DNA

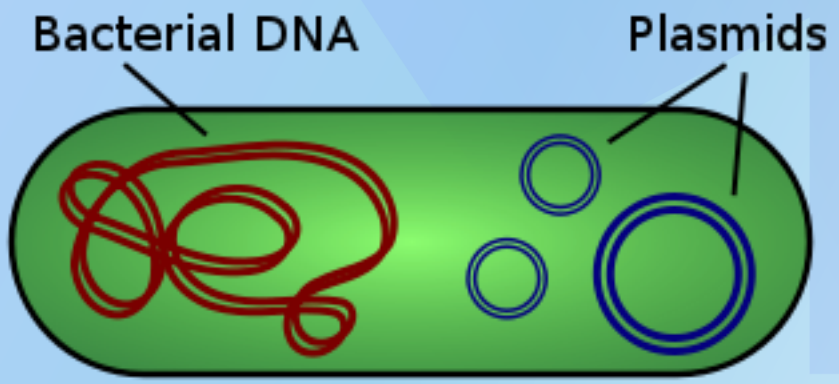
Alexandra Kathryn Vedeler

We will cover...

- ❑ Prokaryotes vs Eukaryotes
- ❑ DNA structure
- ❑ Nucleosides and nucleotides
- ❑ Types of DNA
- ❑ DNA replication
  - ❑ Prokaryotes
  - ❑ Eukaryotes
- ❑ DNA repair

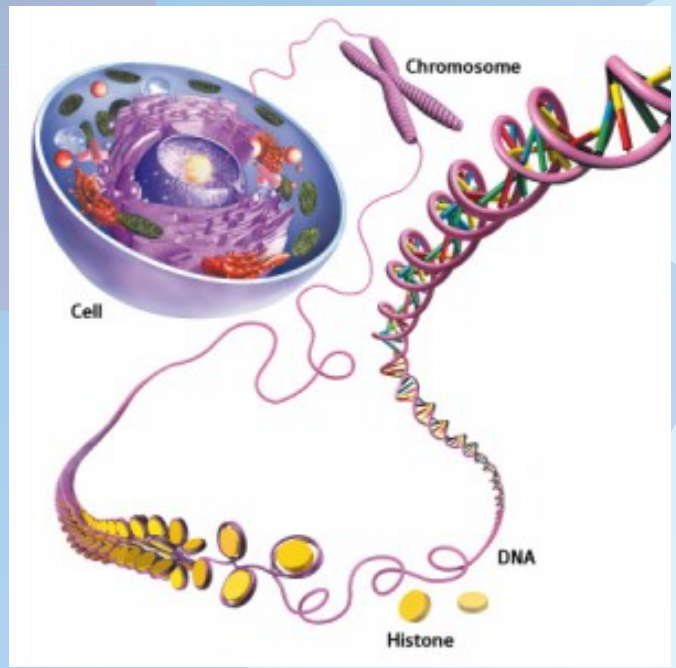
PROKARYOTES

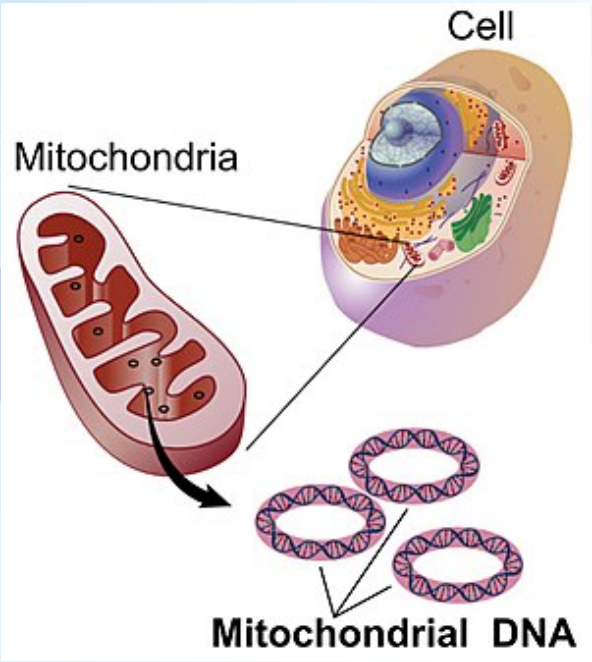
- Circular DNA
- NO nucleus, mitochondria or other organelles
- Plasmids
  - Non-chromosomal DNA
  - Can lead to antibiotic-resistance*



EUKARYOTES

- Linear DNA – nucleus
- Circular DNA - mitochondria

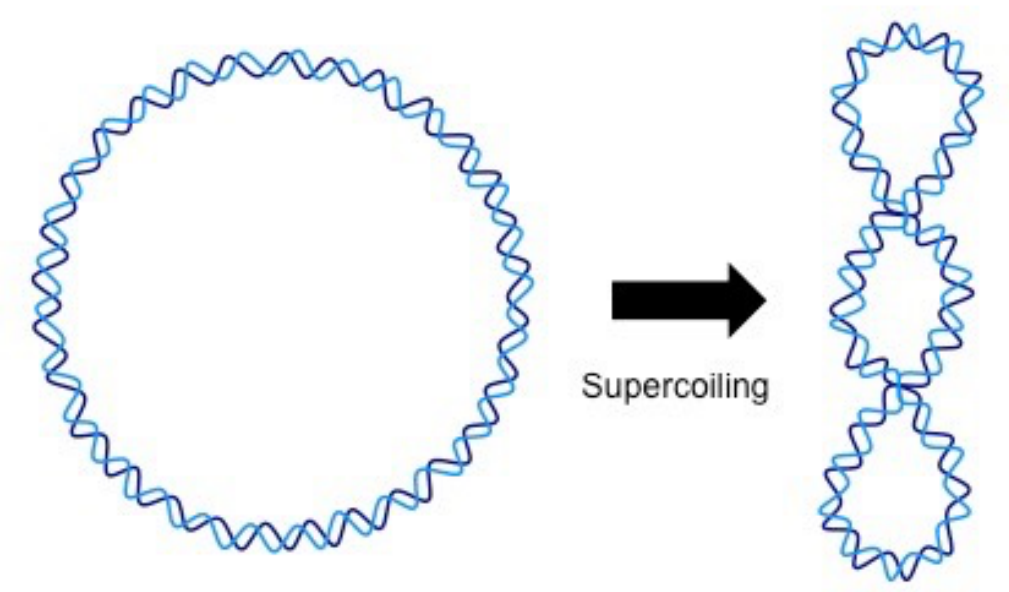




Let’s brake it down

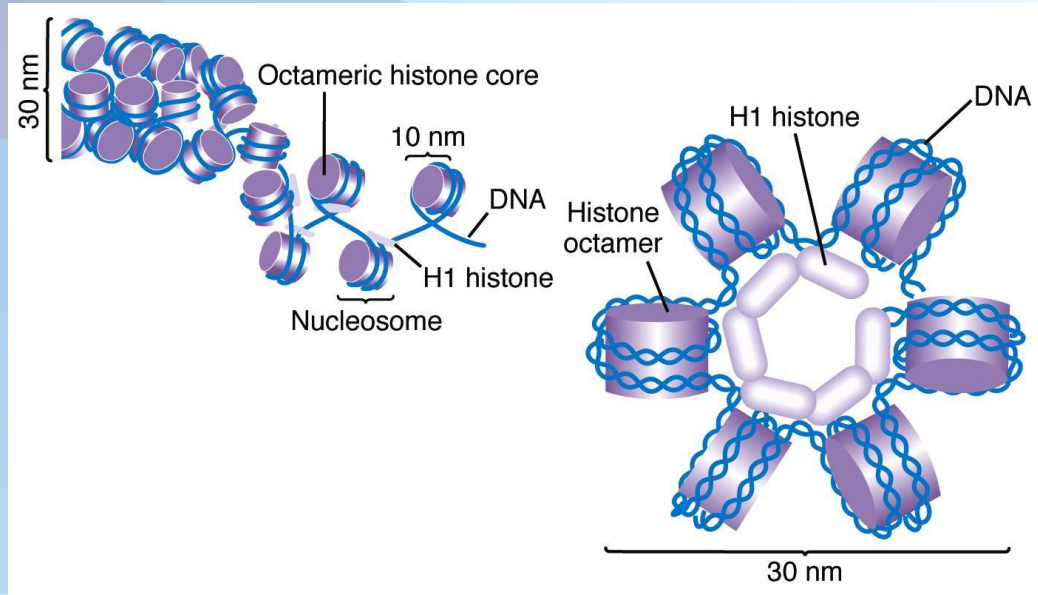
Circular DNA

- Double-stranded DNA (dsDNA)
- Supercoils



Linear DNA

- Double-stranded DNA (dsDNA)
- Histones



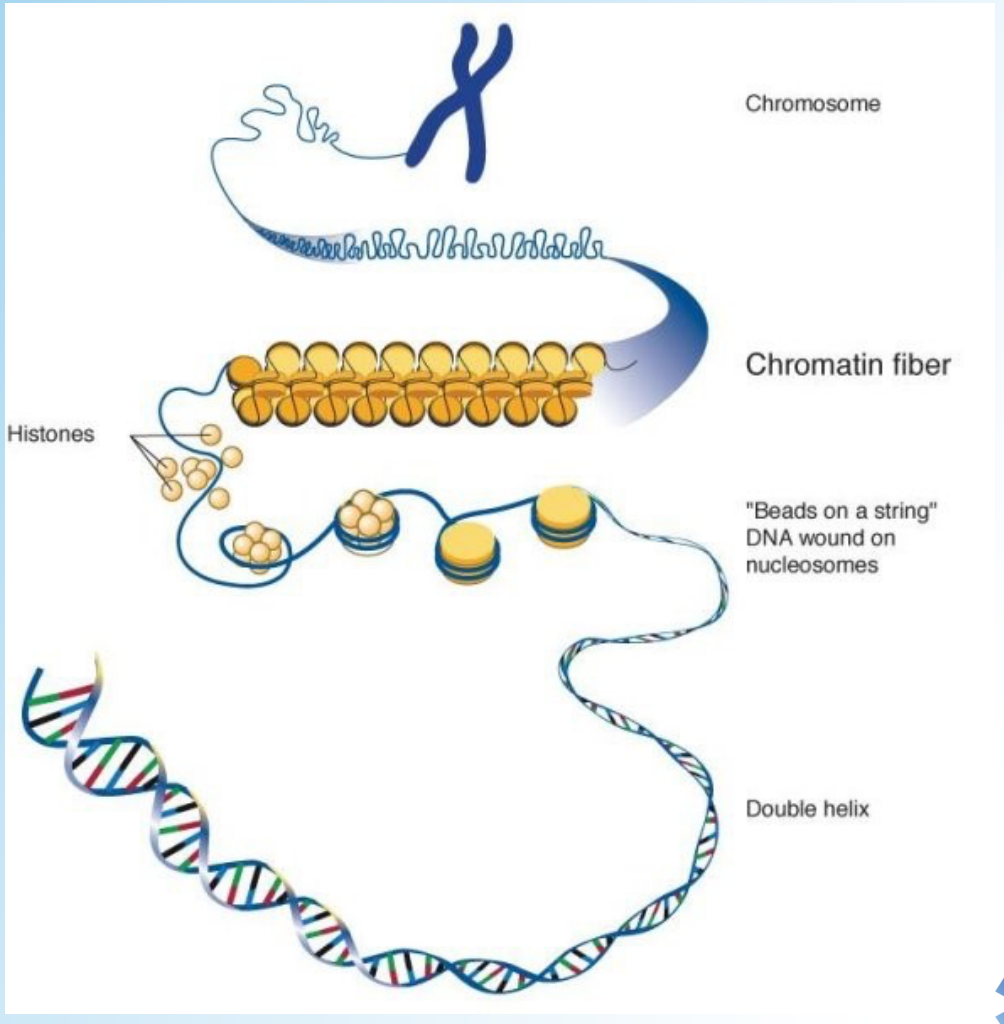
Acetylation = reduces positive charge, decreases bonding
Deacetylation = increases bonding between histones & DNA

Histones

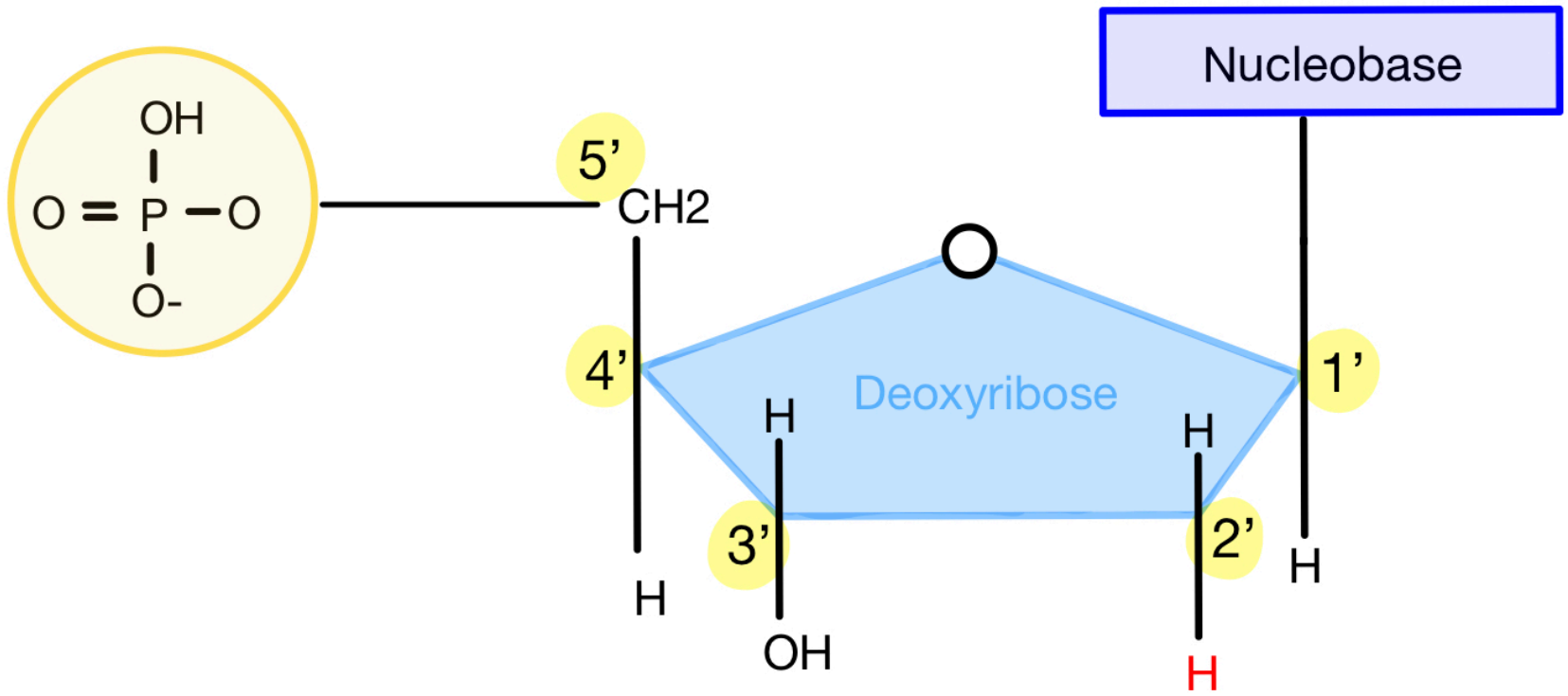
- Classes:
  - H1, H2A, H2B, H3, H4
  - H1 = linker DNA
- Octamer = 2x(H1, H2A, H2B, H3, H4)
- **Positive charge** at physiological pH
  - Pos. charged aa residues → tight bonding of histones to DNA

Nucleosome

= 2x(H1, H2A, H2B, H3, H4)



DNA structure



Deoxyribose
Nucleobase
Phosphate group

- Negative charge

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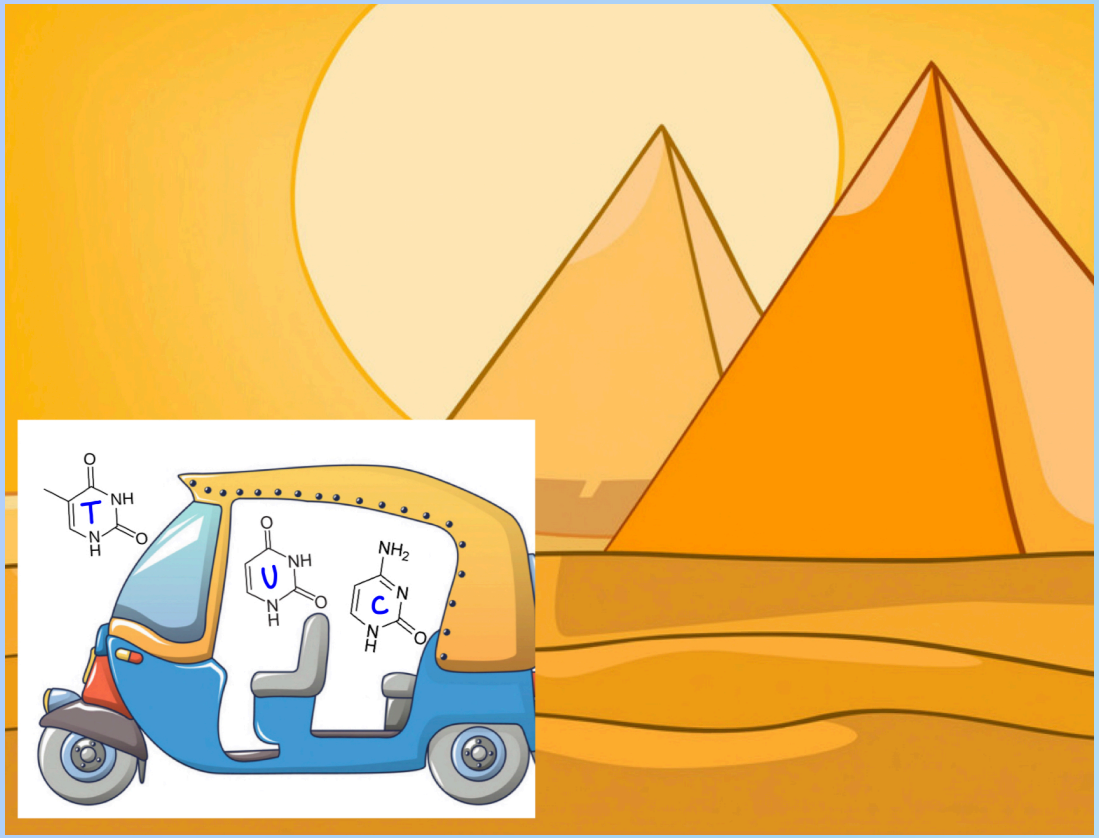
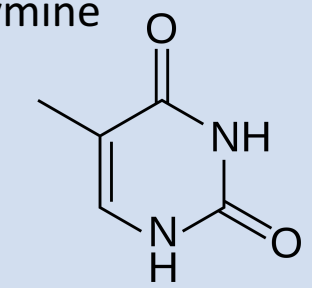
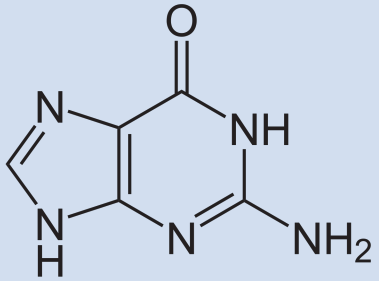
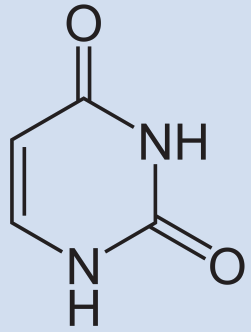
Nucleobases



“TUC-TUC around the pyramids”

PURINES	PYRIMIDINES
Adenine	Thymine 
Guanine 	Cytosine
	Uracil (RNA) 

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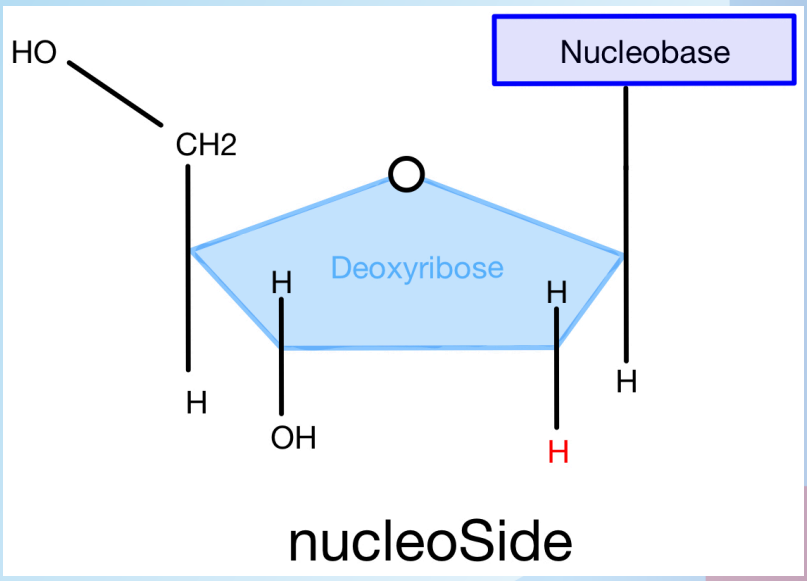
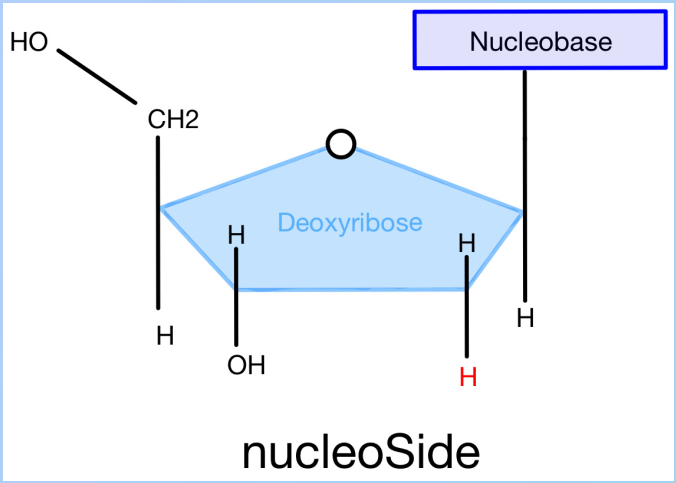
nucleoSide/nucleoTide

Nucleobase + Deoxyribose/ Ribose =





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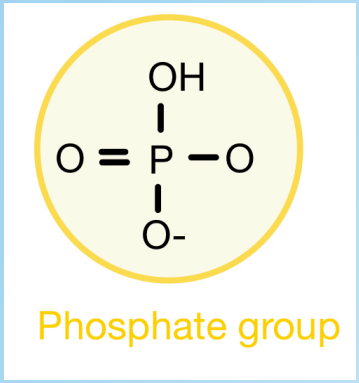

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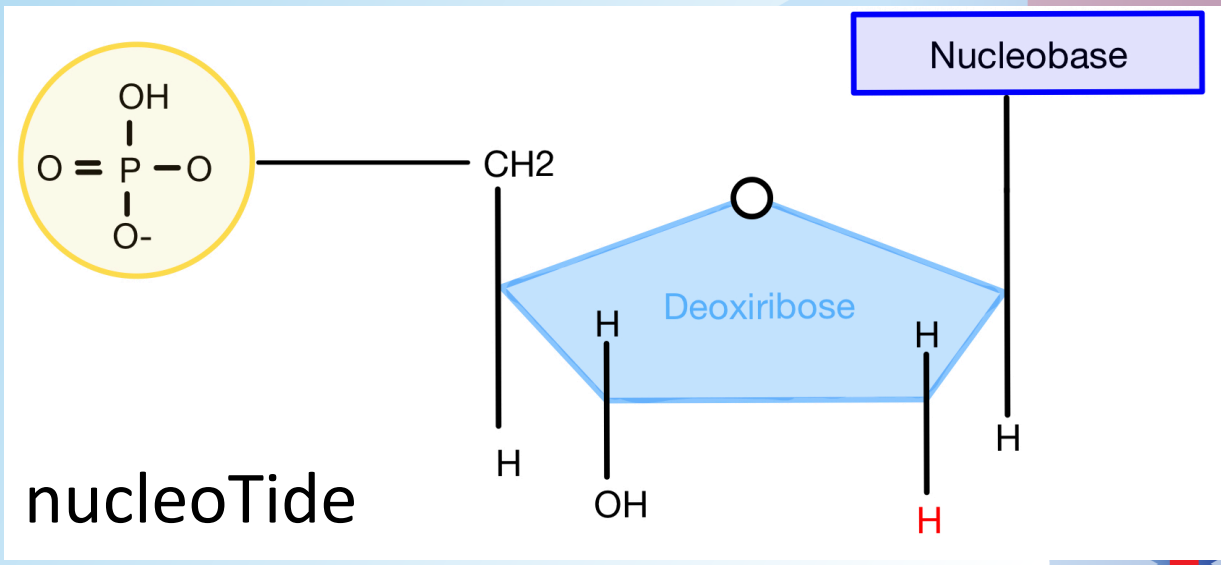

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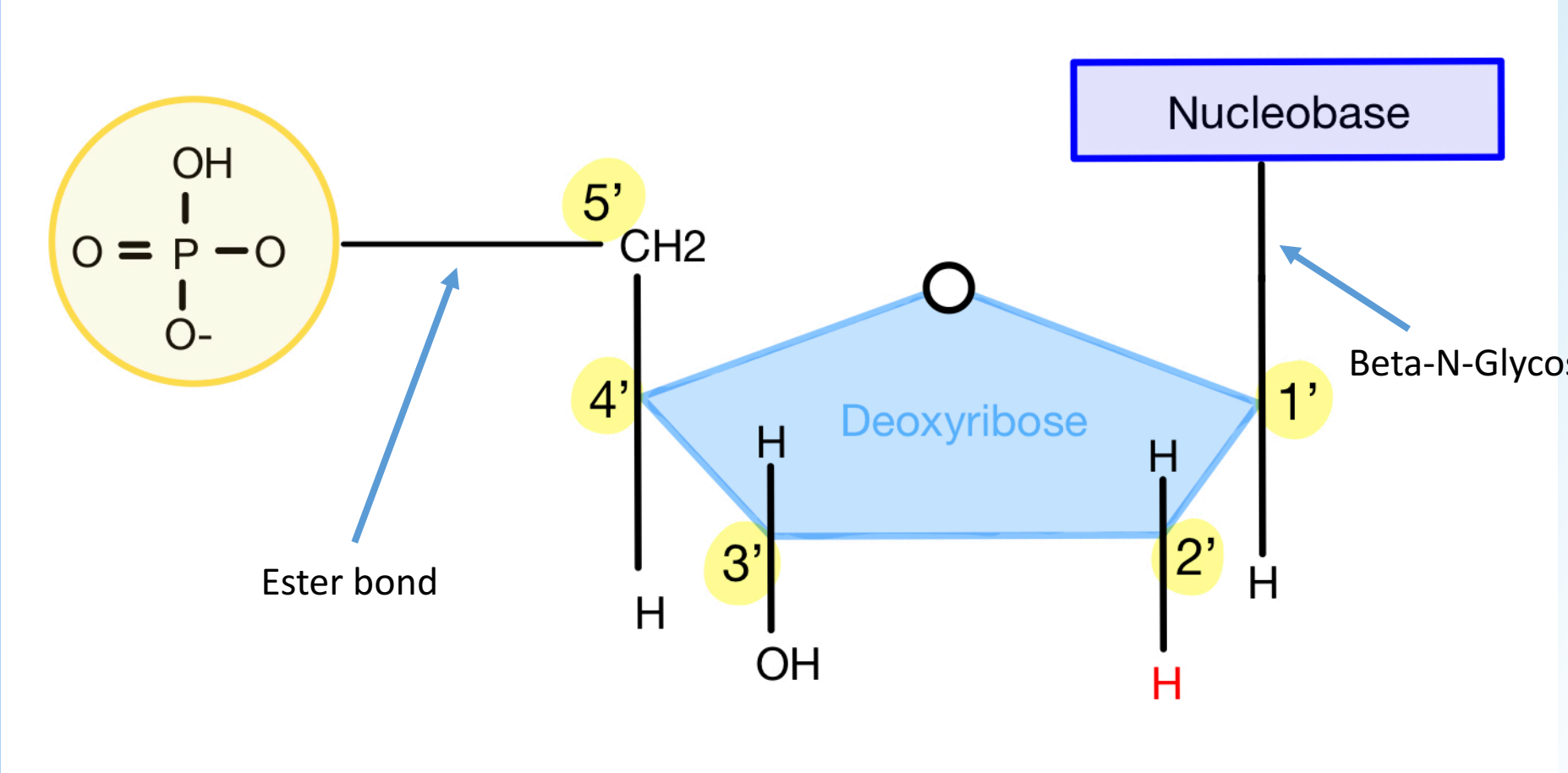
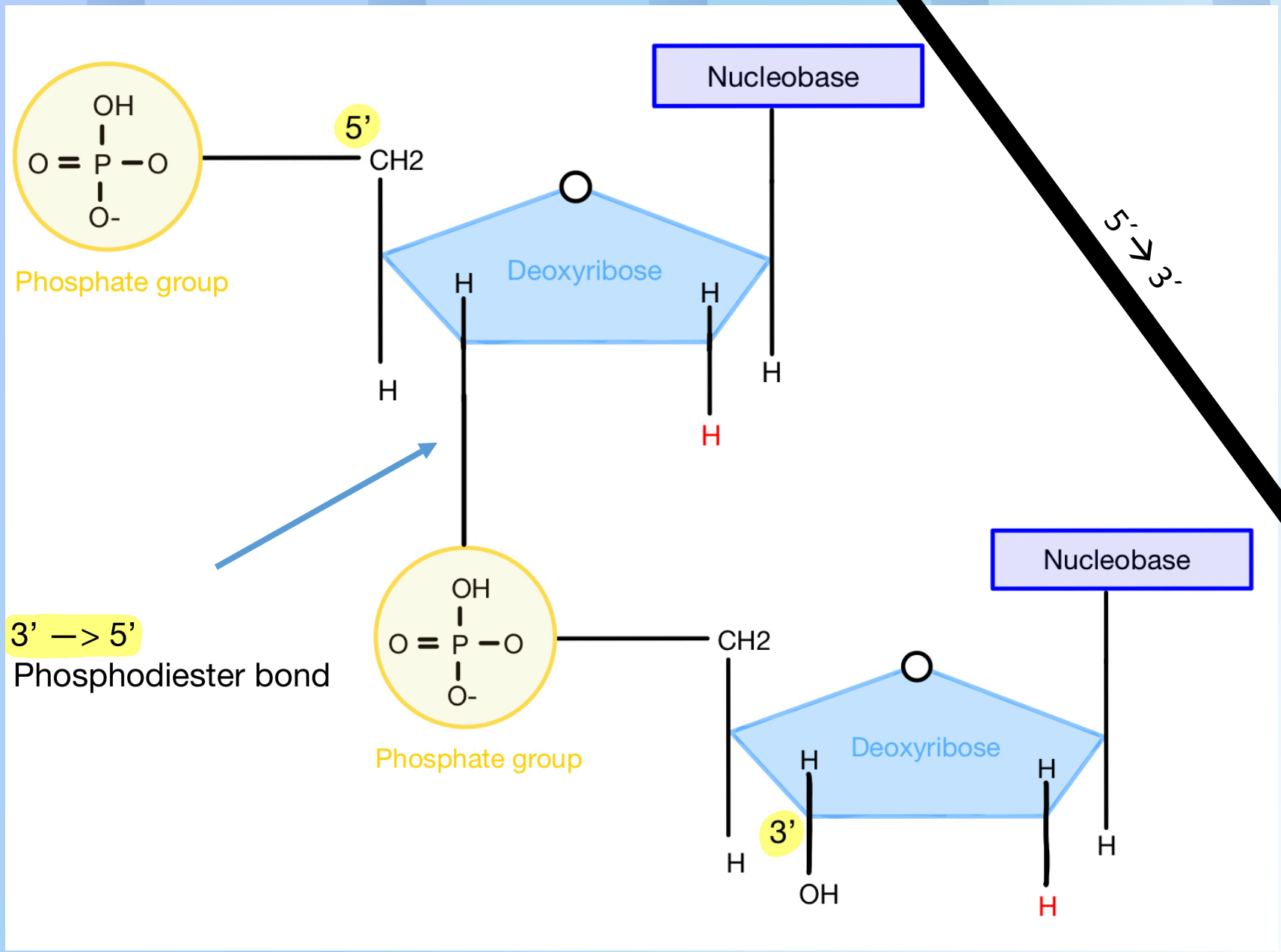
Bonds

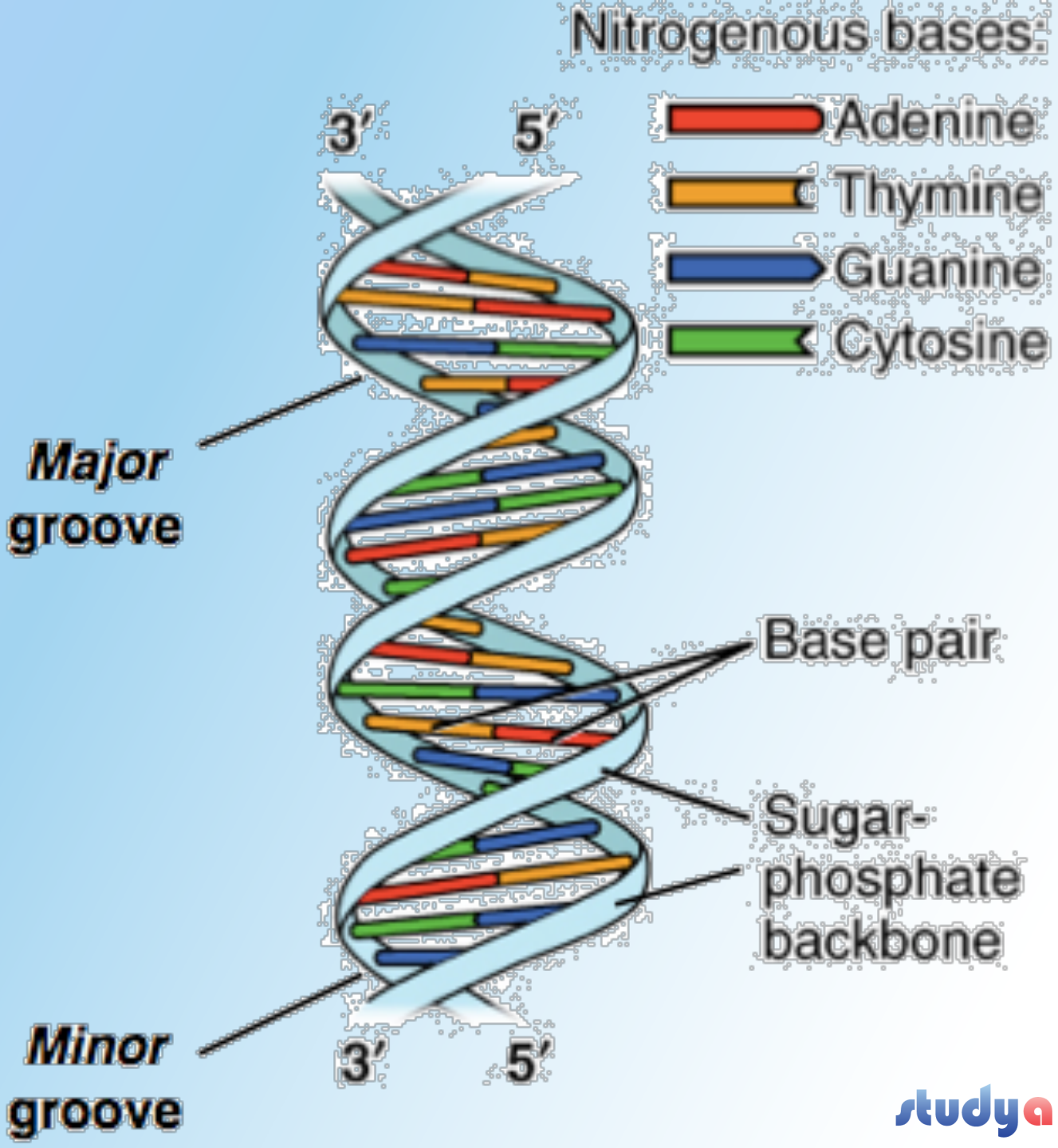


Nucleobase
OH
O = P – O
O-
5'
CH2
4'
Deoxyribose
1'
H
H
3'
2'
H
H
OH
H
Beta-N-Glycosidic bond
Ester bond


OH
O = P – O
O-
Phosphate group
5’
CH2
Deoxyribose
H
H
H
H
H
Nucleobase
5’ → 3’
3’ –> 5’
Phosphodiester bond
OH
O = P – O
O-
Phosphate group
CH2
Deoxyribose
H
H
3’
OH
H
H
H
Nucleobase

The Double Helix

- Deoxyribose-phosphate backbone
  - Hydrophilic
  - Van der Waals interactions
- Base pairs
  - Hydrophobic
  - H-bonds



The Double Helix

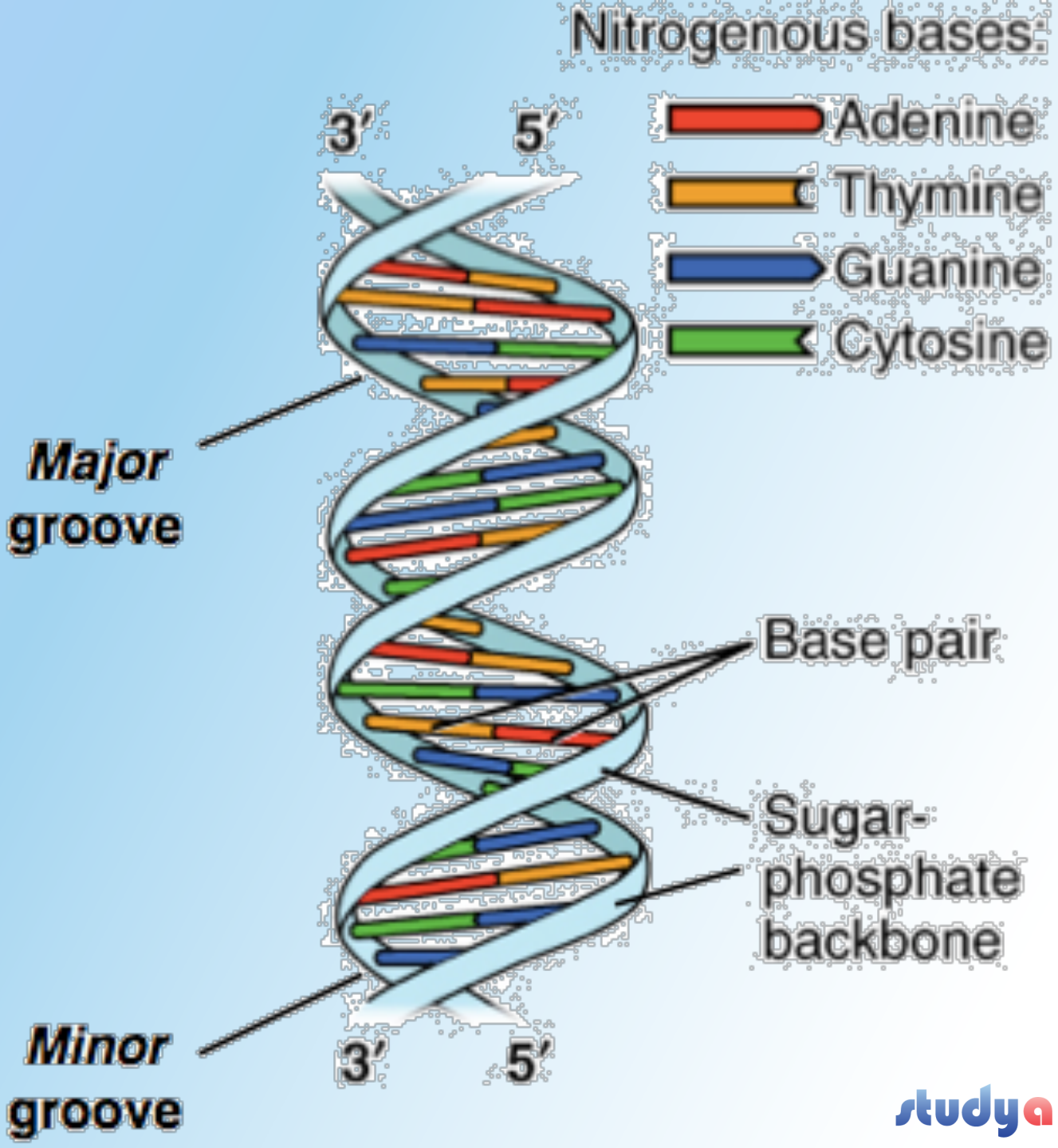
Major & minor grooves

- Provide access for binding of regulatory proteins

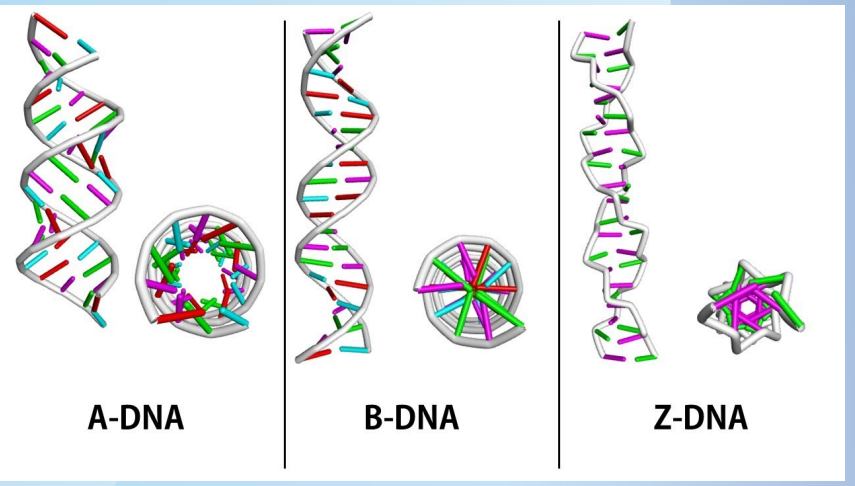
Dactinomycin (anticancer drug) acts on the minor groove → inhibits DNA replication

Chagaff rule

- The amount of A = T
- The amount of G = C
- The amount of purines = pyrimidines



Types of DNA



	DNA B-type *High humidity DNA*	DNA A-type *Dehydrated B-DNA*	DNA Z-type
Helical rotation	Right-handed	Right-handed	Left-handed
Base pairs per 360°	10bp	11bp	12bp
Helical diameter	20Å = 2.0 nm	26Å = 2.6 nm	18Å = 1.8 nm
Characteristics	Chromosomal DNA	DNA-RNA hybrids or double stranded RNA	Influences gene expression and regulation

Denaturation of DNA = loss of helical structure

Absorption at 260nm

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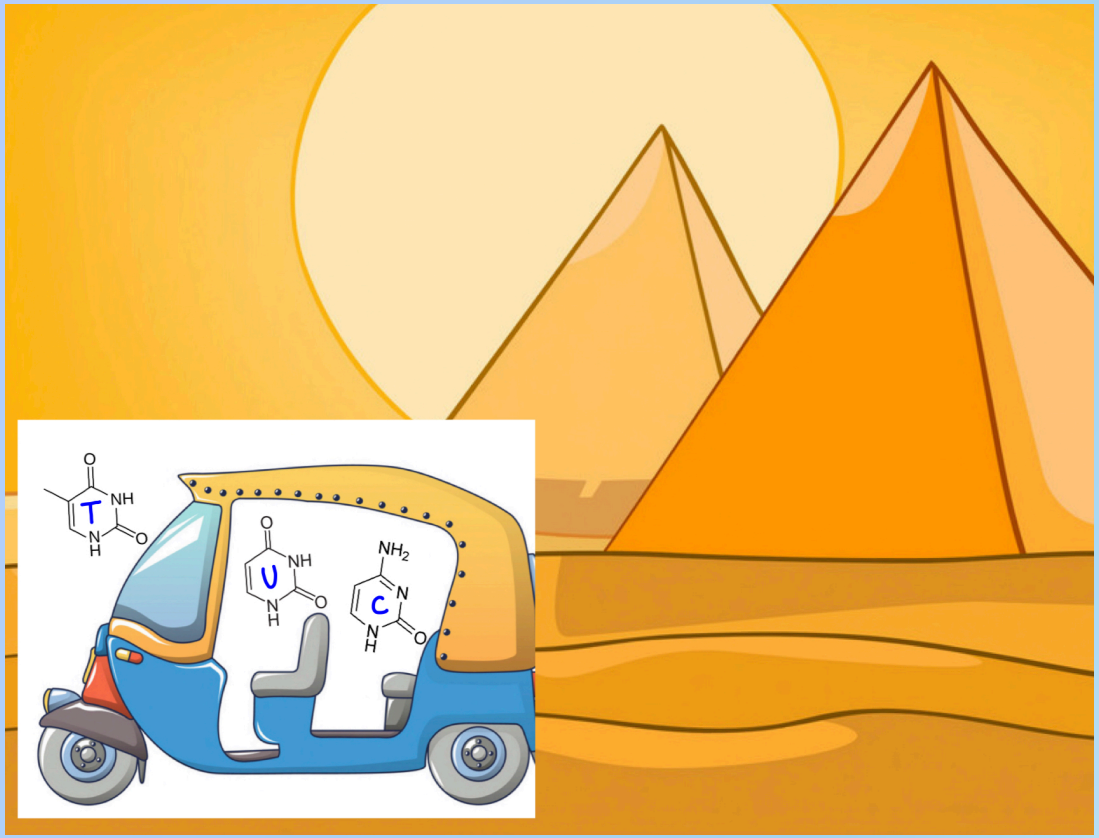
Quiz time

Nucleobases



"TUC-TUC around the pyramids"

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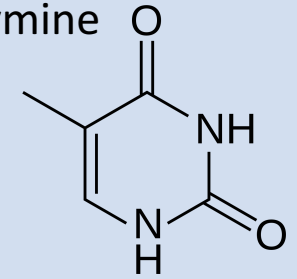
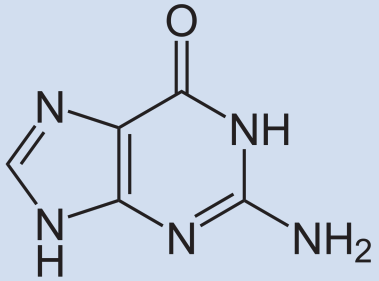
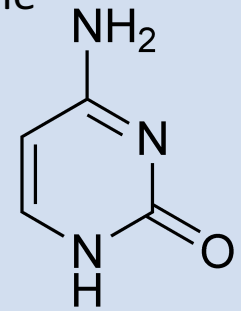
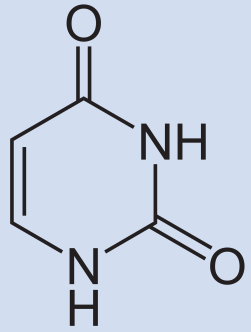
Adenine	Thymine 
Guanine 	Cytosine 
	Uracil (RNA) 

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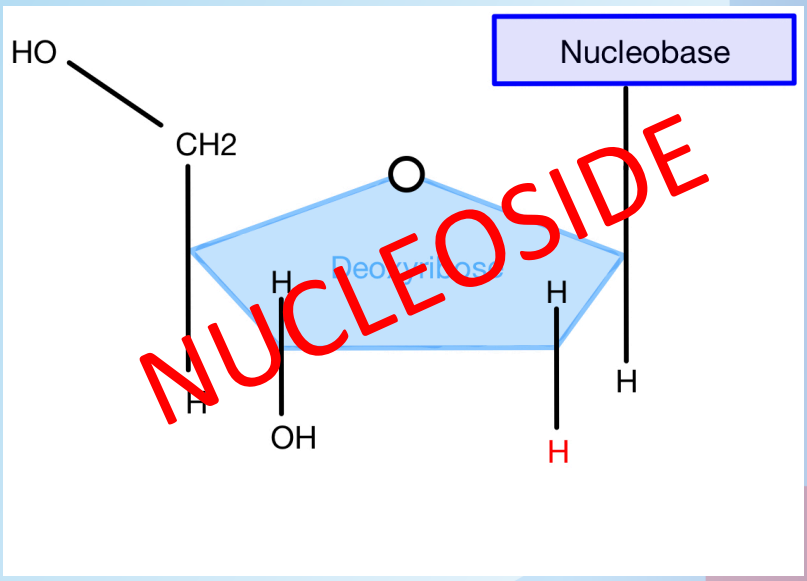
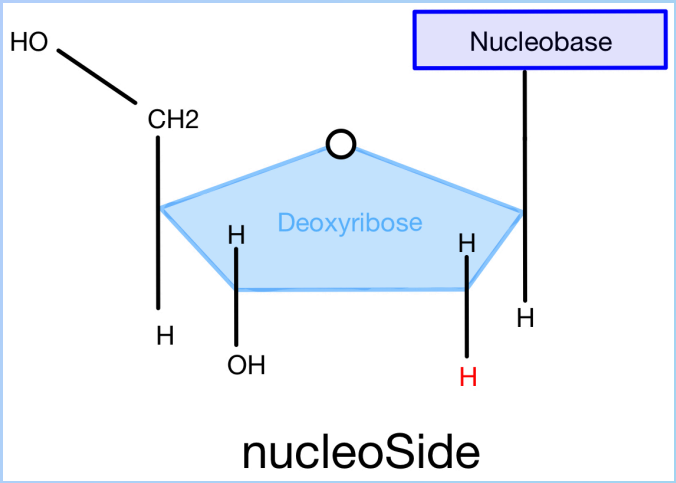
nucleoSide/nucleoTide

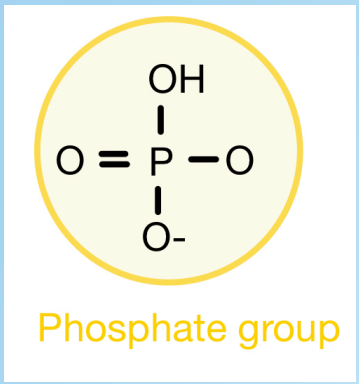
Nucleobase + Deoxyribose/ Ribose =





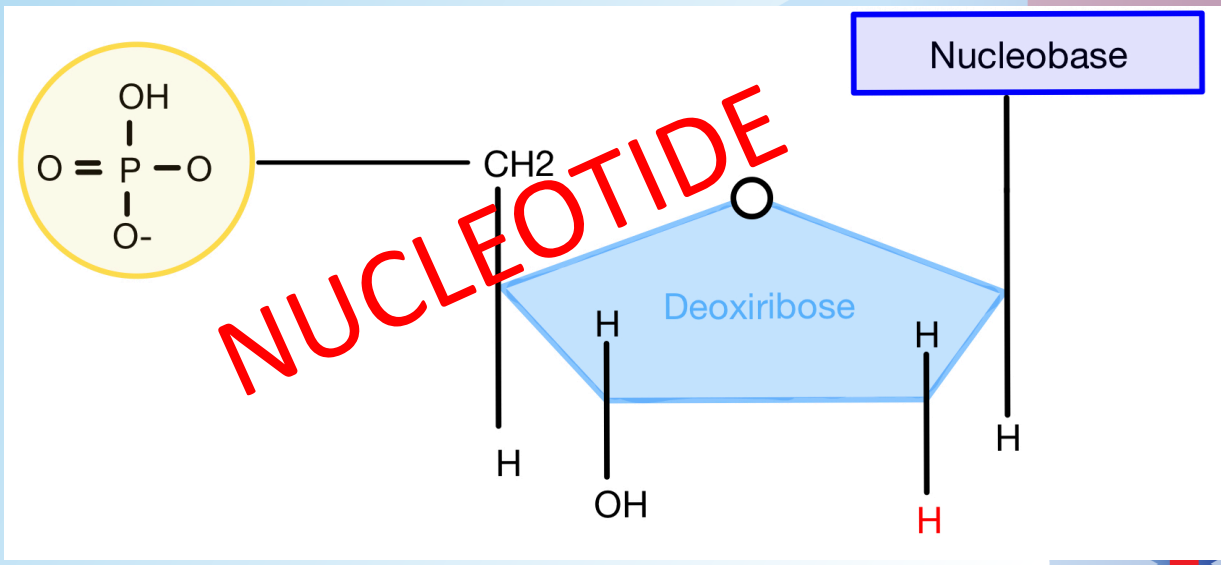
nucleoSide

+



Phosphate group

=



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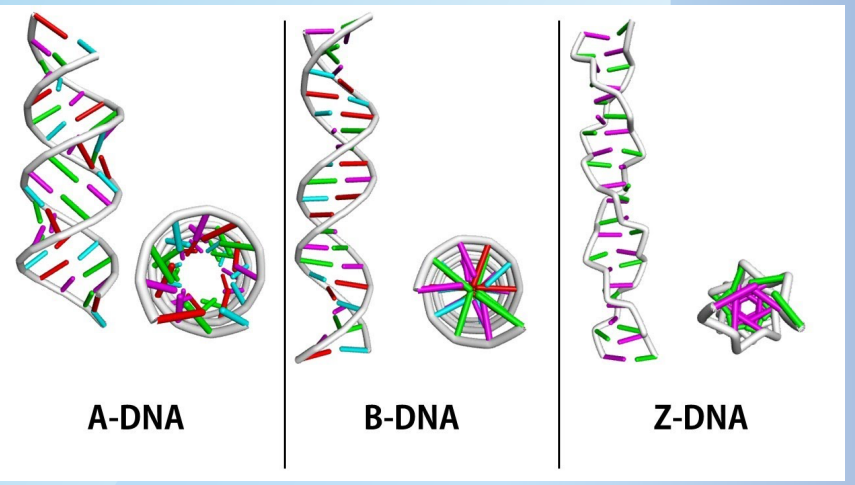
Types of DNA

Most predominant DNA helix



	DNA B-type *High humidity DNA*	DNA A-type *Dehydrated B-DNA*	DNA Z-type
Helical rotation			
Base pairs per 360°	10bp	11bp	12bp
Helical diameter	20Å = 2.0 nm	26Å = 2.6 nm	18Å = 1.8 nm
Characteristics			

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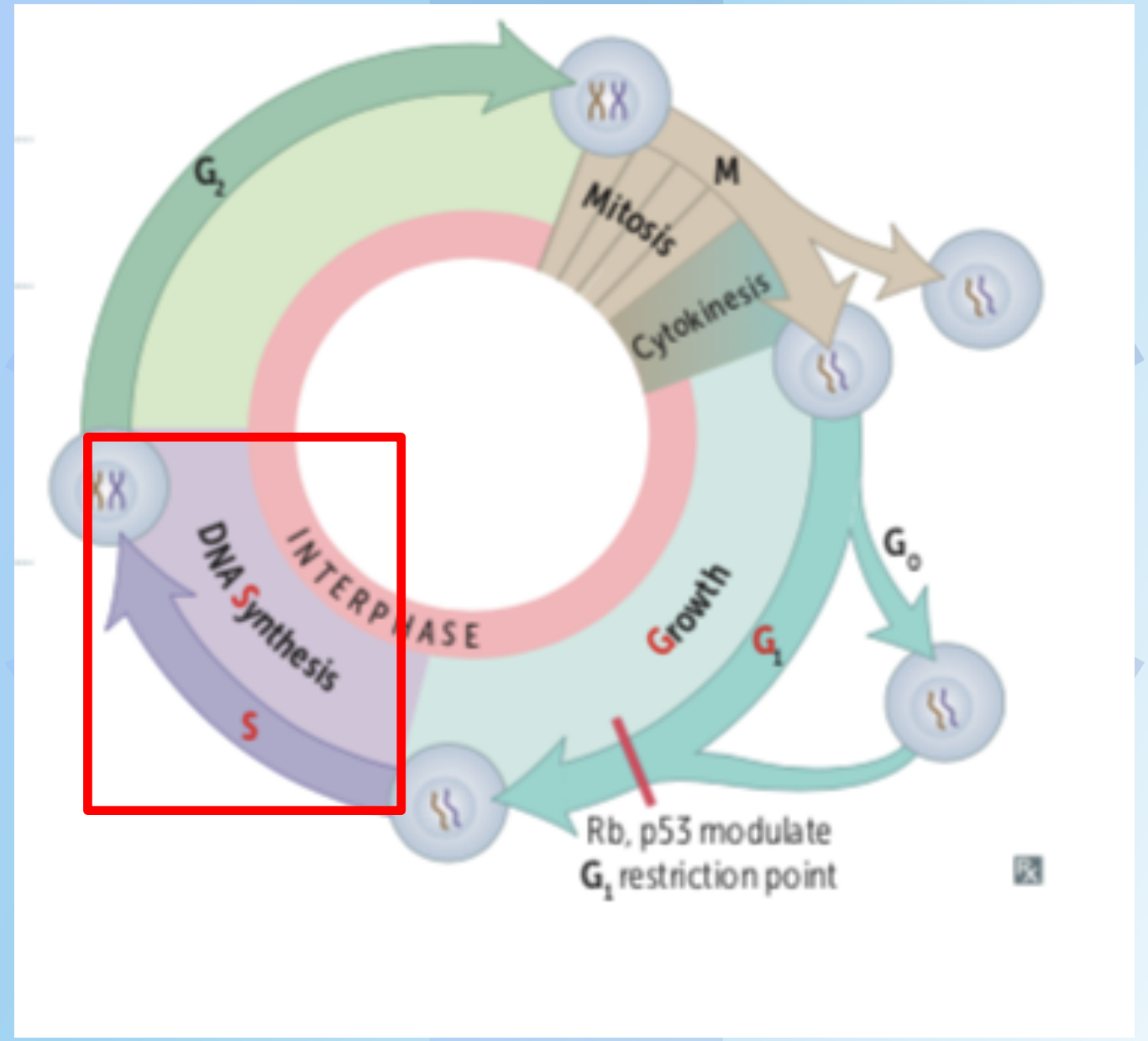
REPLICATION


G2
M
Mitosis
Cytokinesis
INTERPHASE
DNA Synthesis
S
Growth
G1
G0
Rb, p53 modulate
G1 restriction point

Requirements

TEMPLATE	PRIMER	PRECURSORS	ENZYMES
Single strand of original DNA = provides sequence information	Short strand of RNA = provides free 3´hydroxyl groups	5´deoxynucleotiSide triphosphates (dNTP's) = provides energy	DNA Polymerases Sliding clamps Helicases Primases Nucleases Ligases Topoisomerases Single-strand binding proteins (SSB's)

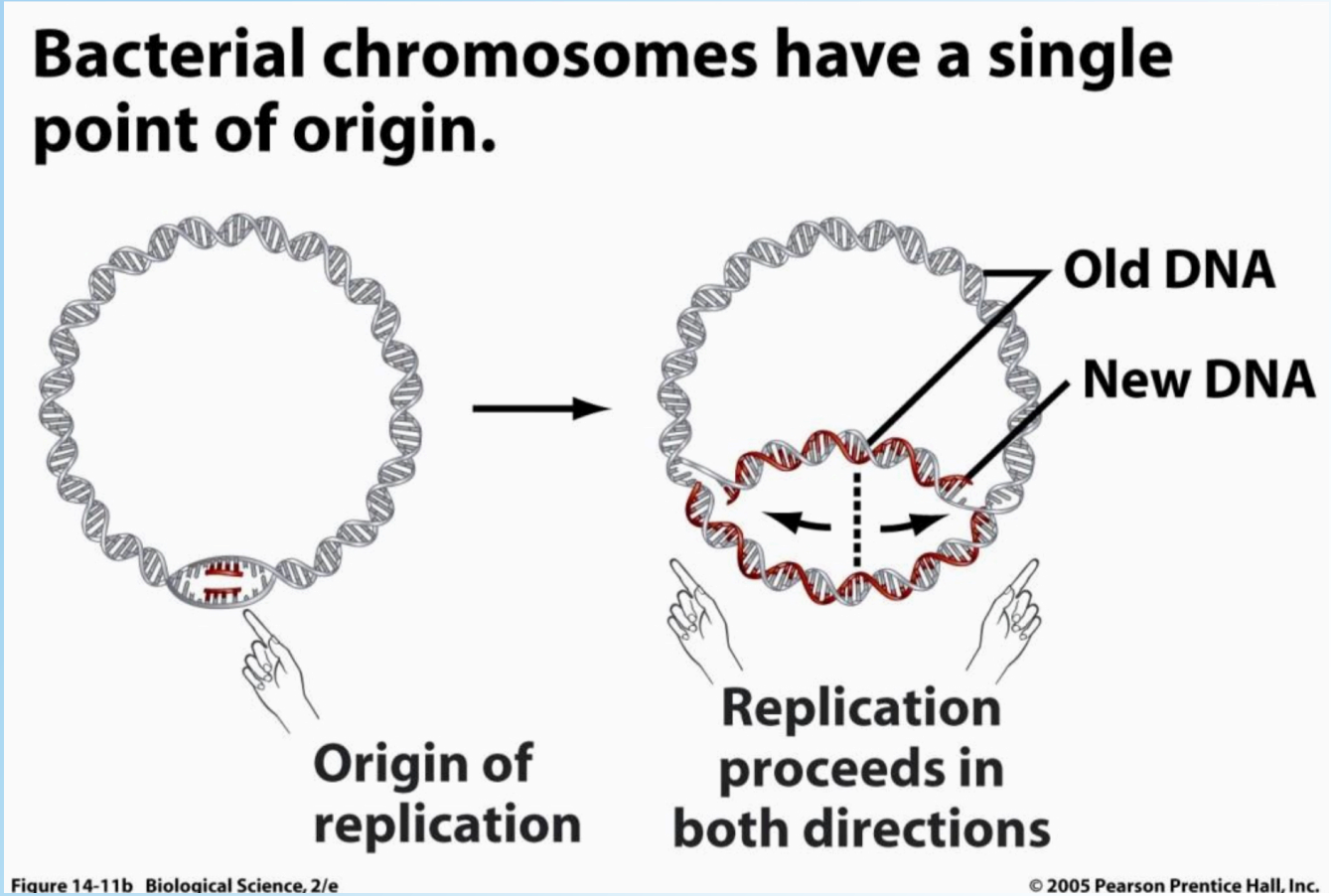
PROKARIOTIC REPLIKATION

Steps

1. Separation
2. Replication fork formation
3. Supercoils
4. Replication
5. Chain elongation
6. Excision of RNA primers
7. Termination

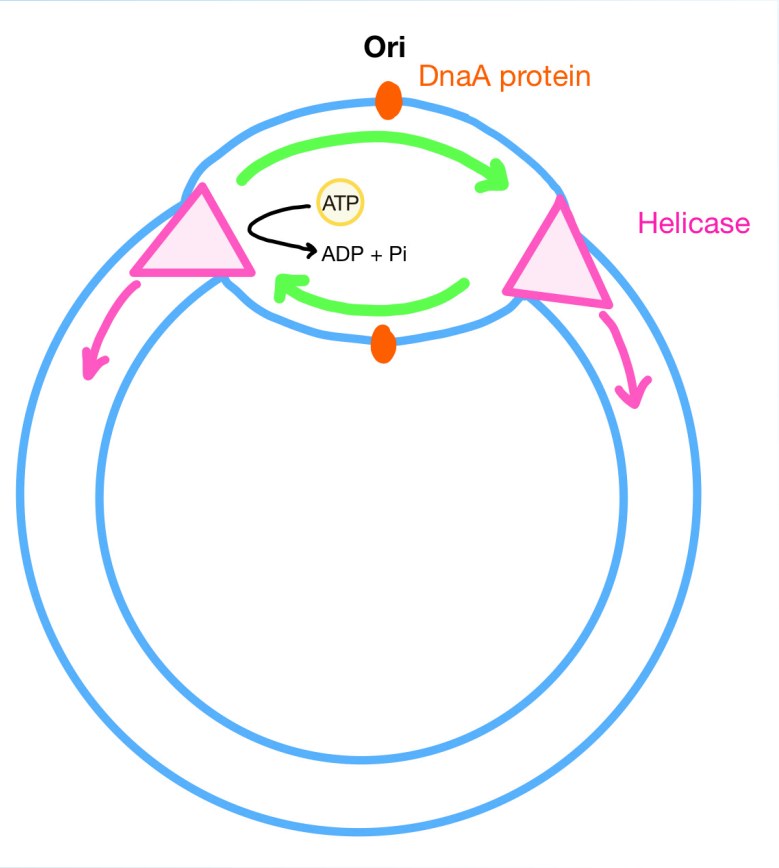
1. Separation

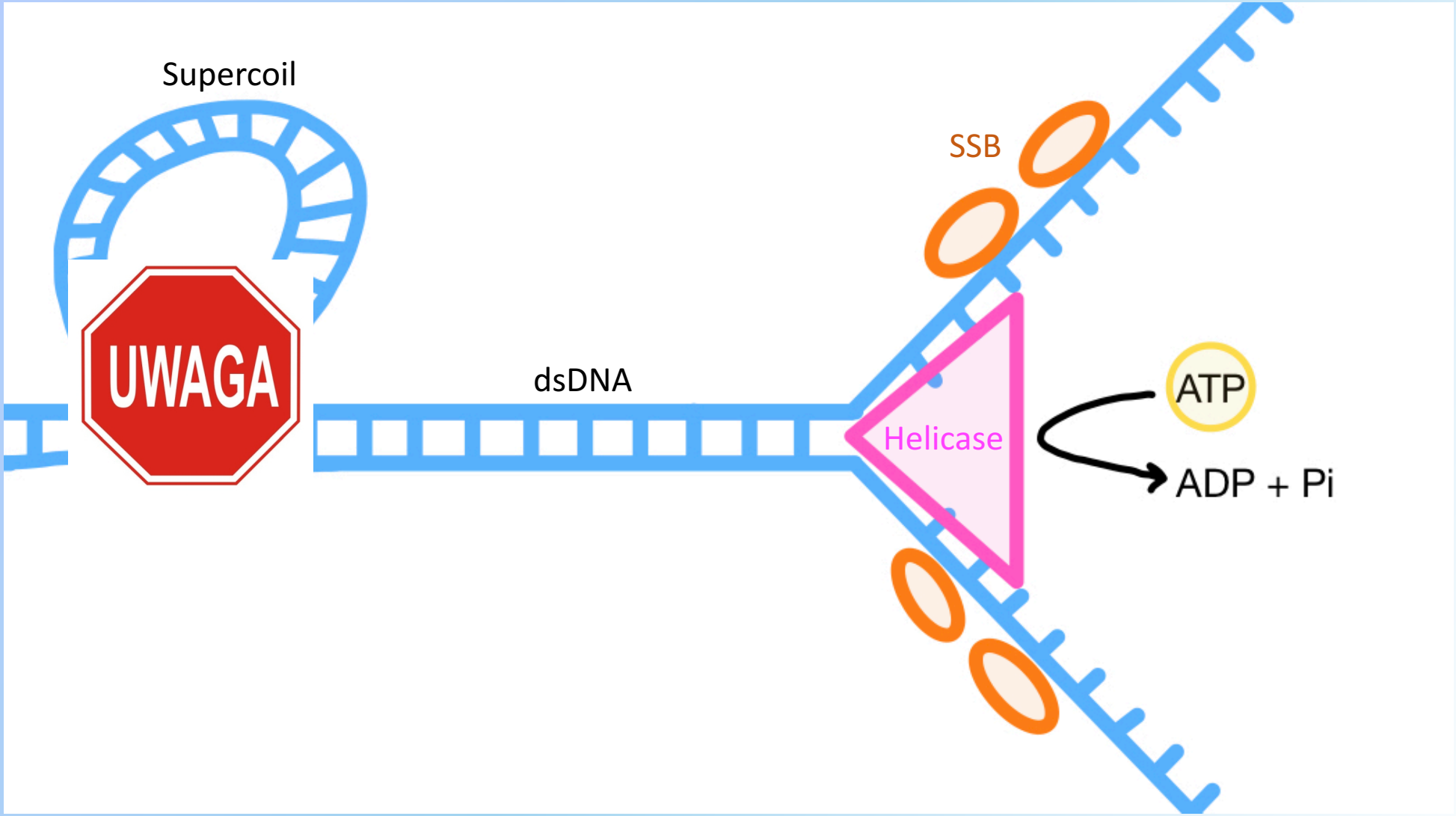
- Origin of replication = Ori
  - AT-rich segment
- E.coli: OriC



2. Replication Fork Formation

DnaA protein	DNA helicase (DnaB)	Single-stranded DNA-binding protein (SSB)
Binds to DnaA boxes ↓ AT-rich segment melts ↓ dsDNA → ssDNA	Binds to ssDNA ↓ Unwinds dsDNA by breaking H-bonds between base pairs ↓ Supercoils *Requires ATP	Binds to ssDNA ↓ Provides ss-template for polymerases + Protects DNA from nucleases IS NOT AN ENZYME

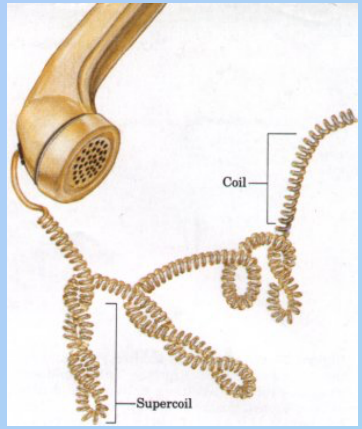


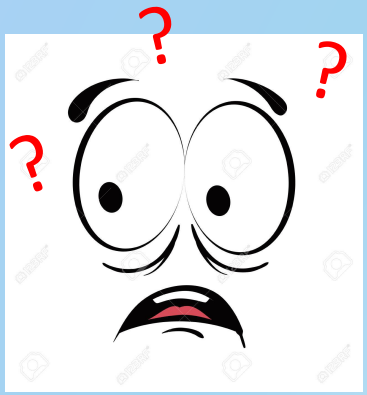

Supercoil
UWAGA
dsDNA
SSB
Helicase
ATP
ADP + Pi

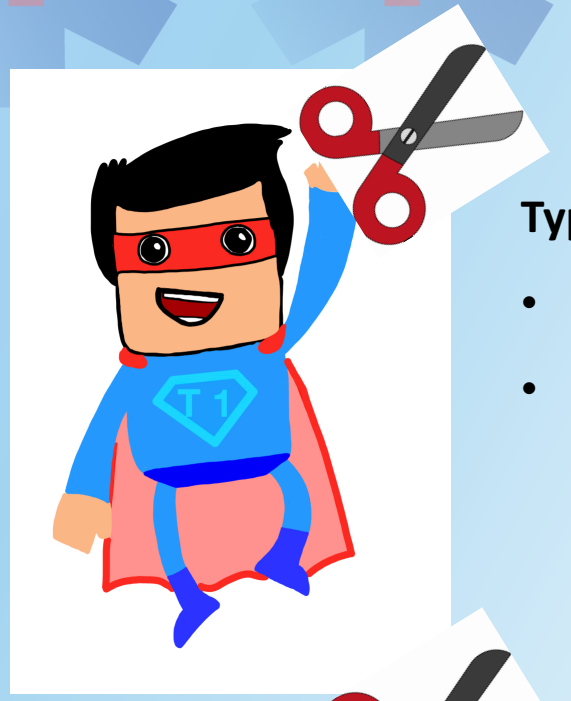
3. Supercoils

Positive supercoils
- DNA region ahead of the replication fork

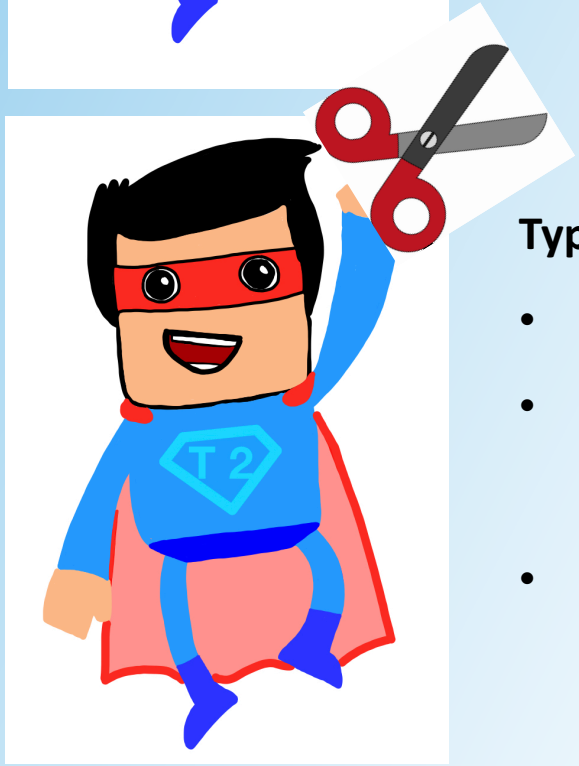
Negative supercoils
- DNA region behind the replication fork







Type 1 DNA topoisomerase
- Cleaves 1 of the DNA strands
- Works for negative supercoils



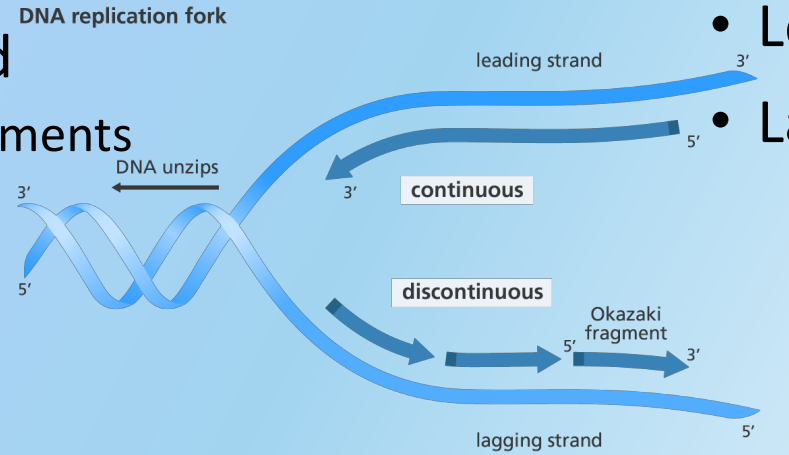
Type 2 DNA topoisomerase
- Cleaves both strands
- Works on negative and positive supercoils
- Required ATP

4. Replication

DNA polymerase

Provides deoxiribonucleoTides for new DNA strand

- 3´→ 5´: reading direction
- 5´→ 3´: synthesizing direction
- Leading strand
- Lagging strand
  - Okazaki fragments



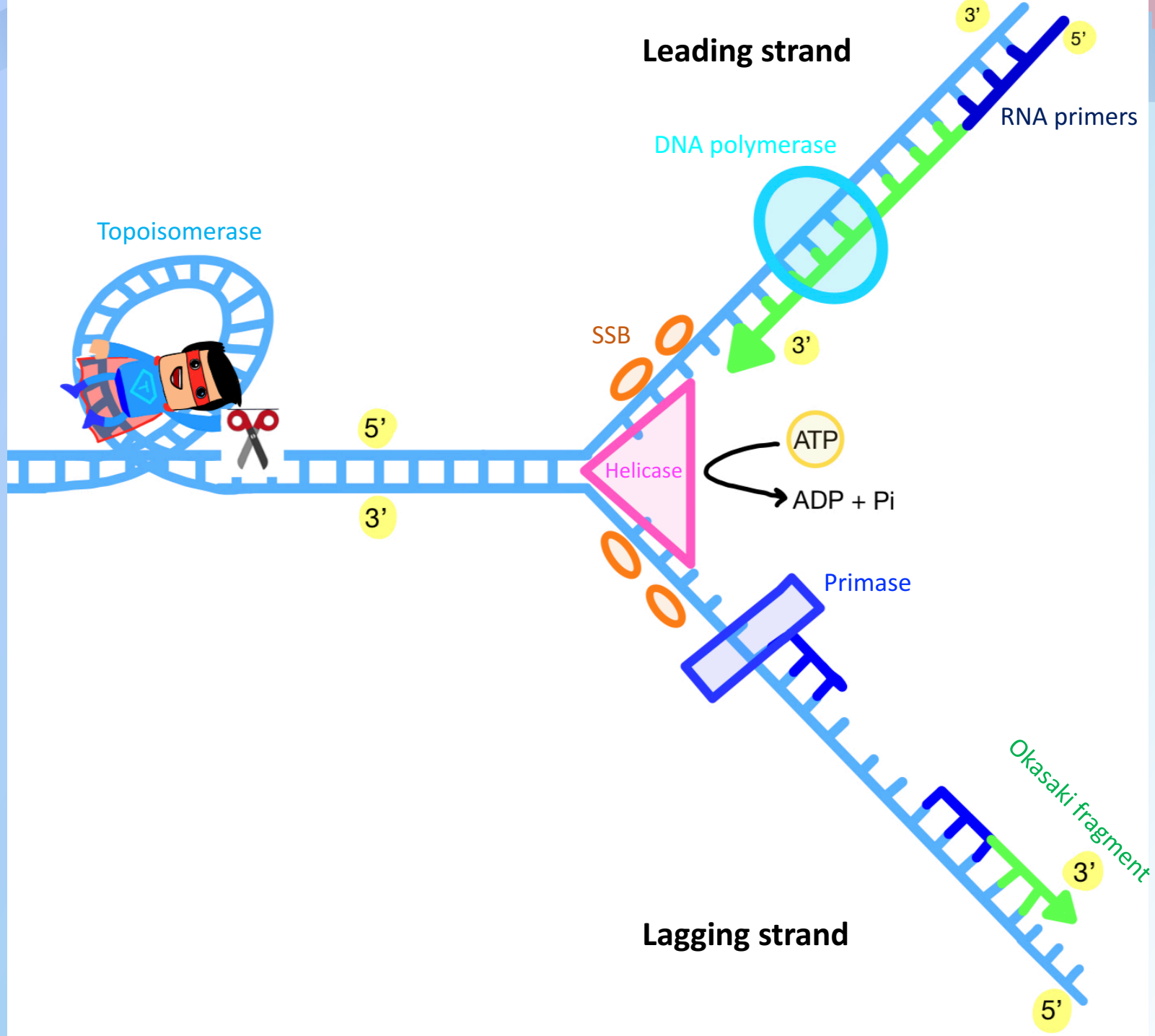
RNA primer

Provides short single strand RNA sequence

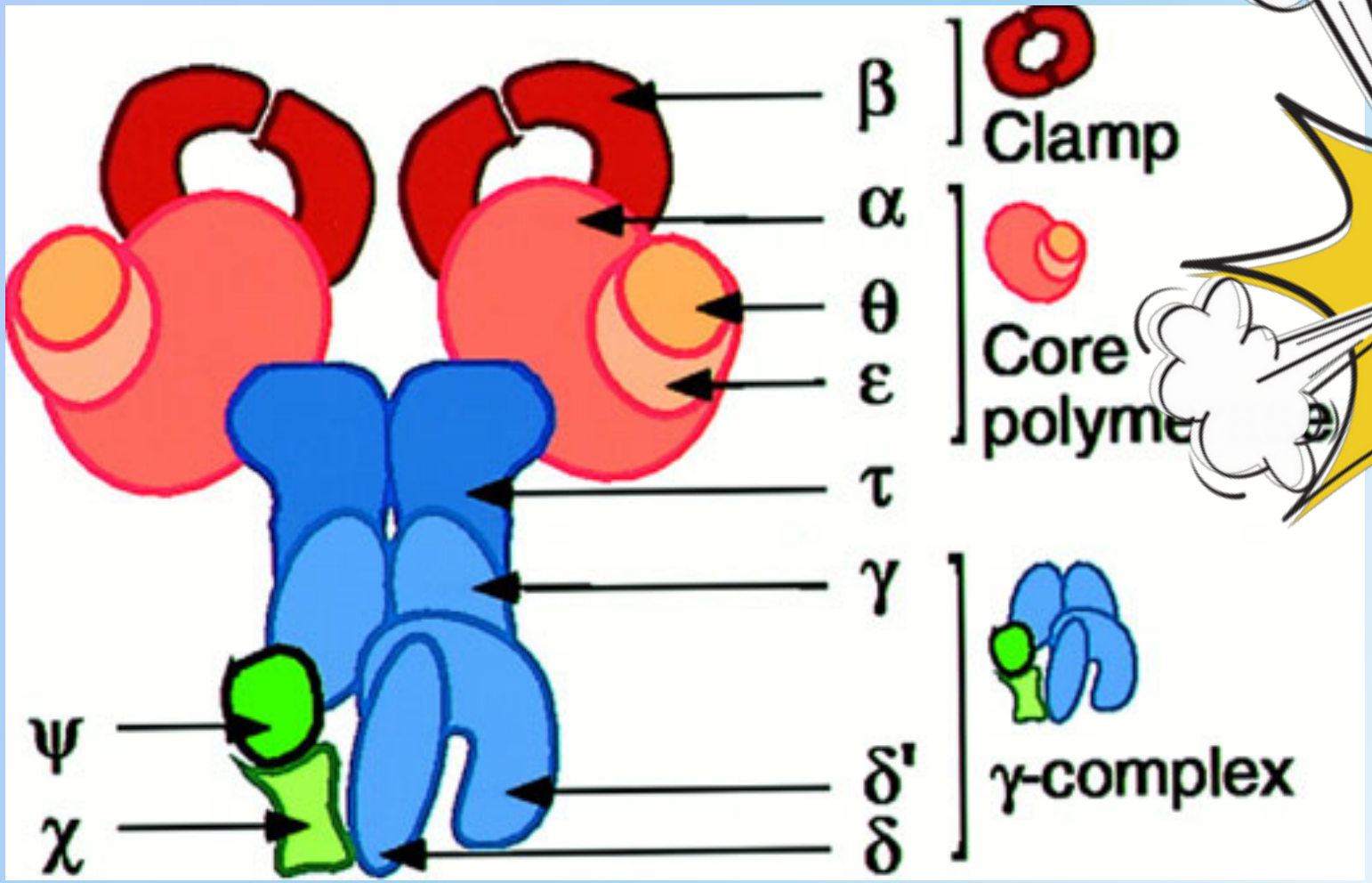
Primase (DnaG)

DNA dependent RNA polymerase

- Synthesize the RNA primer
  - Hybrid duplexes: A – **U**, C – G
- Leading strand: only one
- Lagging strand: constantly synthesized


Leading strand
3'
5'
RNA primers
DNA polymerase
Topoisomerase
SSB
3'
5'
ATP
Helicase
ADP + Pi
3'
Primase
Okasaki fragment
3'
Lagging strand
5'

5. Chain Elongation

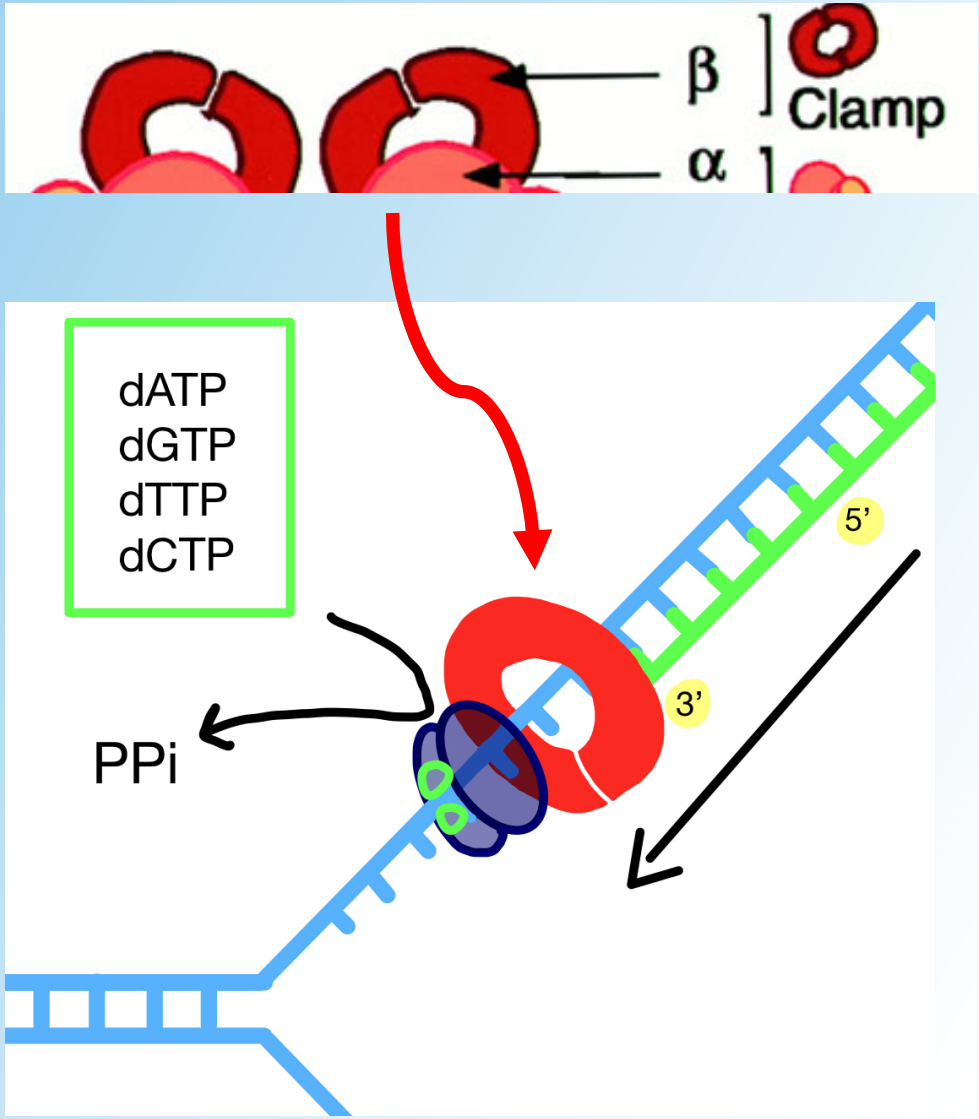


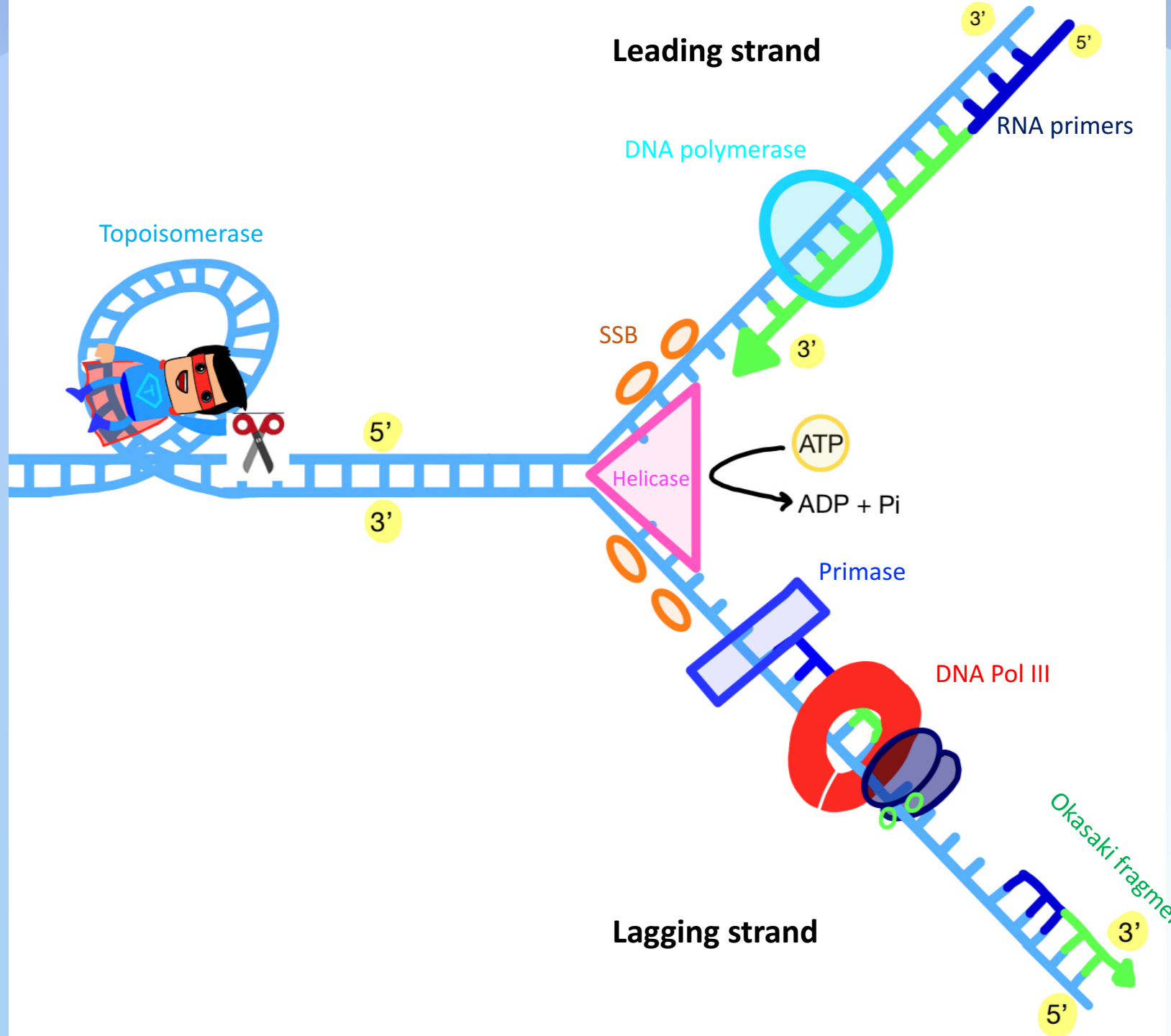
DNA Polymerase III

- Holoenzyme = Active enzyme
- β - subunit = DNA sliding clamps
  - Highly possessive
- 5´-deoxiribonucleoSide triphosphates (dNTP's)

Proofreading

- 3´ → 5´ exonuclease
  - *Removes error in opposite direction*
- 5´→ 3´polymerase
  - *Replaces wrong nucleotides with correct ones*

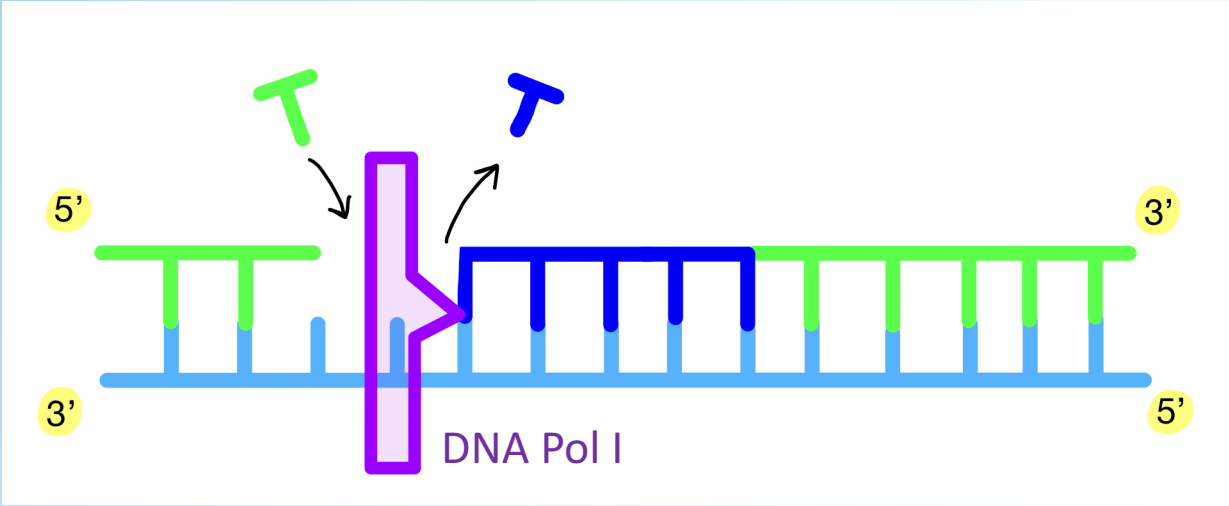



Leading strand
3'
5'
RNA primers
DNA polymerase
Topoisomerase
SSB
3'
5'
ATP
Helicase
ADP + Pi
3'
Primase
DNA Pol III
Okasaki fragment
Lagging strand
3'
5'

6. Excision of RNA Primers

DNA Polymerase I

- Removes RNA primer
  - 5´→ 3´exonuclease activity
- Synthesize new DNA
  - 5´→ 3´polymerase activity
- Proofreading
  - 3´→ 5´exonuclease activity



7. Termination

DNA ligase

- Joins Okasaki fragments
- Final phosphodiester linkage: **3´→ 5´**
- Requires ATP

TUS

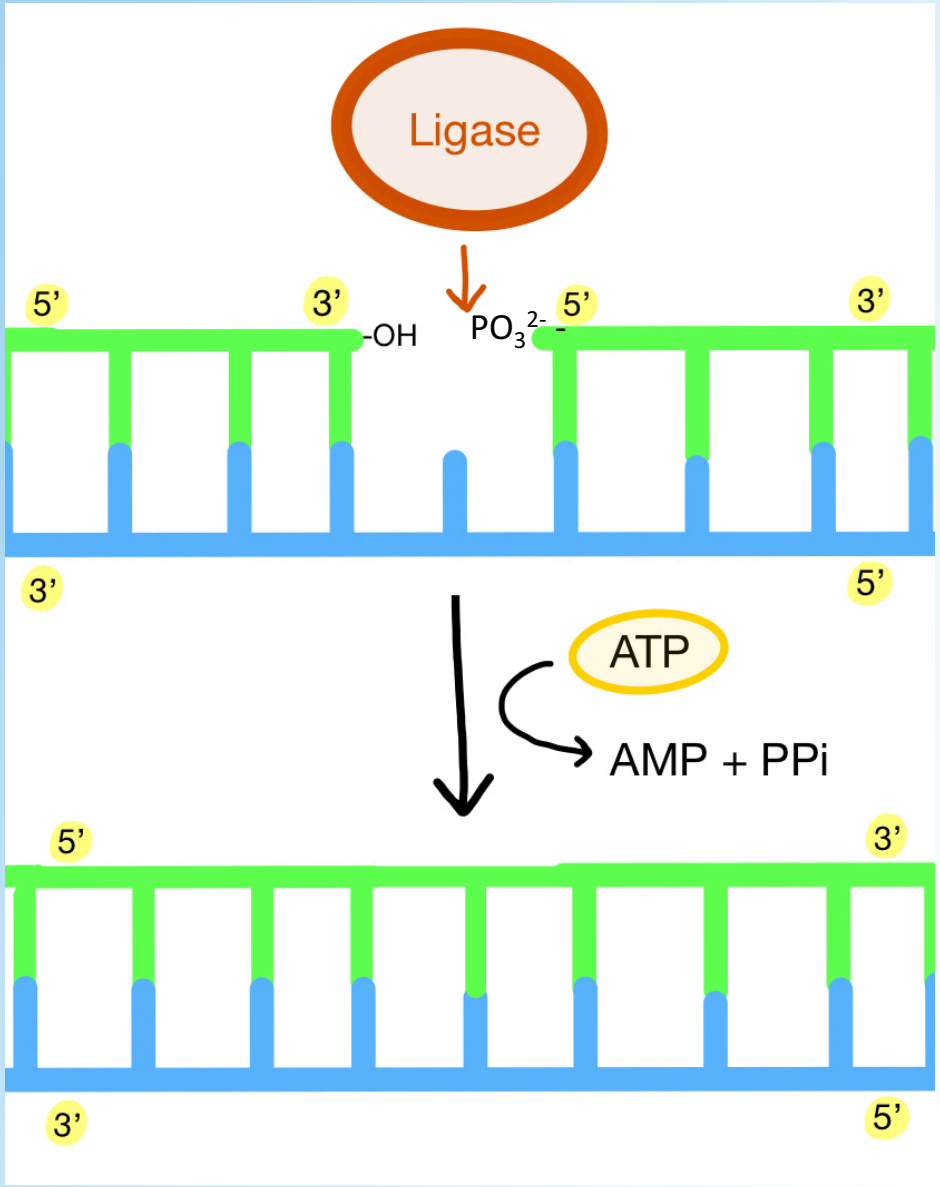
Terminus utilization substance

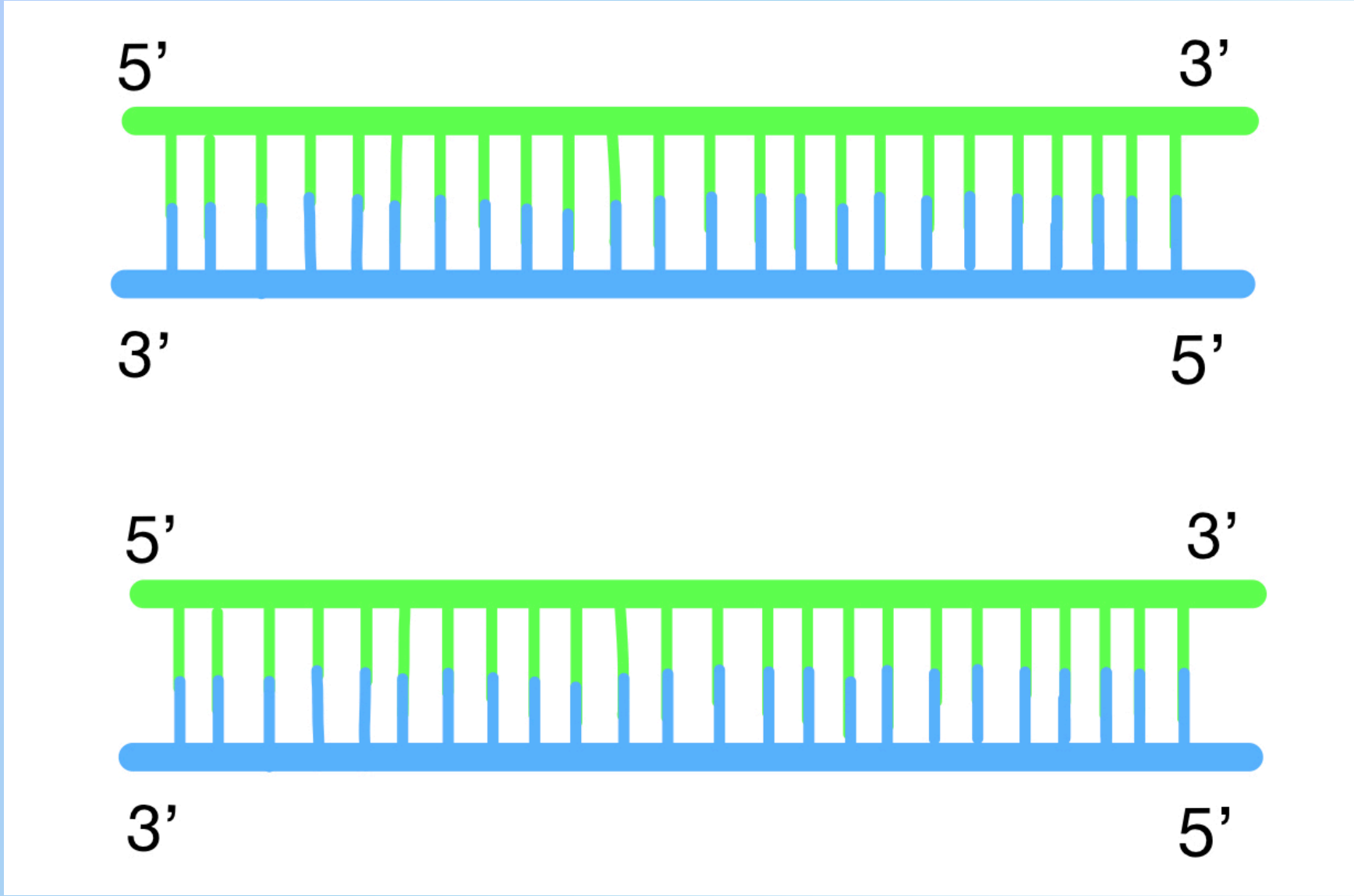
+

Ter

Replication terminator

STOP




5’
3’
3’
5’
5’
3’
3’
5’

Quiz time

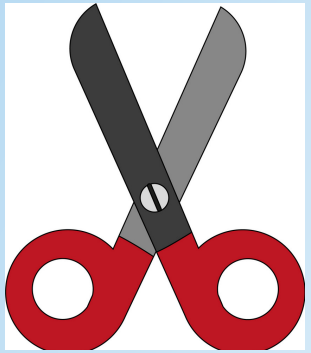
Requirements

Do you remember the steps?

1. Separation
2. Replication fork formation
3. Supercoils
4. Replication
5. Chain elongation
6. Excision of RNA primers
7. Termination

What enzymes remove supercoils?

Topoisomerase I & II



	DNA polymerase I	DNA polymerase III
Exonuclease activity	5´→ 3´ - removes RNA primer 3´→ 5´ - proofreading	3´→ 5´ - proofreading
Polymerase activity	Fills gap after removal of RNA primer, DNA repair	Replication, proofreading and editing

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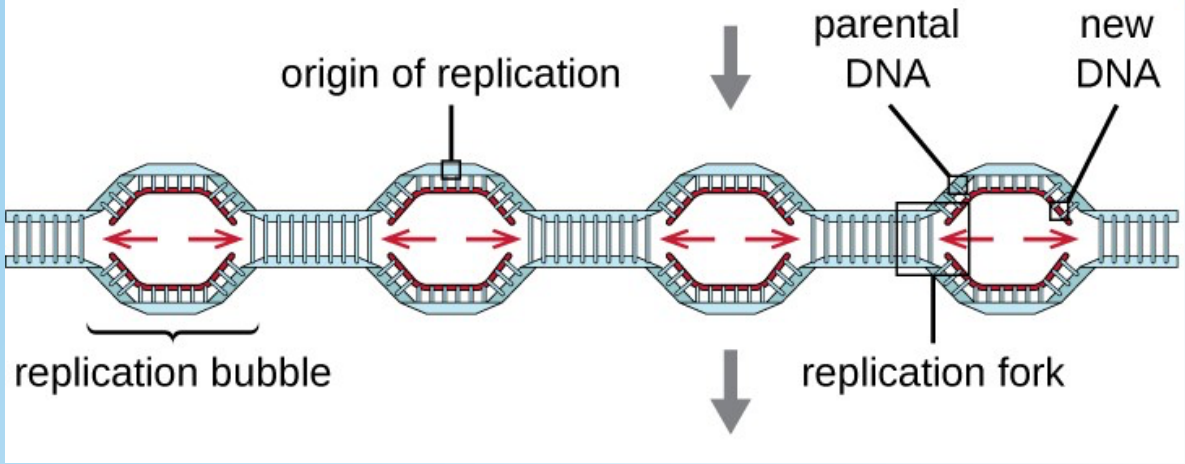
Eukaryotic DNA synthesis

	PROKAROTIC	EUKARYOTIC
Origin	Single	Multiple
Primer excision	DNA pol I	RNase H Flap endonuclease-1 (FEN-1)
DNA polymerases	Numbered **I**, II, **III**, IV, V	Greek letters α, β, δ, ε, γ



DNA polymerases

Polymerase	Function	Proofreading
α *alpha*	Initiates DNA synthesis Primase; RNA primer *5´→3´polymerase*	No
β *beta*	DNA repair *Gap filling*	No
δ *delta*	Complete DNA synthesis on lagging strand	Yes *3´→5´exonuclease*
ε *epsilon*	Complete DNA synthesis of leading strand	Yes *3´→5´exonuclease*
γ *gamma*	Replicates mitochondrial DNA	Yes *3´→5´exonuclease*

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Chromosome

Telomeres

= complexes of non-coding DNA + proteins located at the end of linear chromosomes

- Prevent attack from nucleases
- Noncoding hexametric sequences; AGGGTT
- Telomere shortening

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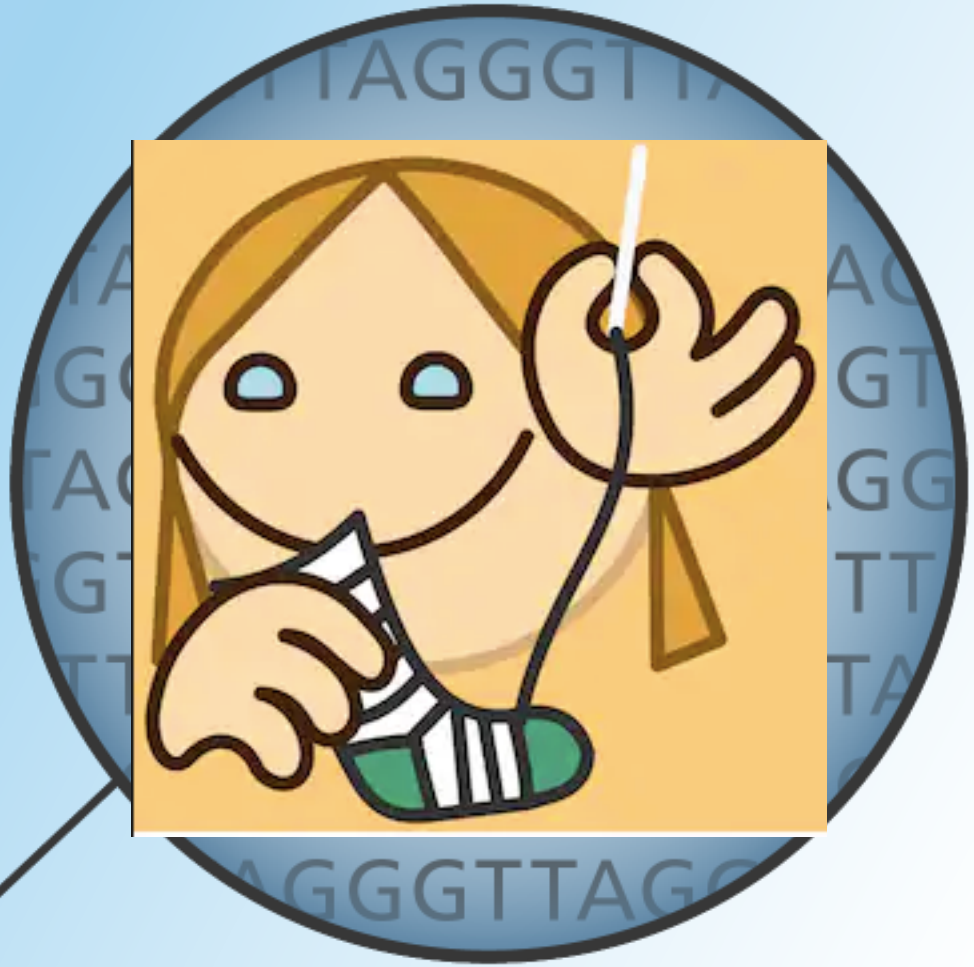
Chromosome

Telomerase

acts as a reverse transcriptase

= RNA dependent DNA polymerase

- Found in germ cells, stem cells and cancer cells
- Maintain telomere length in cell
- DNA pol α



Reverse transcriptase is involved in replication of HIV

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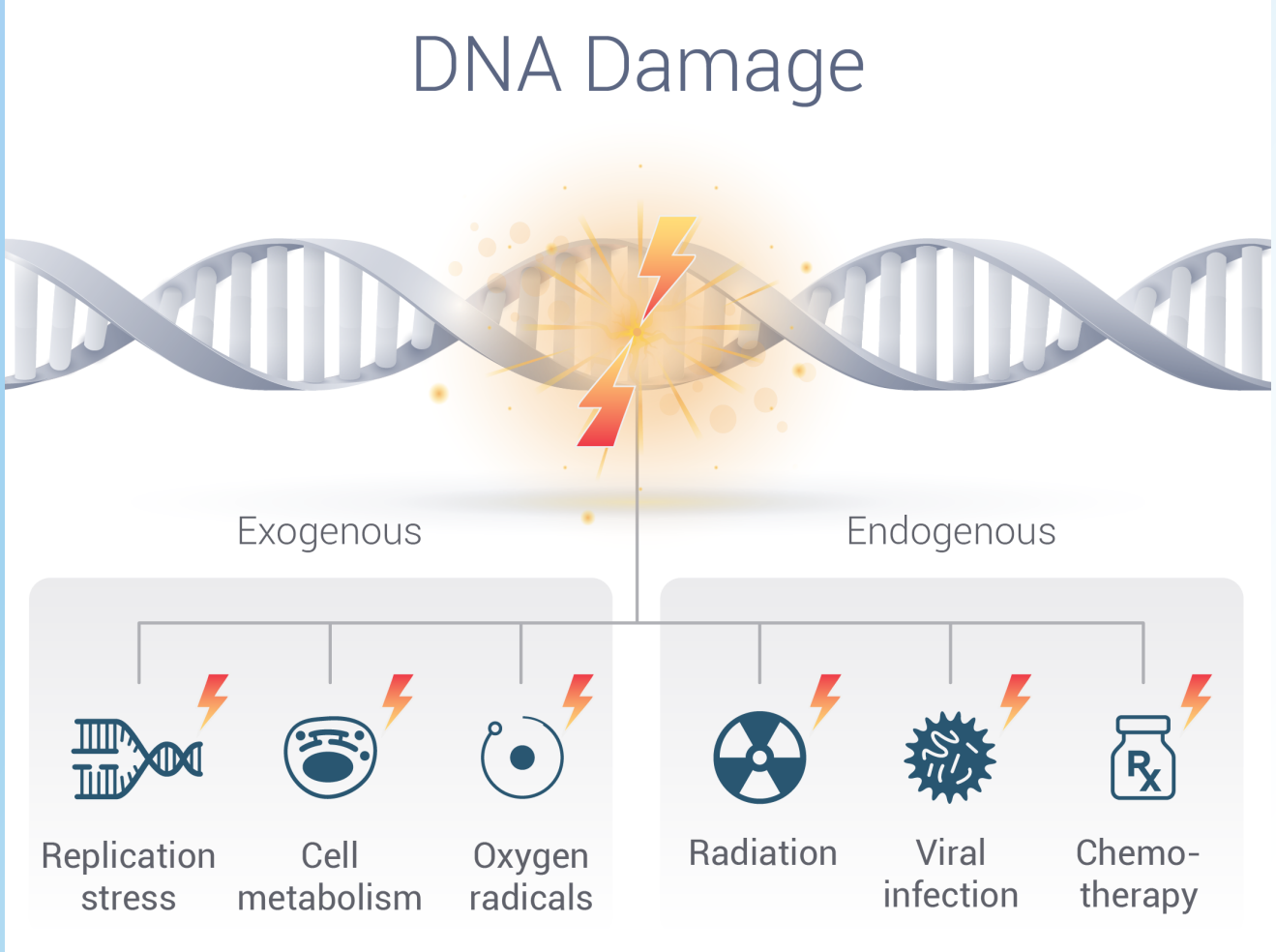
DNA Repair

DNA Repair: The triple R's

- **R**ecognize
- **R**emove
- **R**eplace



Methyl-directed mismatch repair

- *Mut proteins*
  - Parental strand fully methylated = correct
- Endonuclease activity
- Polymerase activity

Base pair excision repair

- *Glycosylases*
- *AP-endonucleases + deoxyribose phosphate lyase*
  - Apyrimidinic / Apurinic = AP-site
- *DNA polymerase + DNA ligase*

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Nucleotide Excision Repair

= Repair of damaged DNA due to **UV-radiation**

UV light → covalent bonding of two pyrimidines (thymine's), **dimer**

DNA polymerase activity

- *uvrABC excinuclease (endonuclease)*
- *DNA polymerase & DNA ligase*

Xeroderma pigmentosum

- Pyrimidine dimers form in skin
- Rare, genetic disorder
- Cannot repair DNA
- Mutations & skin cancer

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Quiz time

DNA polymerases

Polymerase	Function	Proofreading
α *alpha*		
β *beta*		
δ *delta*		
ε *epsilon*		
γ *gamma*		

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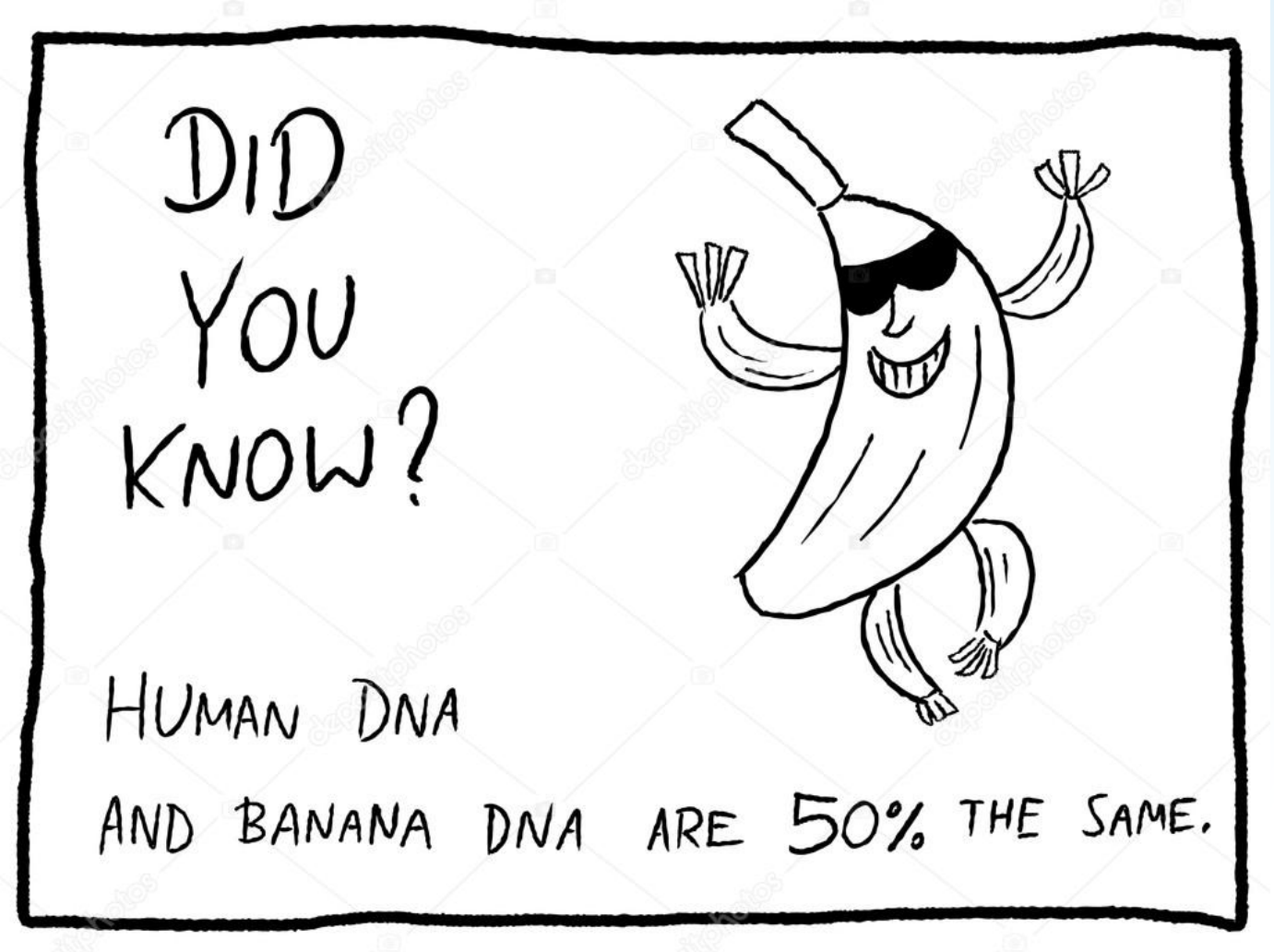
What causes xeroderma pigmentosum?

a. Accumulation of purine dimers
b. Accumulation of pyrimidine dimers
c. Methylation of parental DNA strand
d. Accumulation of AP-sites

GOOD LUCK!