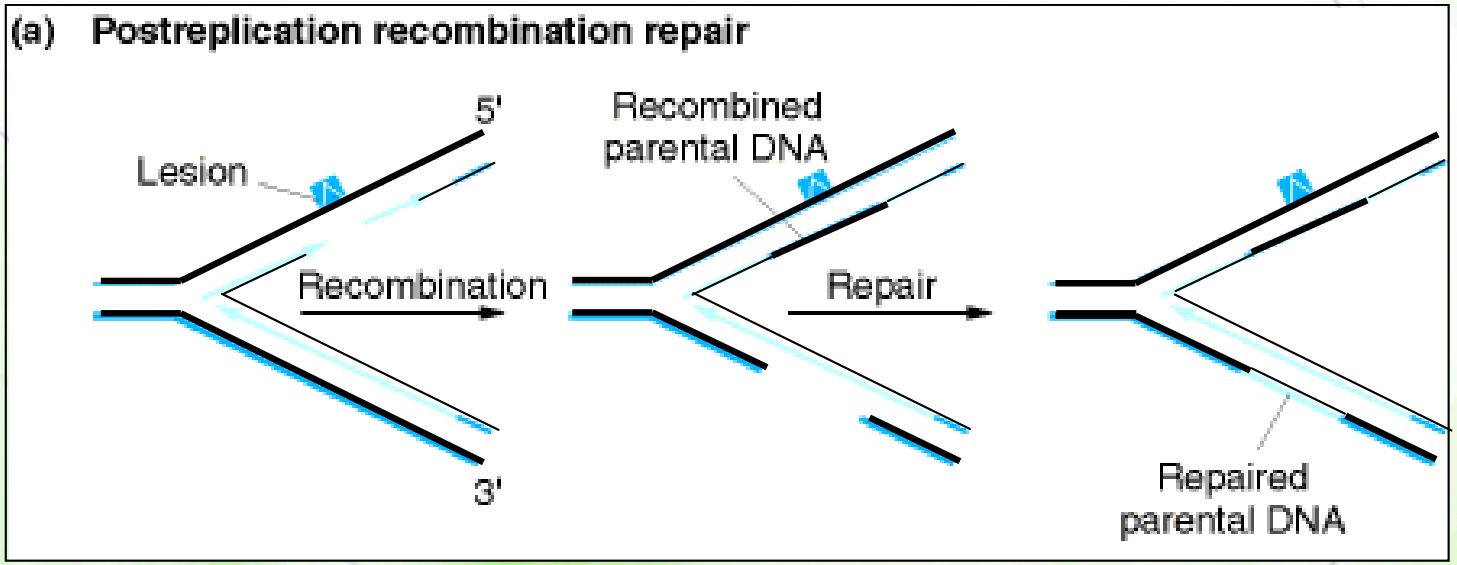
SOS system and mutations

- However, the SOS system inserts the necessary number of bases (often incorrect ones) directly across from the lesion and replication continues without a gap.

- SOS system often generates mutations.

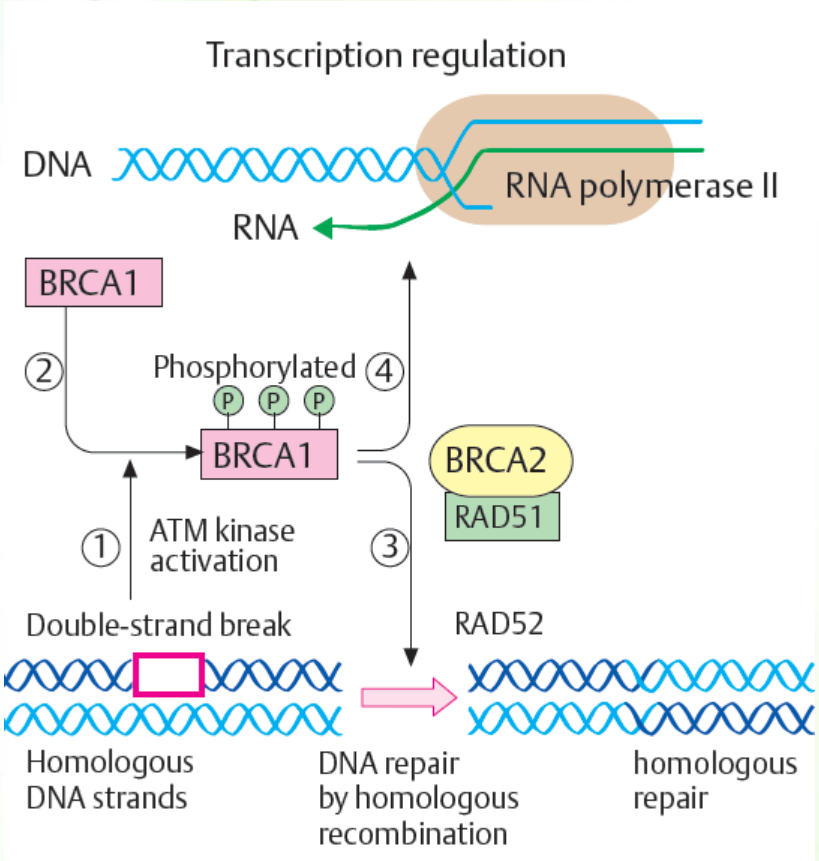
Recombinational repair

- The recA gene also takes part in postreplication repair
- Here the DNA replication system pauses at a UV photodimer or other blocking lesion and then restarts past the block, leaving a single-stranded gap.
- In recombinational repair, this gap is patched by DNA cut from the sister molecule.



Breast cancer

- Mutations in BRCA1 account for 2% of all breast cancers and, at most, 5% of ovarian cancer (men are also at risk for breast cancer).
- BRCA1 activates homologous recombination repair of DNA double-stranded breaks).
- BRCA1 is also involved in transcription and transcription-coupled DNA repair.
- When its function is lost, genetic defects accumulate, which are directly responsible for cancer formation.



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