
بسم الله الرحمن الرحيم

DNA Repair

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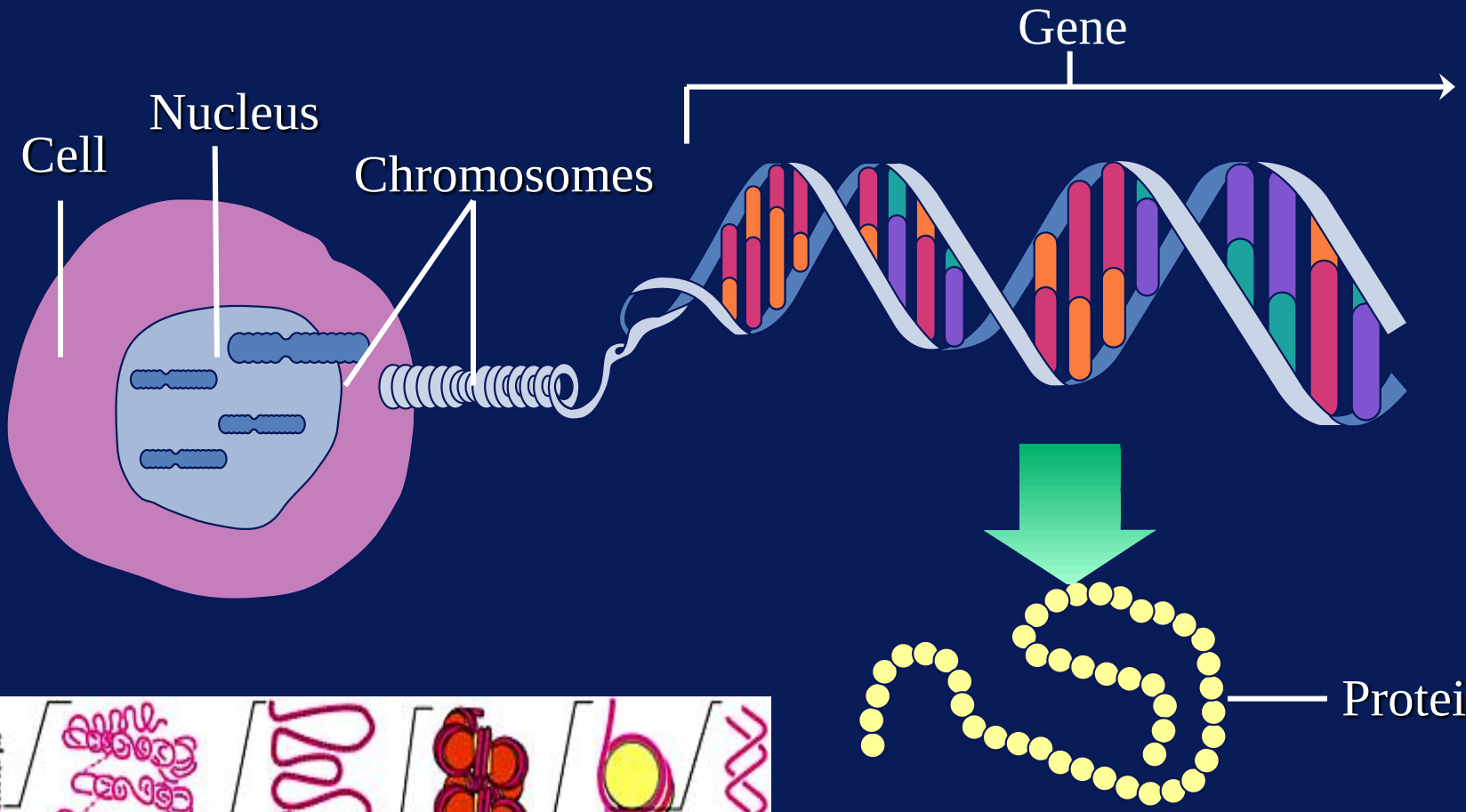
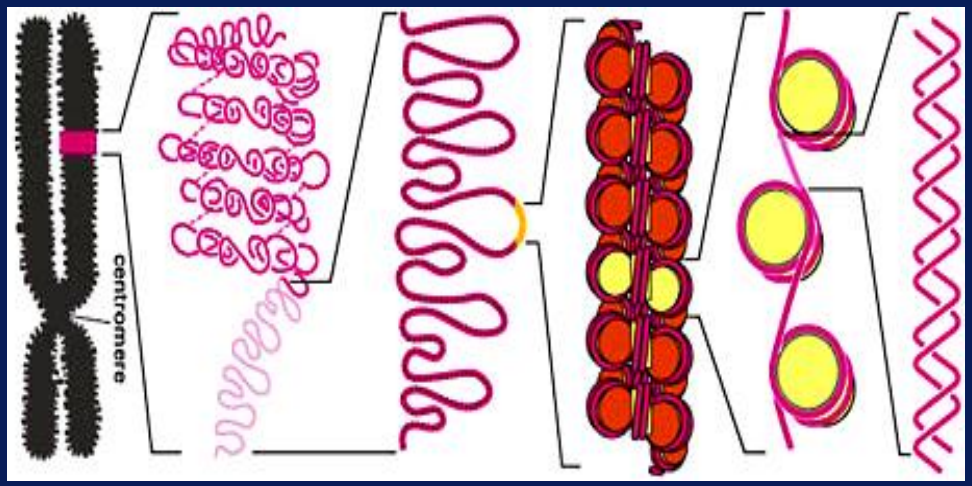
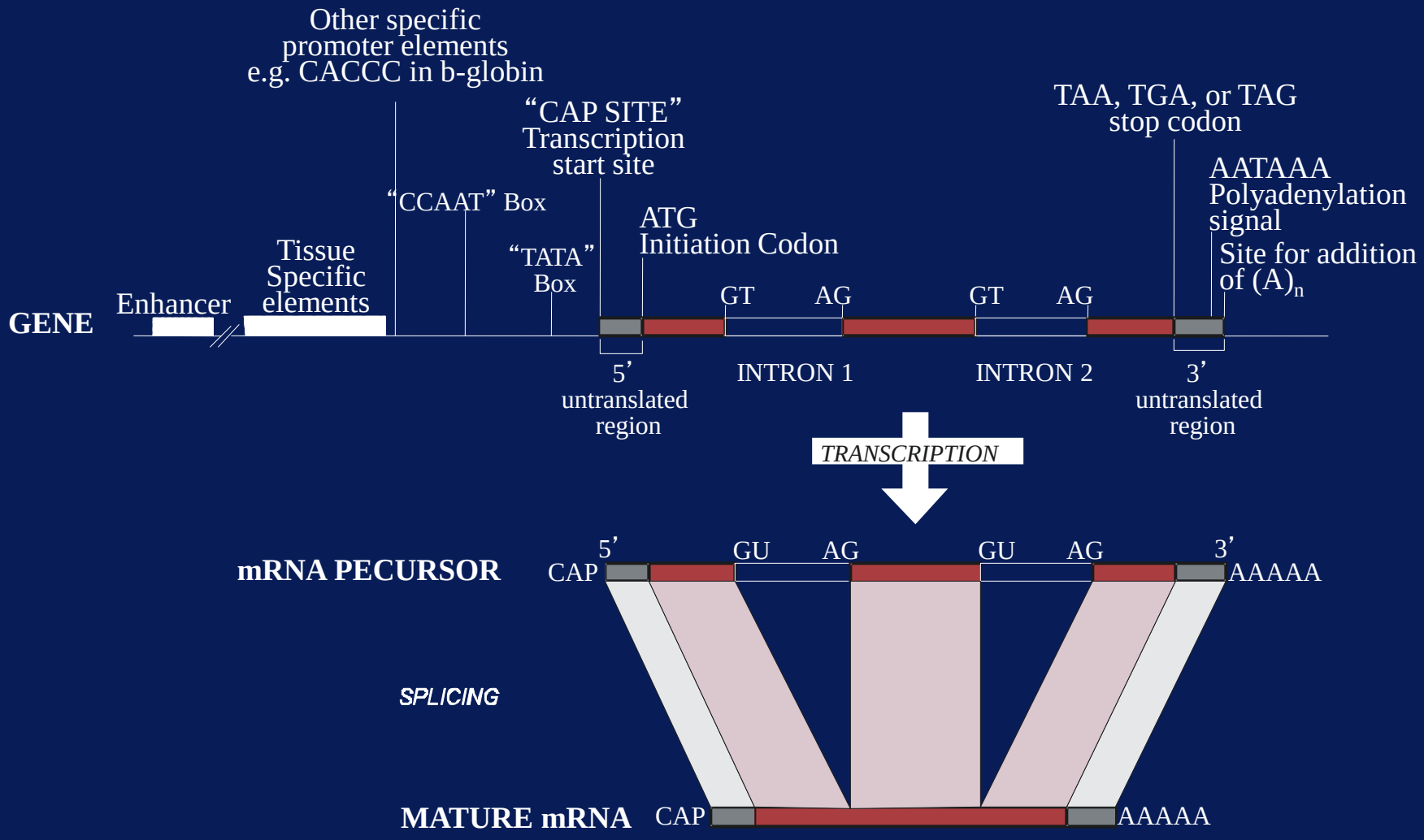
Medical Geneticist

Chromosomes, DNA, and Genes

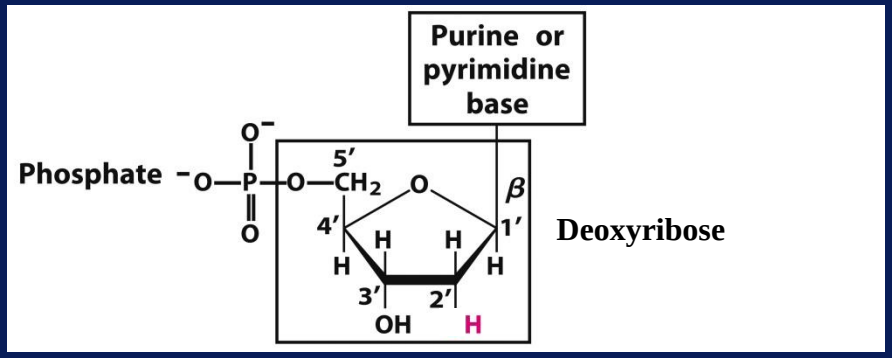




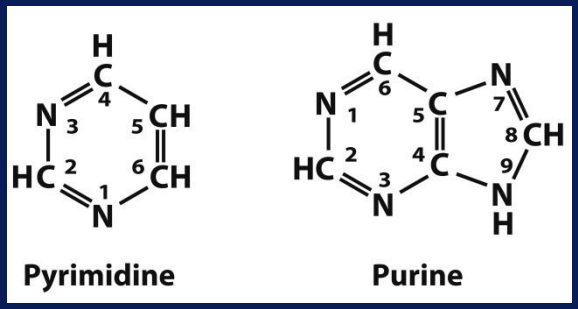

Other specific
promoter elements
e.g. CACCC in b-globin
"CAP SITE"
Transcription
start site
TAA, TGA, or TAG
stop codon
AATAAA
Polyadenylation
signal
"CCAAT" Box
ATG
Initiation Codon
Tissue
Specific
elements
"TATA"
Box
Site for addition
of (A)n
GENE
Enhancer
GT
AG
GT
AG
5'
untranslated
region
INTRON 1
INTRON 2
3'
untranslated
region
TRANSCRIPTION
mRNA PECURSOR
5'
GU
AG
GU
AG
3'
CAP
AAAAA
SPLICING
MATURE mRNA
CAP
AAAAA

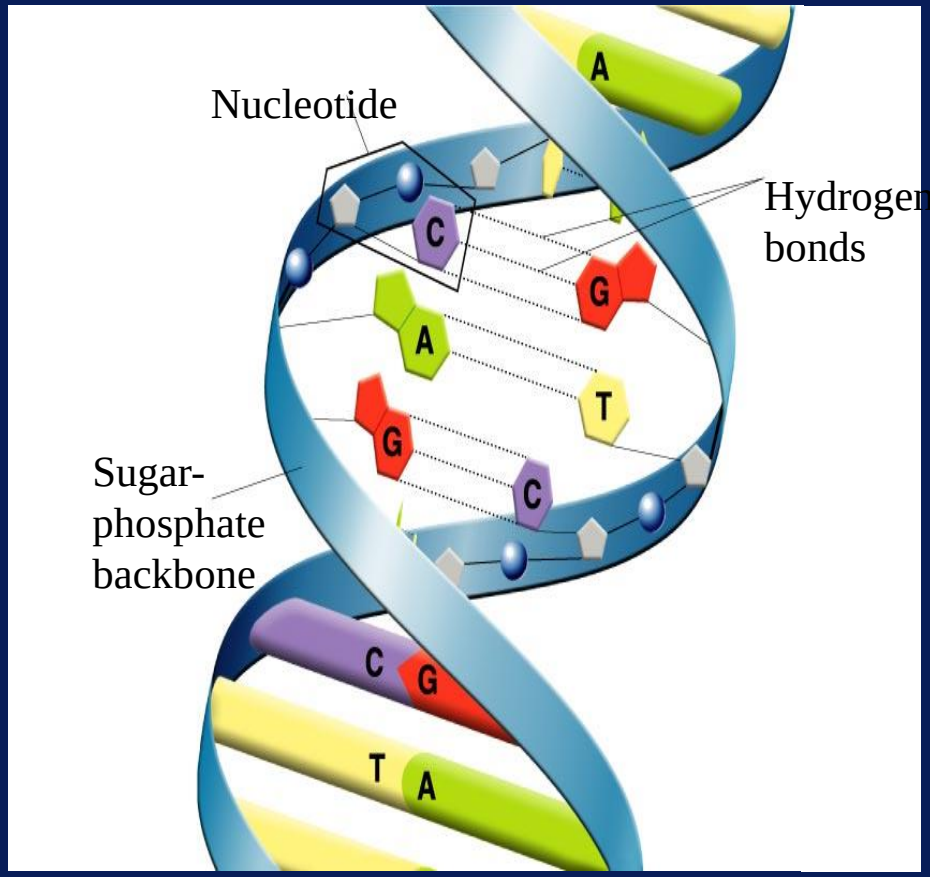
The Double Helix Molecule

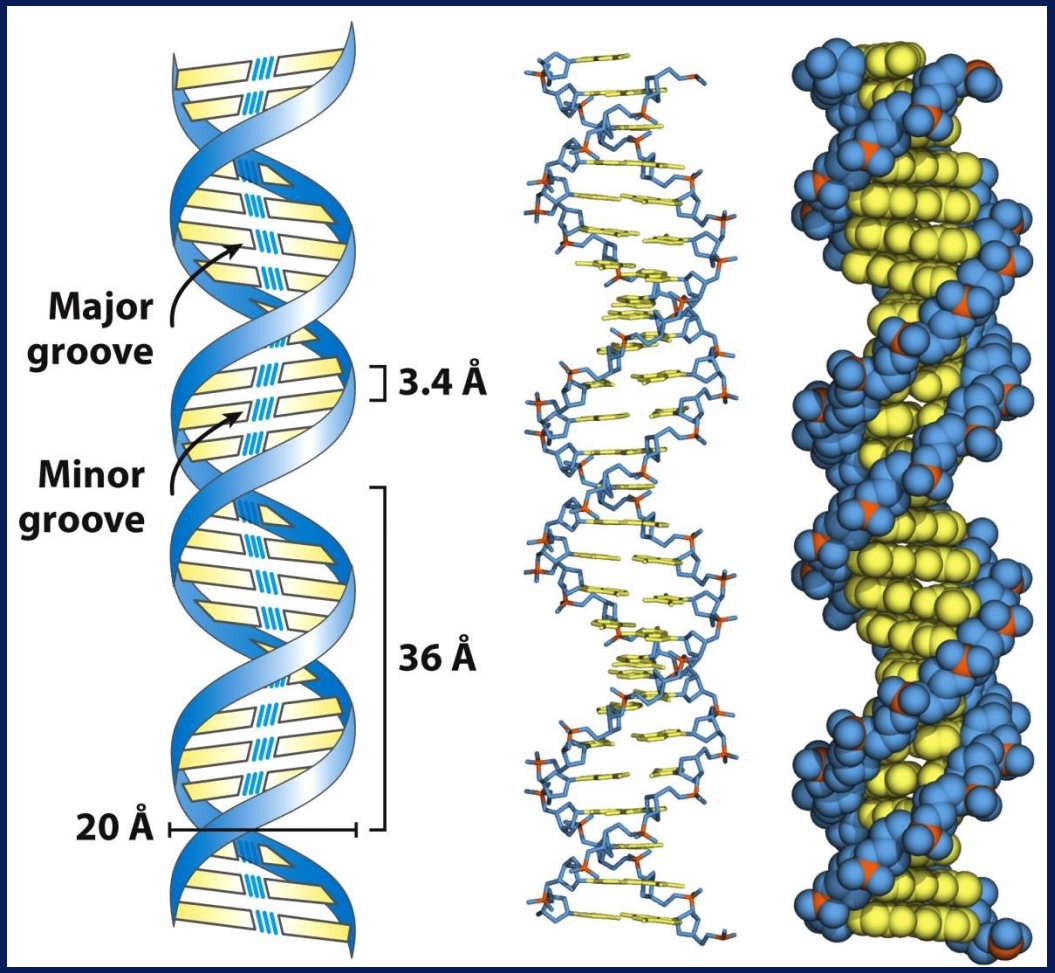
- The DNA double helix has two strands twisted together.

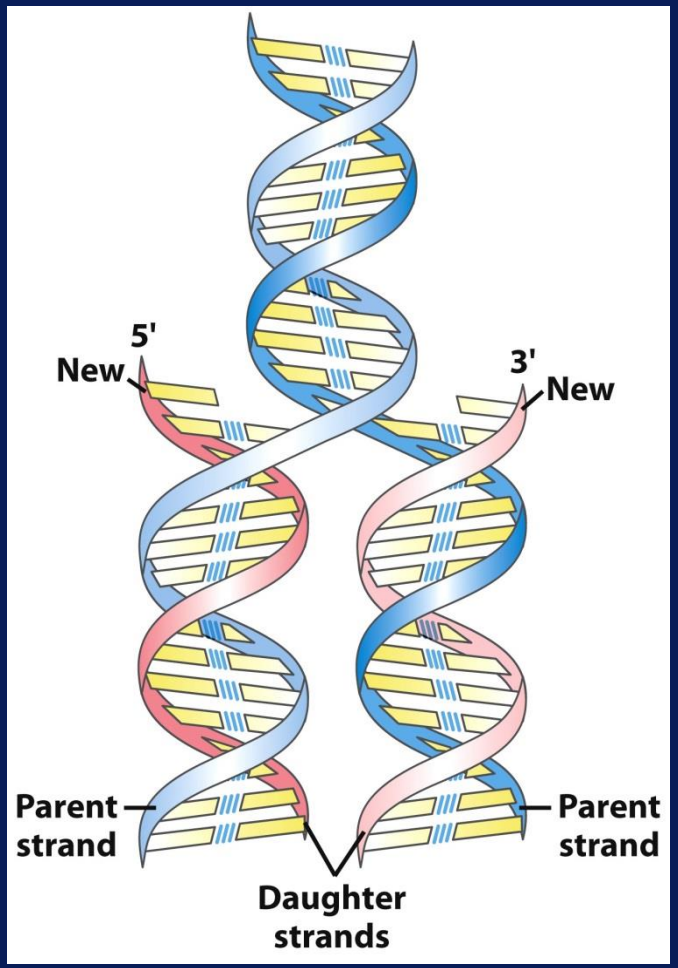


Pyrimidines: Cytosine C; Thymine T;
Purines: Adenine A; Guanine G





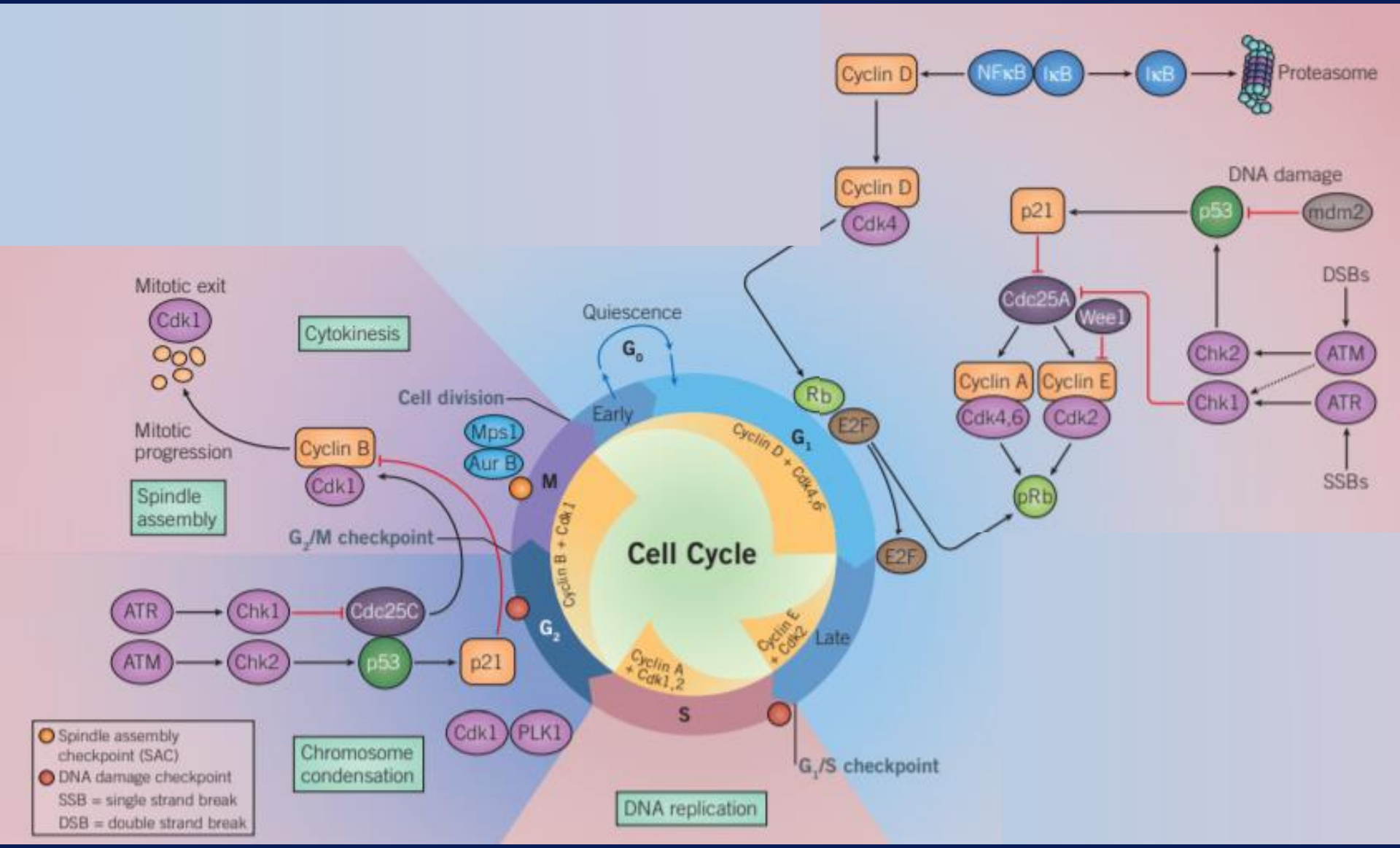




Each time a cell divides it forms 10DSBs, and 50,000 SS!

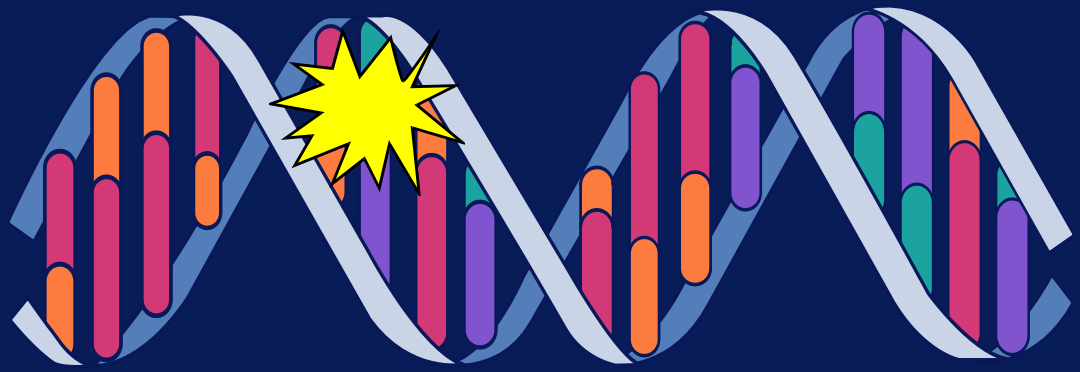
DNA repair enzymes continuously monitor chromosomes to correct damaged nucleotides

- Endogenous mutagens - ROS from cellular respiration, hydrolysis, metabolites that act as alkylating agents
- Exogenous mutagens - U.V., cigarette smoke, dietary factors


Cyclin D
NFκB
IκB
IκB
Proteasome
Cyclin D
Cdk4
DNA damage
p21
p53
mdm2
Cdc25A
Wee1
Cyclin A
Cyclin E
Cdk4,6
Cdk2
Chk2
Chk1
ATM
ATR
DSBs
SSBs
Rb
E2F
pRb
E2F
Mitotic exit
Cdk1
Cytokinesis
Quiescence
G_0
Cell division
Early
Mitotic progression
Cyclin B
Cdk1
Mps1
Aur B
M
Spindle assembly
G_2/M checkpoint
Cyclin D + Cdk4,6
G_1
Cyclin B + Cdk1
Cell Cycle
Cyclin E + Cdk2
Late
Cyclin A + Cdk1,2
S
ATR
Chk1
Cdc25C
ATM
Chk2
p53
p21
G_2
Cdk1
PLK1
Chromosome condensation
G_1/S checkpoint
DNA replication
Spindle assembly checkpoint (SAC)
DNA damage checkpoint
SSB = single strand break
DSB = double strand break

Mutations: Pathogenic variants

A mutation is a change in the “normal” base pair sequence



- Can be:
  - a single base pair substitution
  - a deletion or insertions of 1 or more base pairs (indel)
  - a larger deletion/insertion or rearrangement

- A mutation is a change in the nucleotide sequence of a short region of a genome, including point mutations, insertion or deletion of one or a few nucleotides.
- Transitions, transversions.
- All cells possess DNA-repair enzymes that attempt to minimize the number of mutations that occur.

DNA Sequence Variation

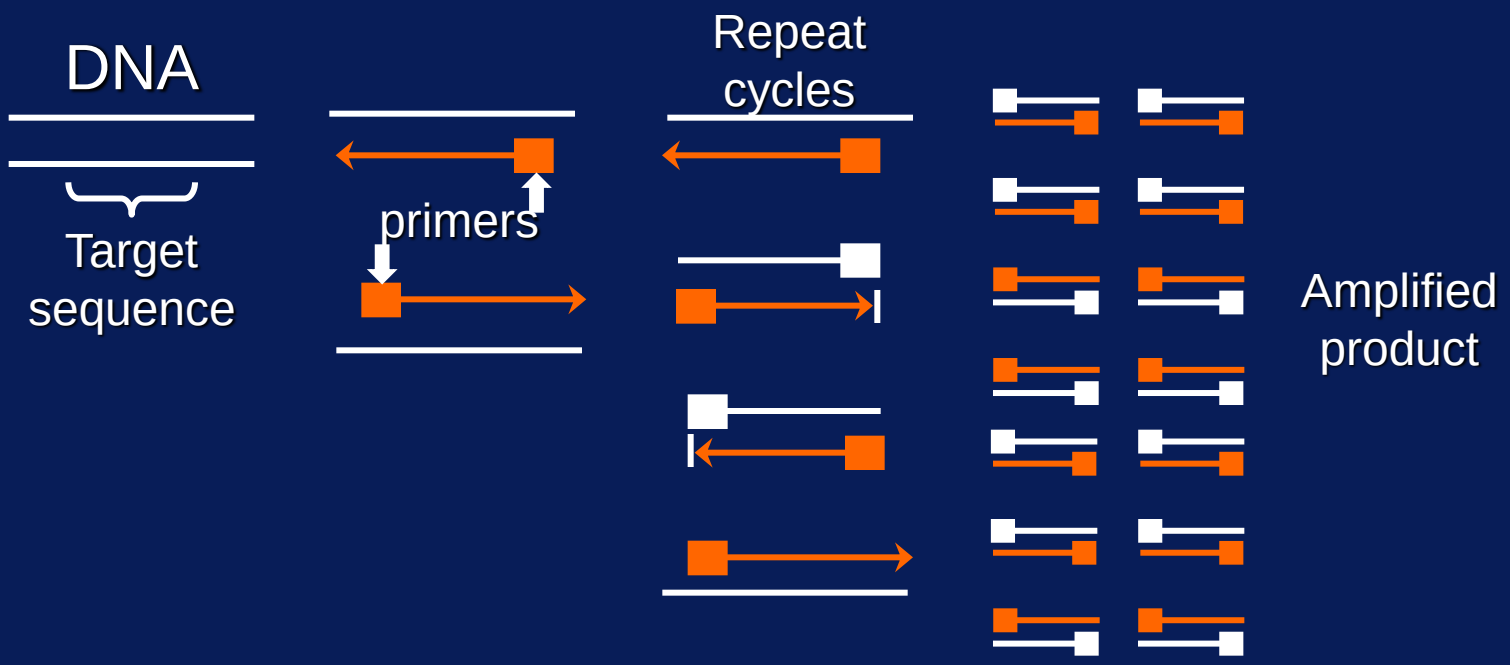
- DNA Sequence Variation:
  - Human to human: ~0.1% (1:1000 bp)
    - Human genome = $3X10^9$ bp X 0.1% =~$3X10^6$ DNA common variants
  - More common in “junk” DNA: introns, intergenic regions

DNA Samples and PCR

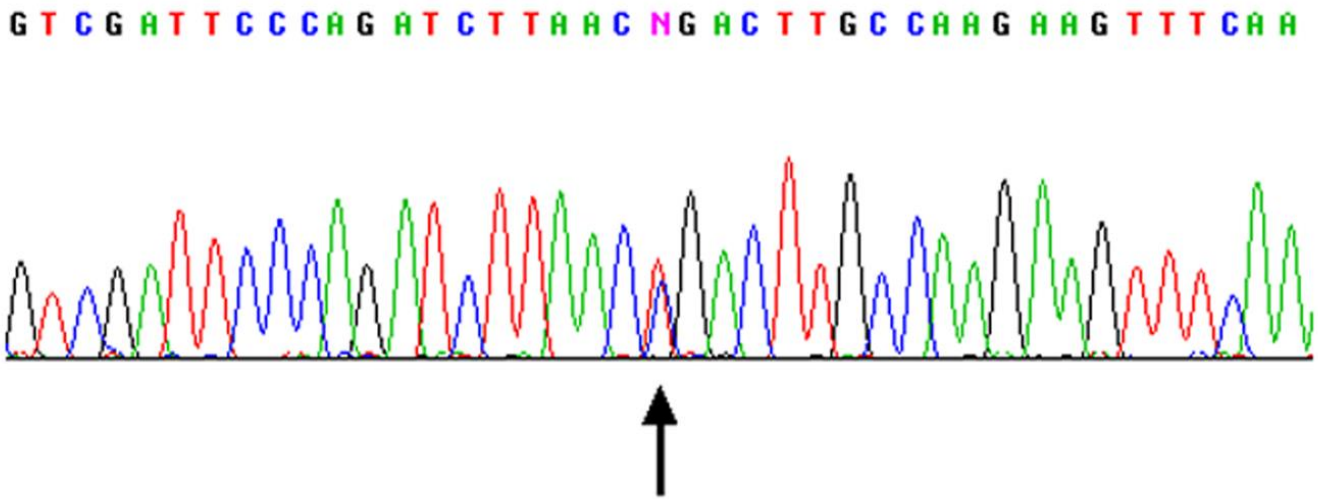
Blood sample

cheek swab
urine sample
Forensic specimens

DNA for analysis



DNA Sequencing



The causes of mutations

- Spontaneous errors in replication
  - that evade the proofreading function of the DNA polymerases.
- Physical and chemical agents
  - arise because a mutagen has reacted with the parent DNA, causing a structural change that affects the base-pairing capability of the altered nucleotide.

Physical mutagens

- Ultraviolet radiation.
  - provokes a reaction between two contiguous bases, which form cytosine or thymine dimers. This stops these bases being able to combine with the complementary base and, furthermore, paralyses replication.

- Ionising radiations.
  - X-rays, γ rays, etc., can lead to the breaking up of the DNA structure. They affect all types of tissues.

It is important to protect oneself from ultraviolet radiation from the sun; the effect of this radiation on DNA is associated with the appearance of skin cancer.

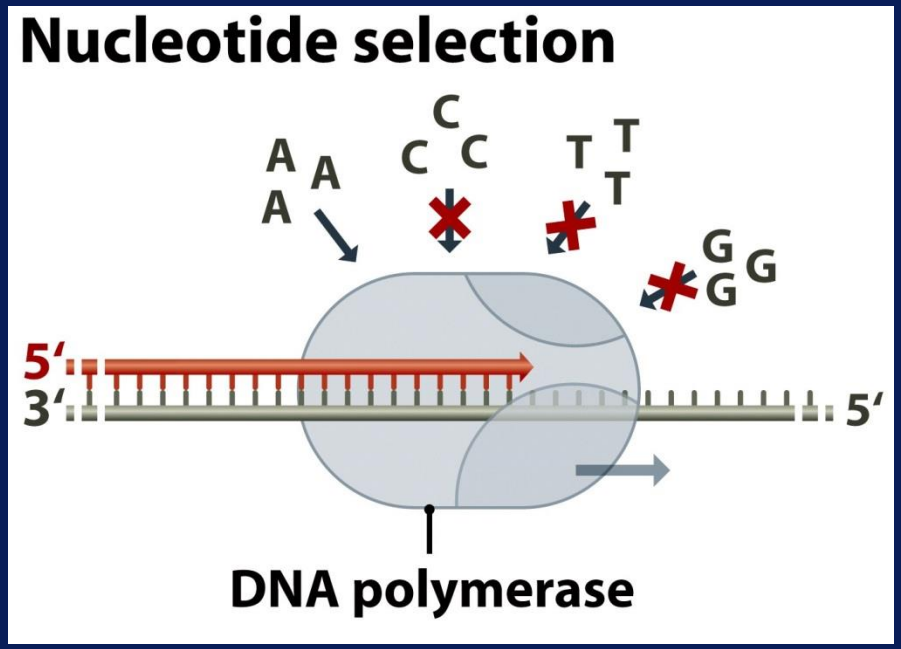
Chemical mutagens

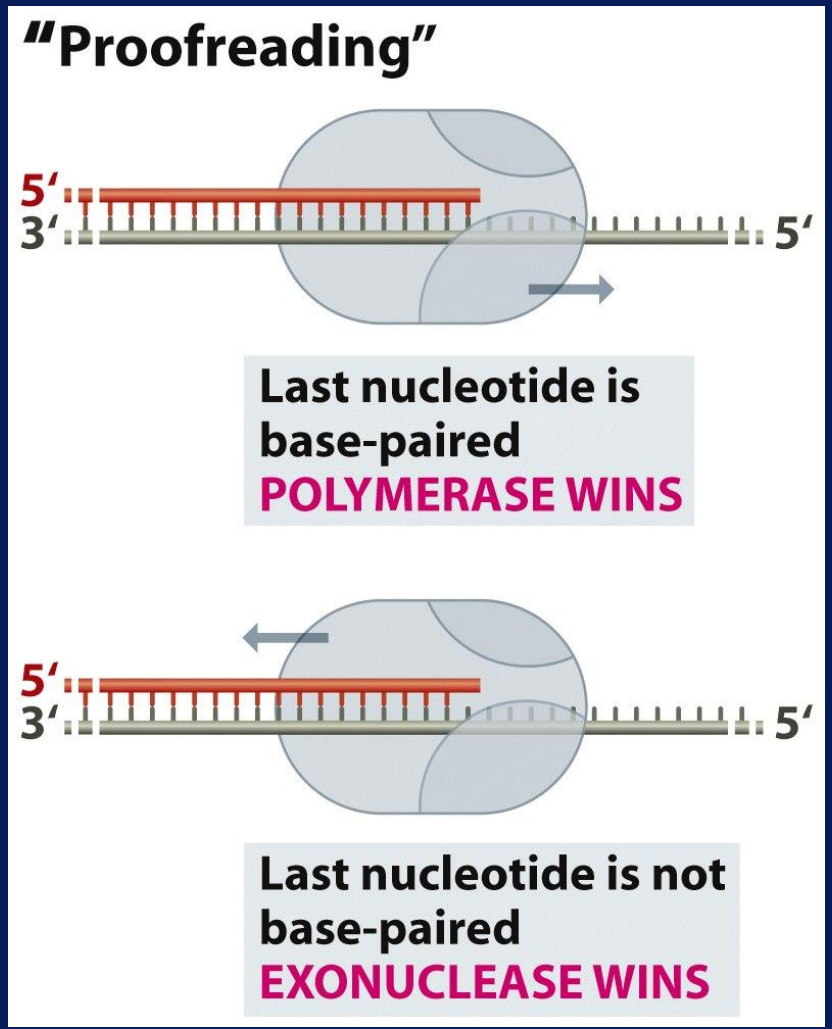
There are many chemical agents which produce mutations, and they have diverse effects too.

Some of these compounds transfer chemical groups to the nitrogen bases, leading to alterations in replication.
Others interleave themselves among the pairs of DNA bases and deform the double helix structure.
And there are others which, given their analogy with a base, substitute the base and provoke errors in the translation of the proteins

Errors in replication are a source of point mutations

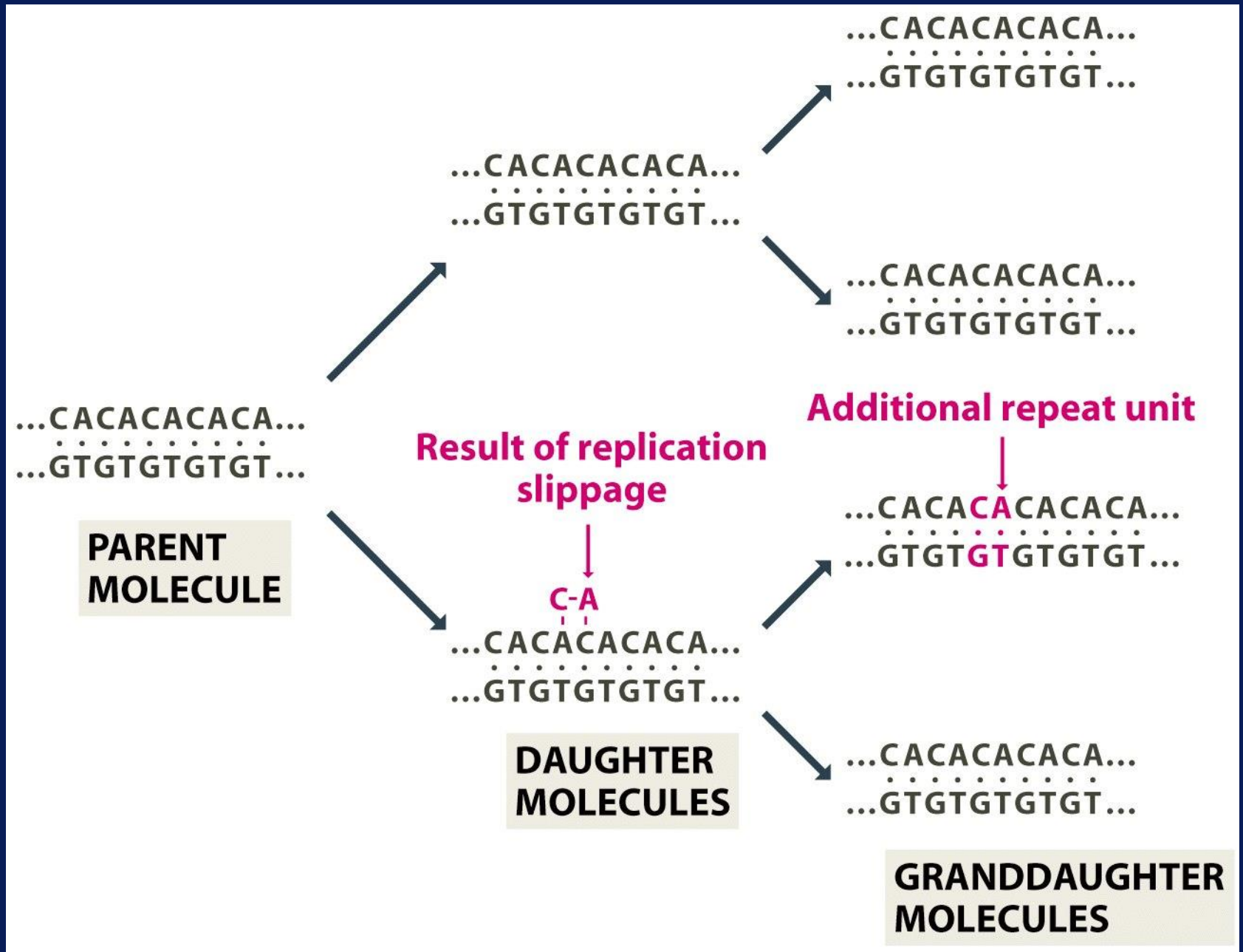
- Without enzymes, the error rate would be 5%-10%.





Replication errors can also lead to insertion and deletion mutations

- Frameshift mutations
- Replication slippage:
  - Insertion and deletion mutations are particularly prevalent when the template DNA contains short repeated sequences, such as those found in microsatellites.
  - Template strand and its copy shift their relative positions so that part of the template is either copied twice or missed out.
  - probably also responsible for the trinucleotide repeat expansion diseases.
  - Once the expansion reaches a certain length it appears to become susceptible to further expansion in subsequent rounds of replication, so that the disease becomes increasingly severe in succeeding generations.


...CACACACACA...
...GTGTGTGTGT...
PARENT MOLECULE
...CACACACACA...
...GTGTGTGTGT...
...CACACACACA...
...GTGTGTGTGT...
Result of replication slippage
C-A
...CACACACACA...
...GTGTGTGTGT...
DAUGHTER MOLECULES
...CACACACACA...
...GTGTGTGTGT...
...CACACACACA...
...GTGTGTGTGT...
Additional repeat unit
...CACACACACACA...
...GTGTGTGTGTGT...
...CACACACACA...
...GTGTGTGTGT...
GRANDDAUGHTER MOLECULES

Examples of human trinucleotide repeat expansions

Locus	Repeat sequence		Associated disease
	Normal	Mutated	
Polyglutamine expansions (all in coding regions of genes)			
HD	$(CAG)_{6–35}$	$(CAG)_{36–121}$	Huntington's disease
AR	$(CAG)_{9–36}$	$(CAG)_{38–62}$	Spinal and bulbar muscular atrophy
DRPLA	$(CAG)_{6–35}$	$(CAG)_{49–88}$	Dentatorubral-pallidoluysian atrophy
SCA1	$(CAG)_{6–39}$	$(CAG)_{39–82}$	Spinocerebellar ataxia type 1
SCA3	$(CAG)_{12–40}$	$(CAG)_{55–84}$	Machado–Joseph disease
Fragile site expansions (both in the untranslated leader regions of genes)			
FRM1	$(CGG)_{6–53}$	$(CGG)_{60–\text{over } 230}$	Fragile X syndrome
FRM2	$(GCC)_{6–35}$	$(GCC)_{61–\text{over } 200}$	Fragile XE mental retardation
Other expansions (positions described below)			
DMPK	$(CTG)_{5–37}$	$(CTG)_{50–3000}$	Myotonic dystrophy
X25	$(GAA)_{7–34}$	$(GAA)_{34–\text{over } 200}$	Friedreich's ataxia

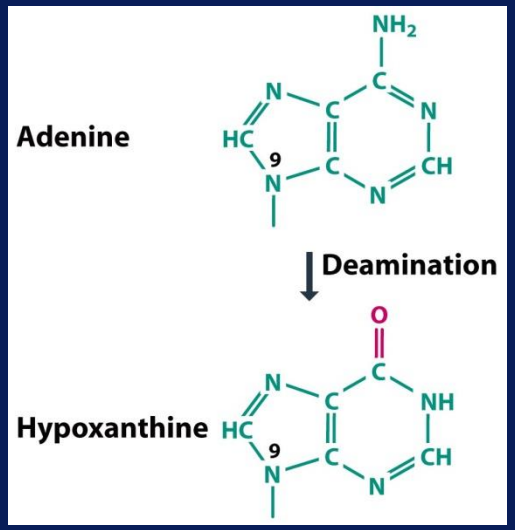
The *DMPK* and *X25* expansions are in the trailer and intron regions of their genes, respectively, and are thought to affect RNA processing. The also a few disease-causing mutations that involve expansions of longer sequences, for example progressive myoclonus epilepsy caused by a $(CCCCGCCCCGCG)_{2–3}$ to $(CCCCGCCCCGCG)_{\text{over } 12}$ expansion in the promoter region of the *EPM1* locus.

Categories of environmental agent that cause damage to living cells

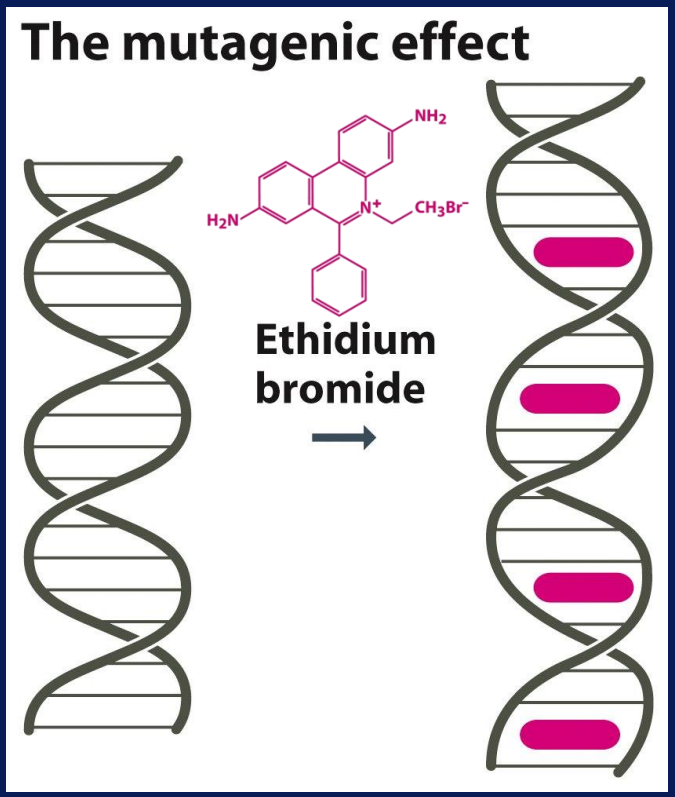
Agent	Effect on living cells
Carcinogen	Causes cancer—the neoplastic transformation of eukaryotic cells
Clastogen	Causes fragmentation of chromosomes
Mutagen	Causes mutations
Oncogen	Induces tumor formation
Teratogen	Results in developmental abnormalities

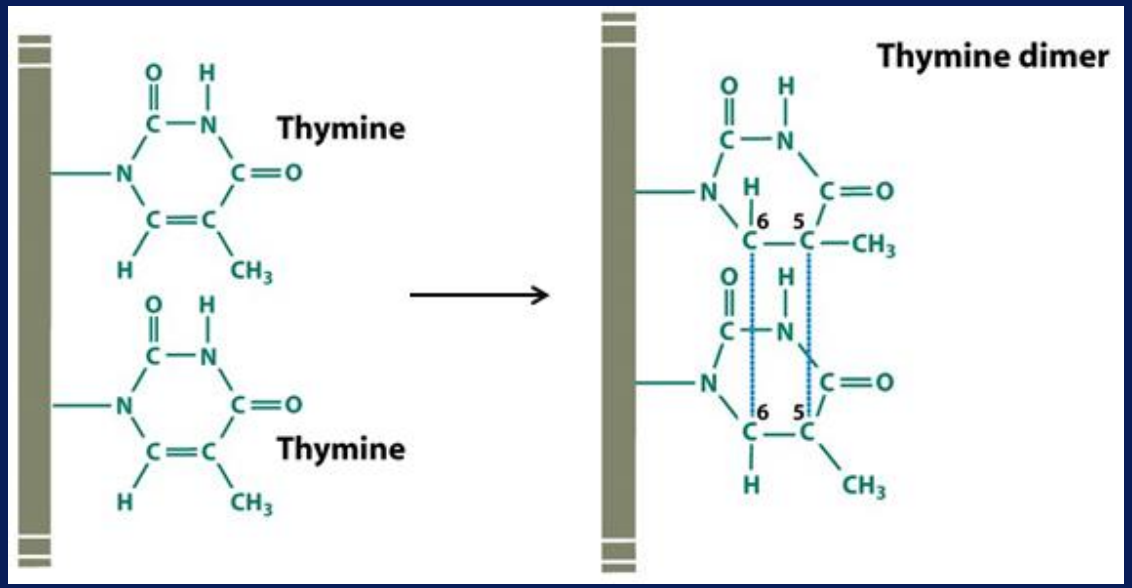
Mutagens cause mutations in three different ways

1. act as base analogs;
2. react directly with DNA (base deamination, alkylation, flat molecules that can slip between base pairs in the double helix);
3. do not themselves affect DNA structure, but instead cause the cell to synthesize chemicals such as peroxides that have a direct mutagenic effect.

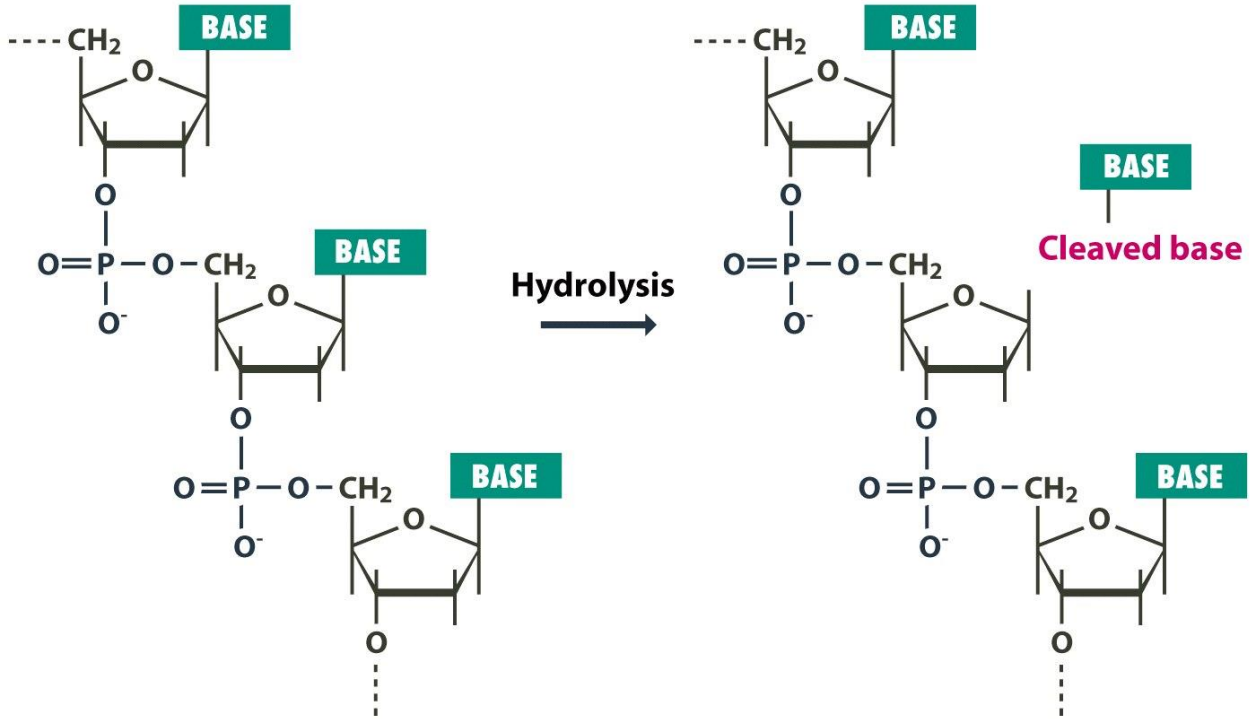


A certain amount of base deamination occurs spontaneously, with the rate being increased by chemical mutagens.

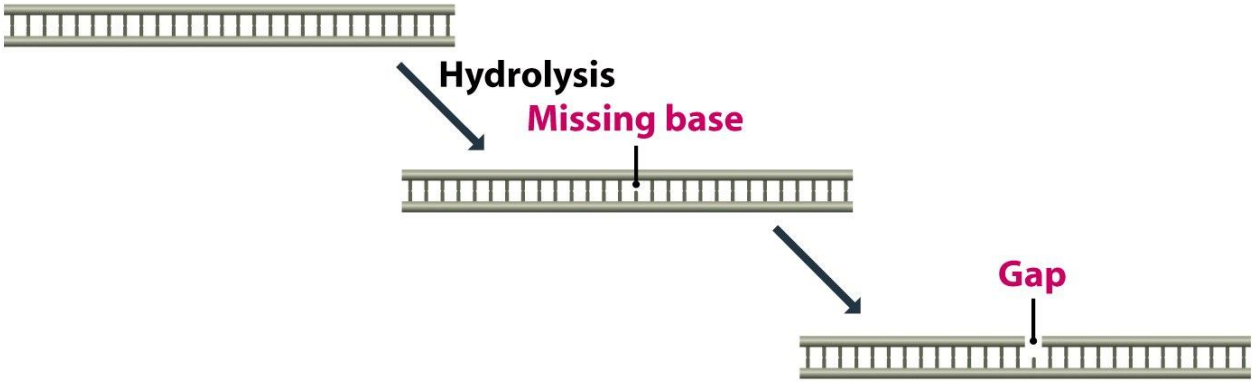




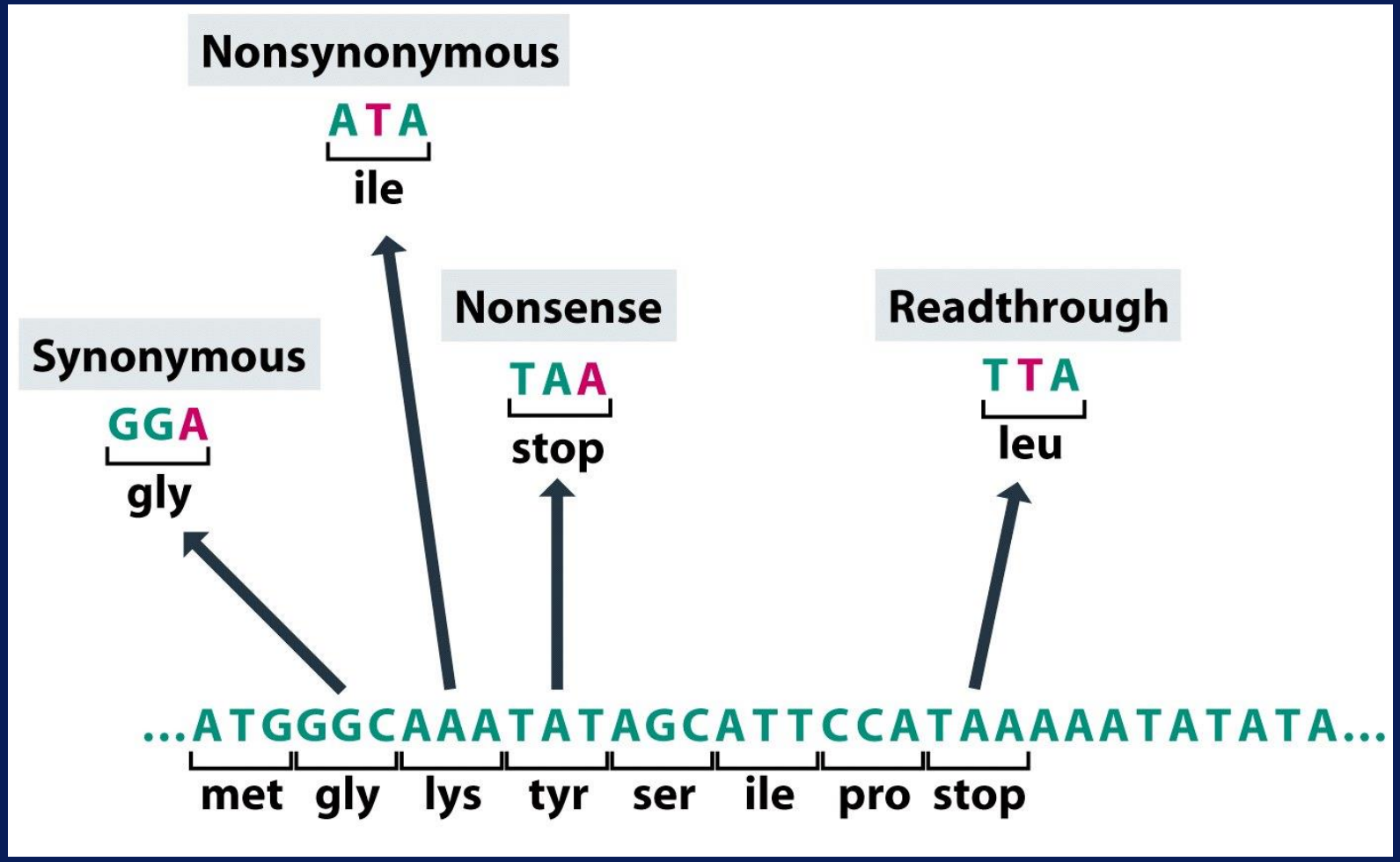
Heat-induced hydrolysis of a β-*N*-glycosidic bond



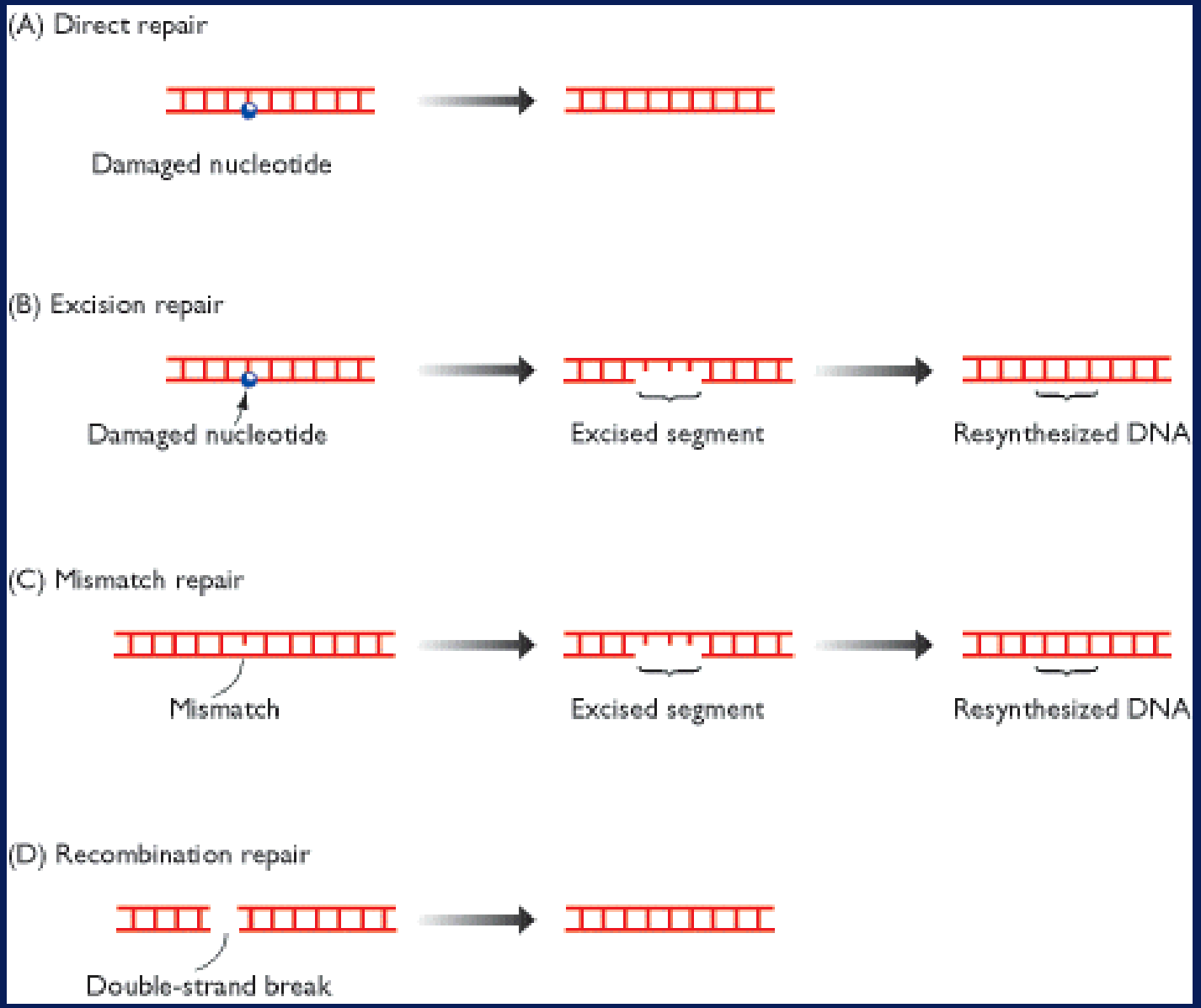
The effect of hydrolysis on double-stranded DNA



The effects of mutations on genomes



DNA Repair


(A) Direct repair
Damaged nucleotide
(B) Excision repair
Damaged nucleotide
Excised segment
Resynthesized DNA
(C) Mismatch repair
Mismatch
Excised segment
Resynthesized DNA
(D) Recombination repair
Double-strand break

Direct repair systems fill in nicks and correct some types of nucleotide modification

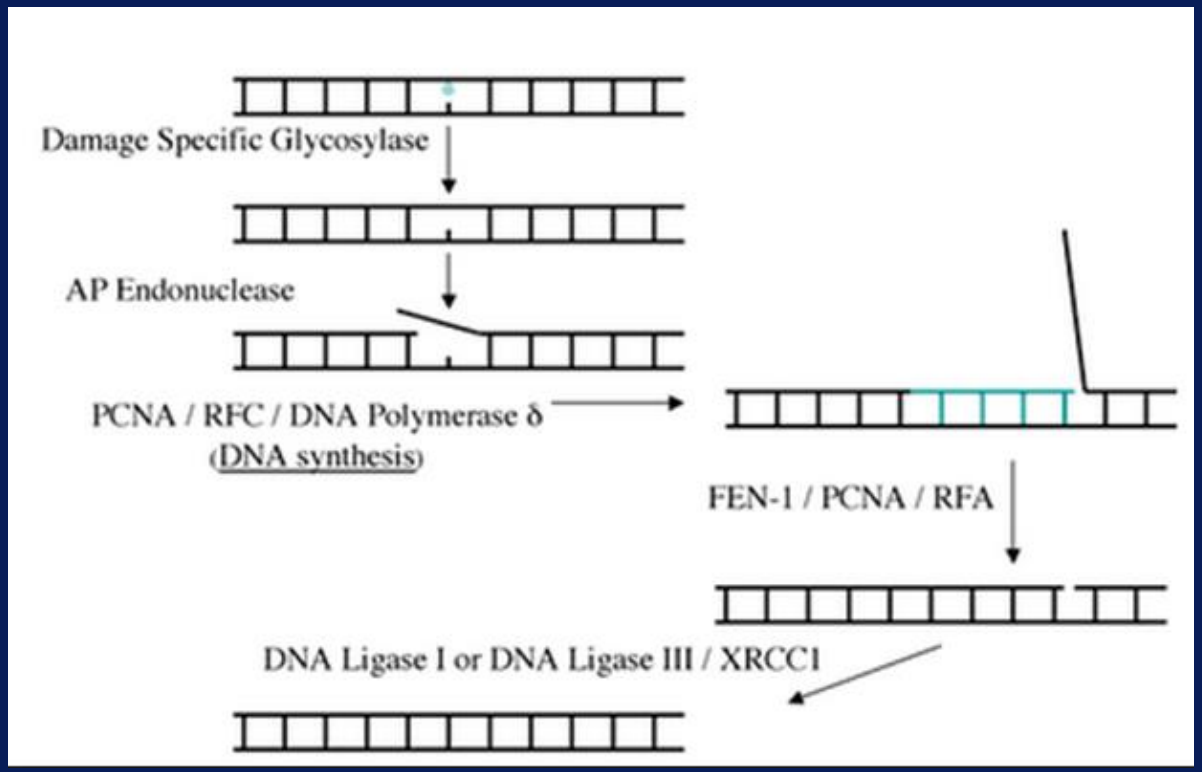
- Only a few types of damaged nucleotide can be repaired directly:

1. Nicks
2. Some forms of alkylation damage
3. Cyclobutyl dimers

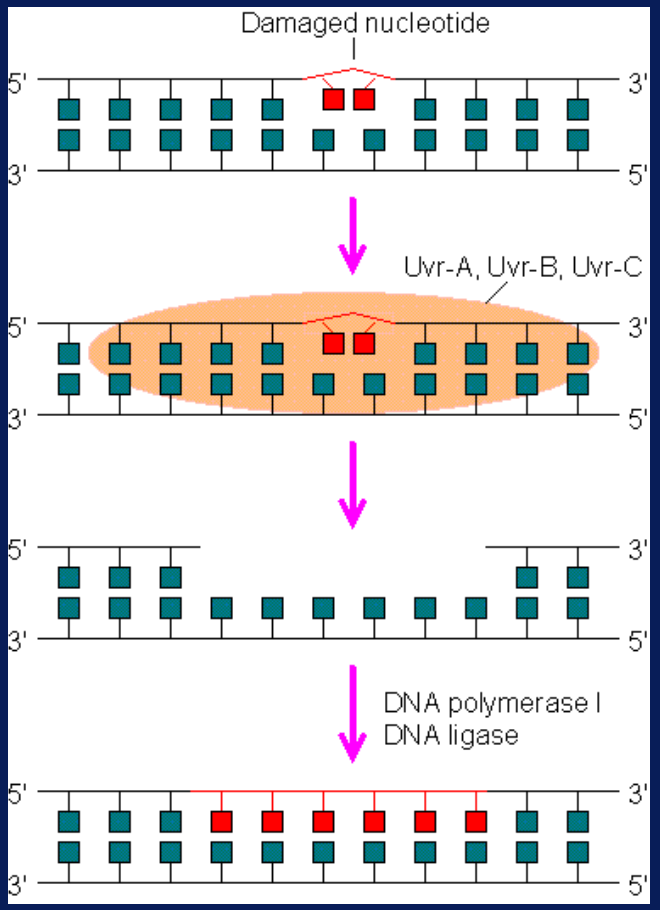
Excision repair

- Human genome sequences contain just a single gene coding for a protein involved in direct repair, but have at least 40 genes for components of the excision repair pathways.
- Base excision repair
- Nucleotide excision repair

Excision repair



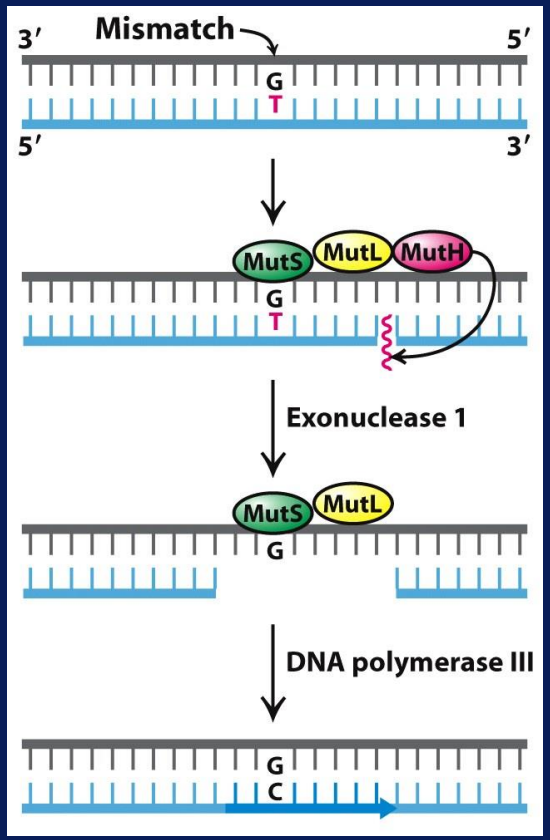
Base excision repair



Nucleotide excision repair

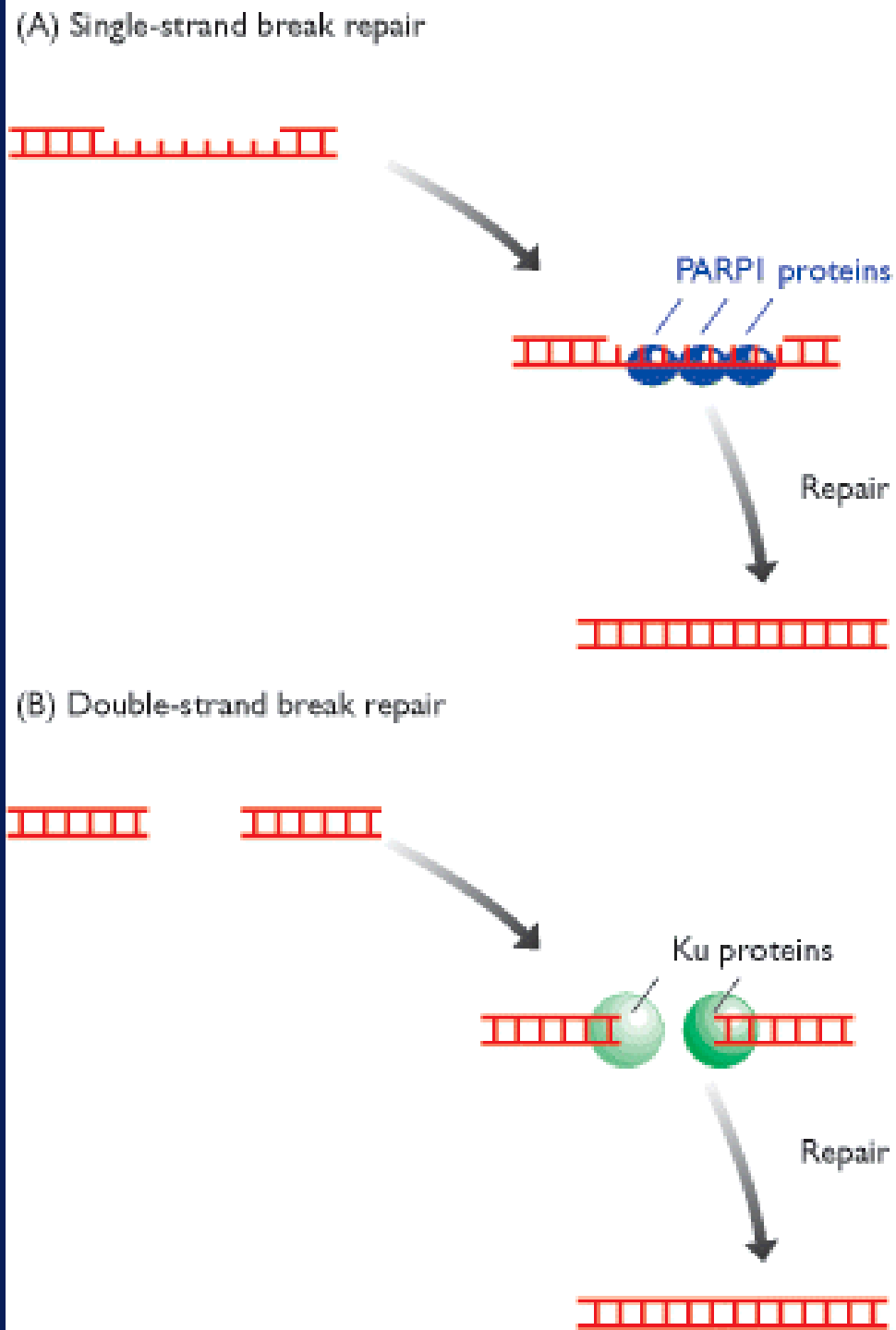
Mismatch repair: correcting errors of replication

- The repair must be made in the daughter polynucleotide because it is in this newly synthesized strand that the error has occurred; the parent polynucleotide has the correct sequence.
- How does the repair process know which strand is which?
- E.Coli: at least three mismatch repair systems
  - 'long patch', 'short patch and 'very short patch'
- Eukaryotes: possibilities include an association between the repair enzymes and the replication complex, so that repair is coupled with DNA synthesis, or use of single-strand binding proteins that mark the parent strand.



Repair of double-stranded DNA breaks

non-homologous endjoining (NHEJ)

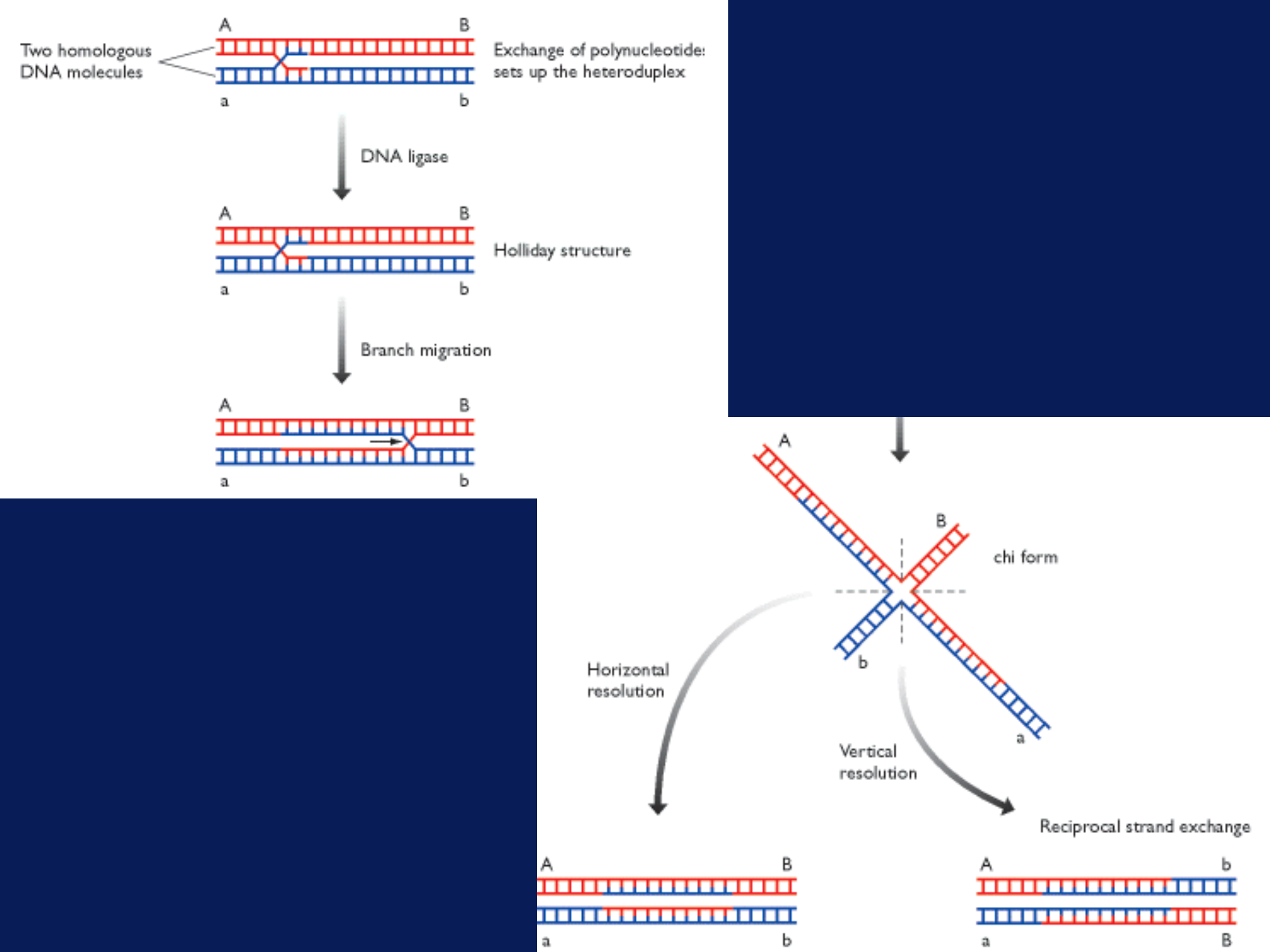


Recombination

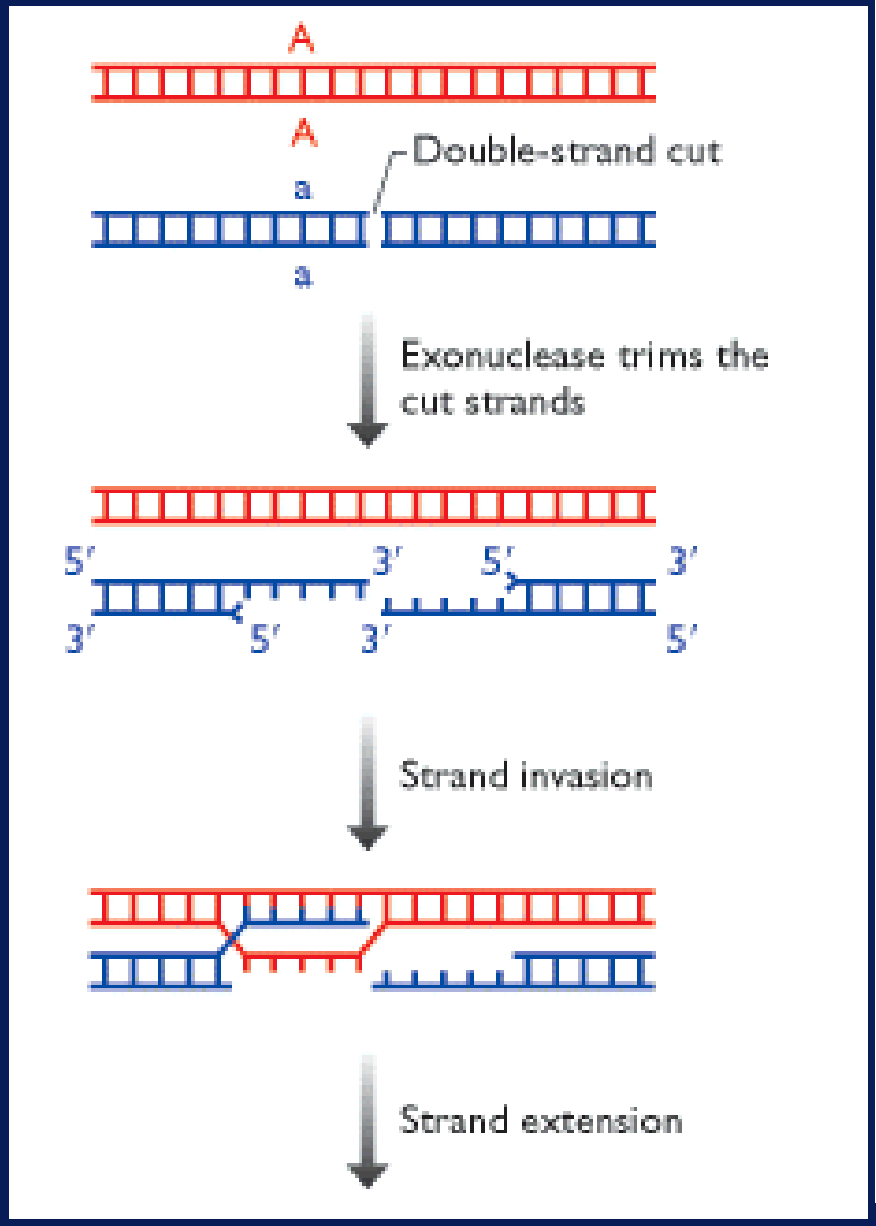
- Recombination was first recognized as the process responsible for crossing-over and exchange of DNA segments between homologous chromosomes during meiosis of eukaryotic cells, and was subsequently implicated in the integration of transferred DNA into bacterial genomes after conjugation, transduction or transformation.
- More diversity, but usually not deleterious

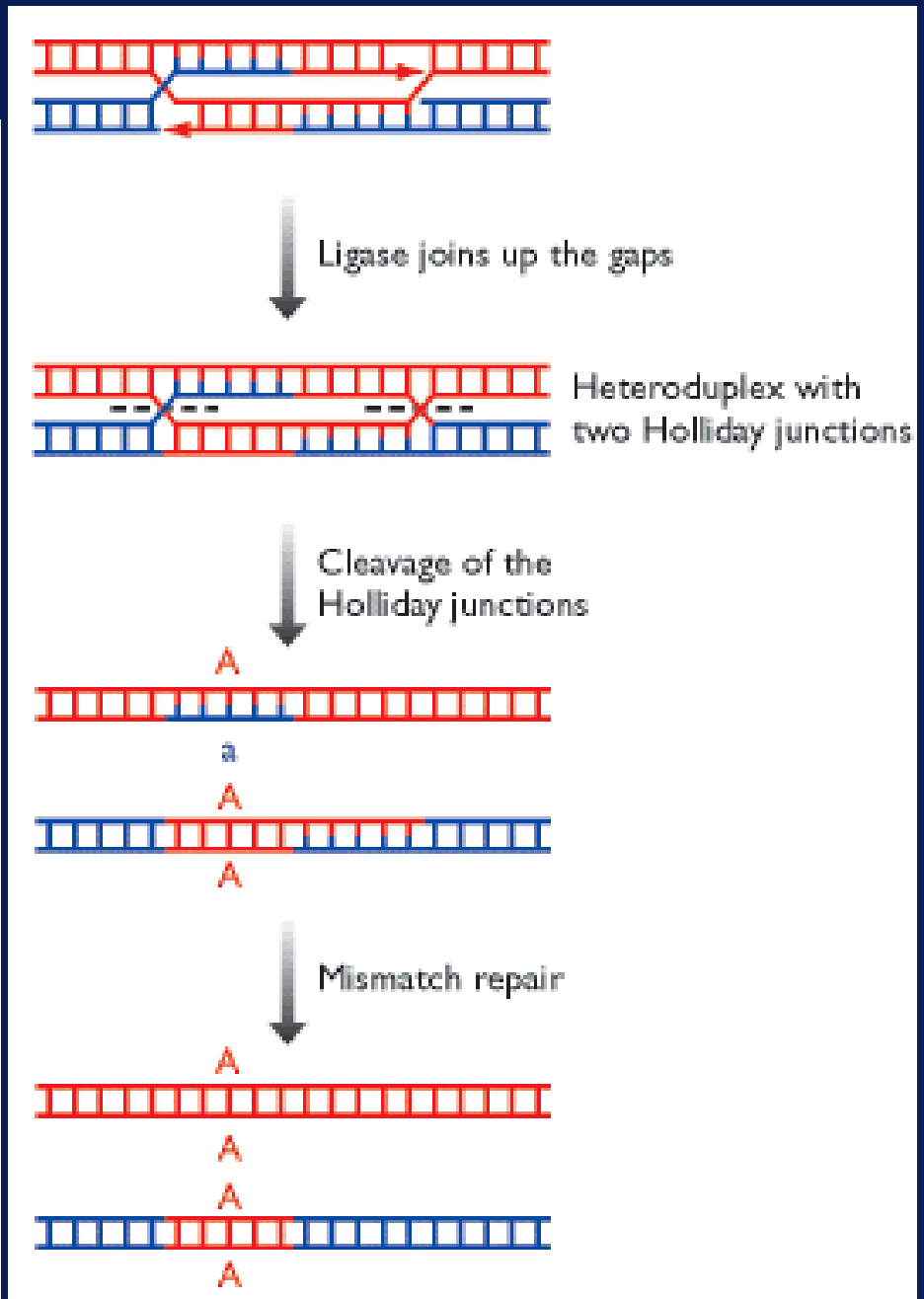
Homologous recombination

- Holliday model
- Two homologous double-stranded molecules, ones with identical or nearly identical sequences, but is equally applicable to two different molecules that share a limited region of homology, or a single molecule that recombines with itself because it contains two separate regions that are homologous with one another.


A
B
Two homologous DNA molecules
Exchange of polynucleotides sets up the heteroduplex
a
b
DNA ligase
A
B
Holliday structure
a
b
Branch migration
A
B
a
b
A
B
chi form
b
a
Horizontal resolution
Vertical resolution
Reciprocal strand exchange
A
B
a
b
A
b
a
B

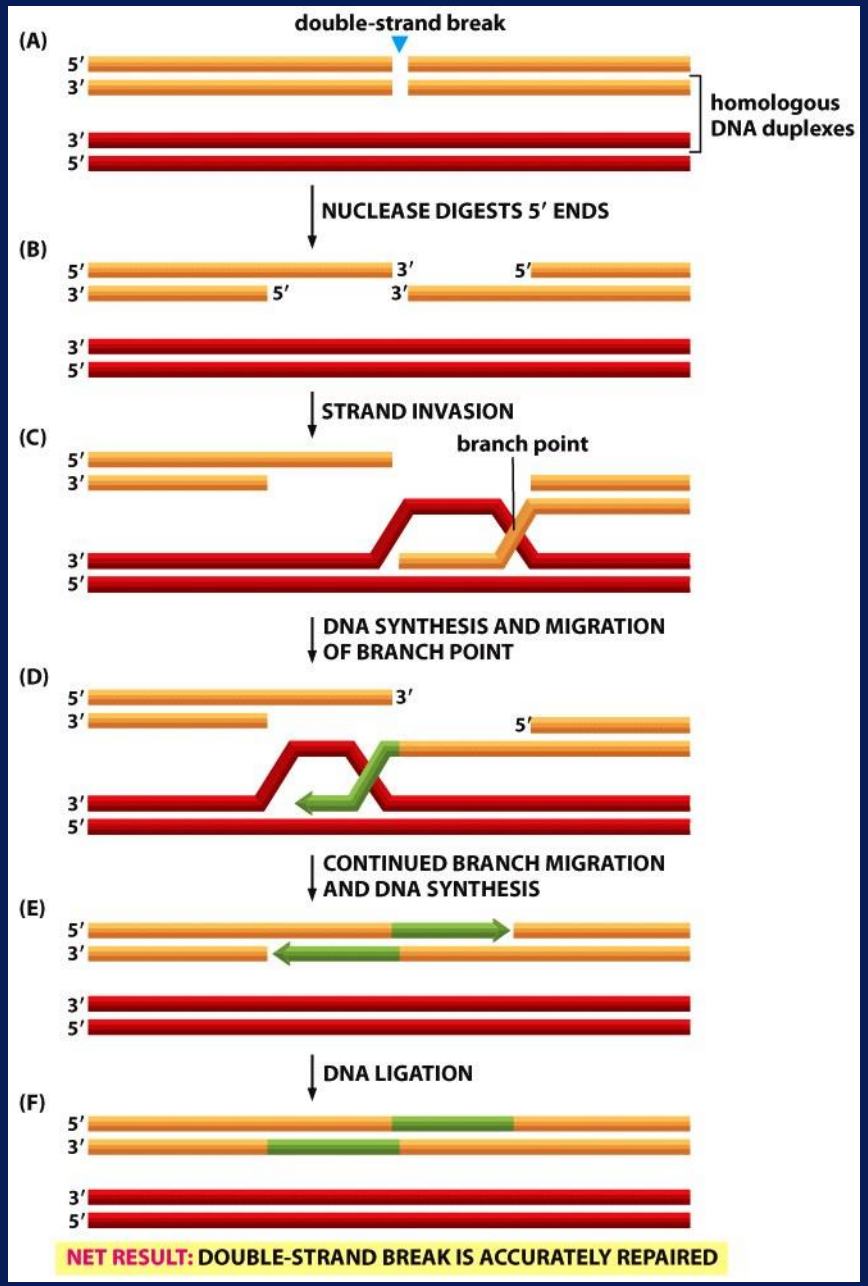
Gene conversion

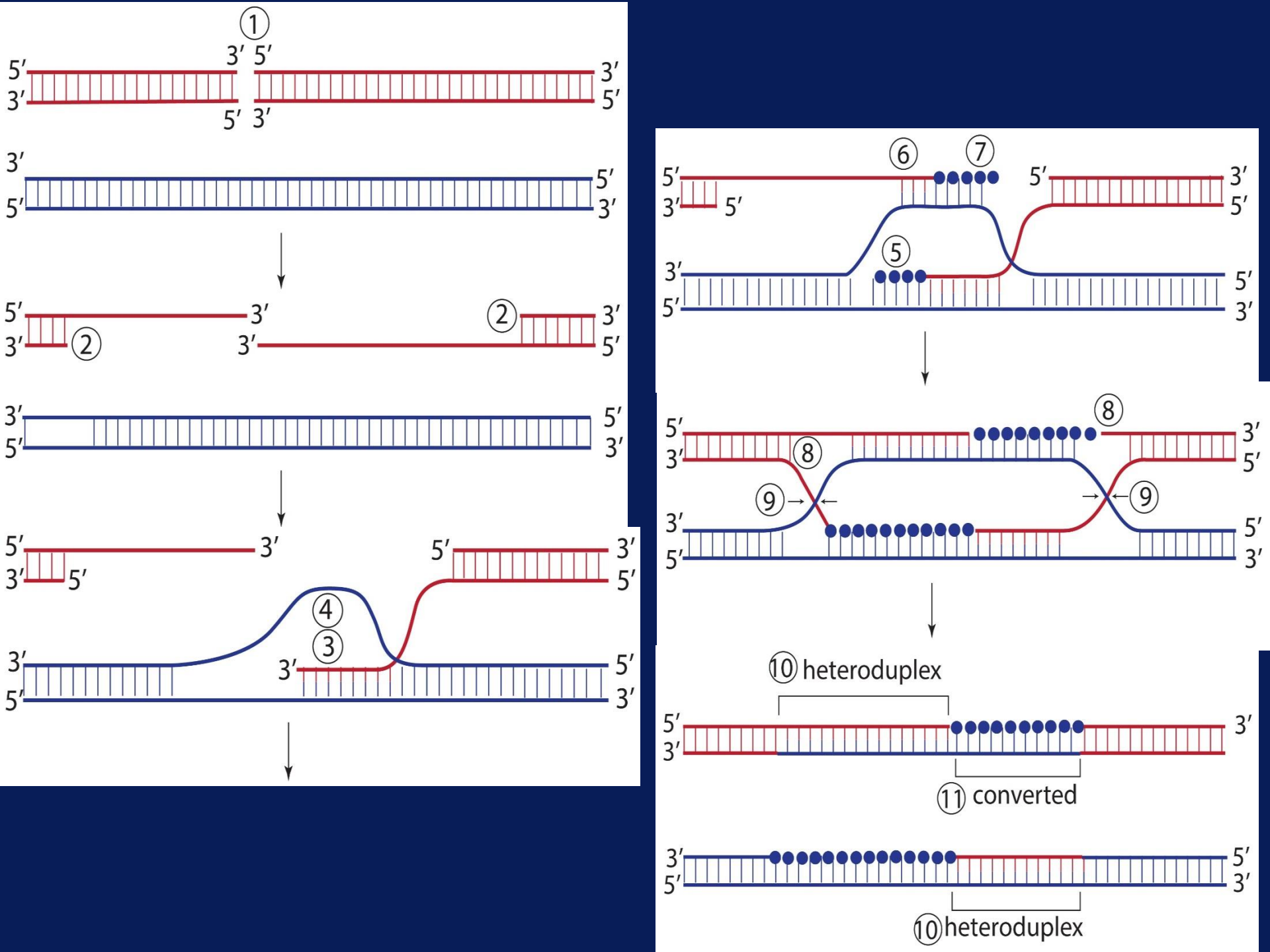




Repair by Homologous Recombination

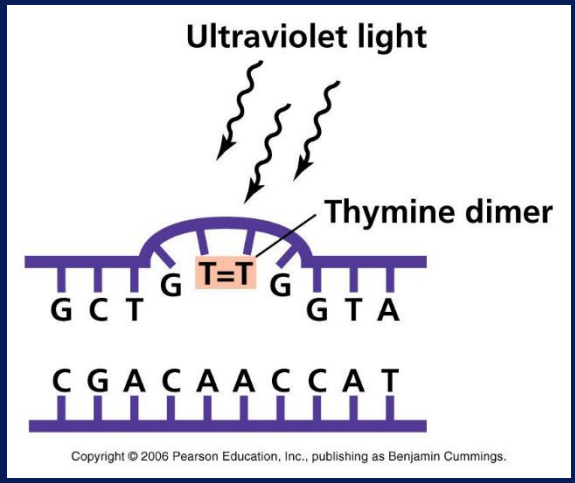
- The first step is exonuclease activity that trims back the 5’ ends on both sides of the break, creating long single stranded ends with free 3’ -OH groups.
- Next, the key step, **strand invasion**: the broken end of one DNA strand invades the unbroken double helix of the homologous chromosome and base pairs with it.
- This broken end acts as a primer and a DNA polymerase extends it.
- After some DNA synthesis, the elongated broken end is long enough to invade the other broken piece of DNA, and more DNA polymerase activity resynthesizes all the bases in the broken region.
- DNA ligase then seals up the nicks.




1
2
3
4
5
6
7
8
9
10 heteroduplex
11 converted
10 heteroduplex

Defects in DNA repair underlie human diseases, including cancers

- One of the best characterized of these is xeroderma pigmentosum, which results from a mutation in any one of several genes for proteins involved in nucleotide excision repair.
- The symptoms of xeroderma pigmentosum include hypersensitivity to UV radiation, patients suffering more mutations than normal on exposure to sunlight, which often leads to skin cancer



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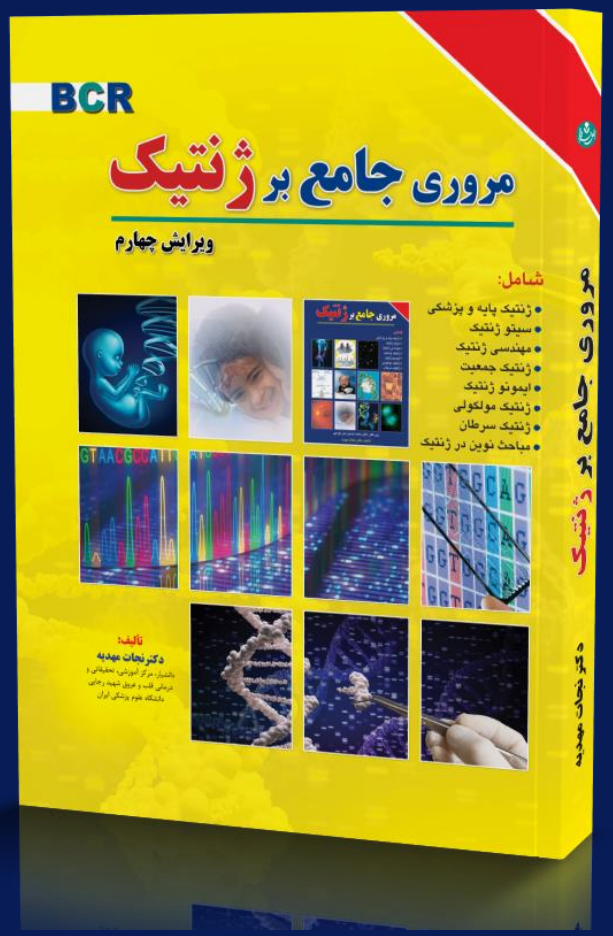

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