molecular biology of The Gene ch 8. pg. 199
Molecular biology of The Cell ch 5 pg. 237

Replication of DNA

objectives

what is the basis/dogma of this process?

- to explain the prineiples of DNA replication
- to describe the chemistry of DNA synthesis ← what rxns take place for the dogma to occur?
- to describe the functions of enzymes involved in DNA synthesis ← how do these complex molecular structures help achieve the dogma?
- to explain the mechanisms of catalysis by DNA polymerase ← how do the rxns occur for efficient achievement of the dogma?
- To describe process of DNA replication ← what occurs step by step for the dogma to be achieved?

Key Terms

- biological growth
- repair
- transmission of genetic info
- Primer
- genetic mutations
- recombination
- Crick's central Dogma
- Semi-conservative replication
- Bidirectional replication
- dNTPs
- ATP
- polymerase
- primase
- helicase
- ligase
- topoisomerase
- ss Binding Protein
- Initiator protein
- Phosphodiester Bond formation
- The Replication forK
- Initiation
- unwinding
- primer synthesis
- Elongation
- leading strand
- lagging strand

NUCLEIC ACIDS

STRUCTURE & FUNCTION, GENOME DIVERSITY & ORGANISATION

- Describe the 3 main components of nucleic acids
- Describe the main functions of DNA & RNA
- Demonstrate how the structures of DNA & RNA account for their functions
- Illustrate & by drawing monomeric structure of DNA & RNA
- Describe the conformations of DNA
- Explain how DNA molecules are packaged into the cell's nucleus when they are many times longer than the cell.

Key Terms

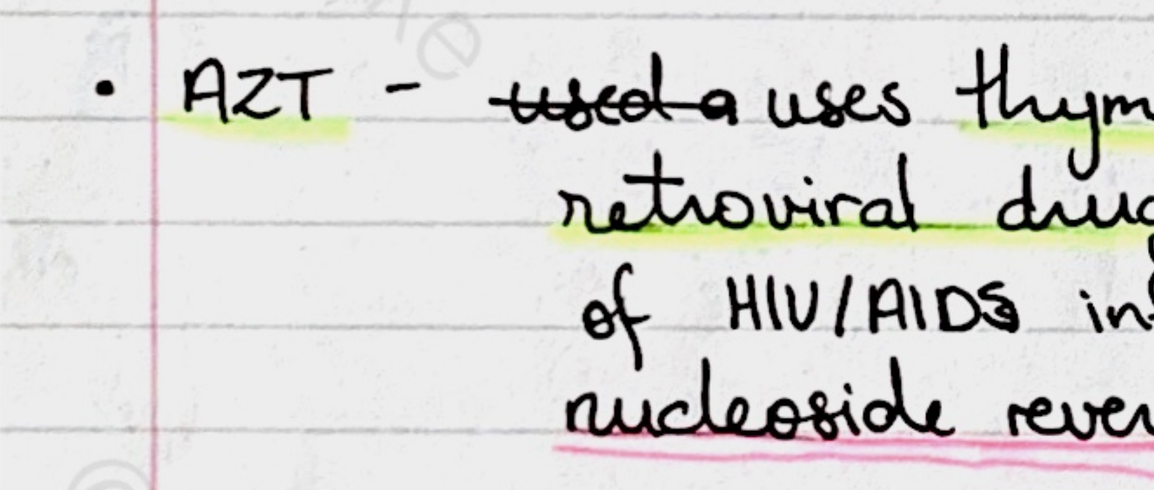
- purines
- pyrimidines
- nucleoside
- nucleotide
- Chargaff's Rules
- Base composition
- Structural backbone
- Secondary Structure
- Doble helix
- DNA helix axis
- Conformations
- Supercoil
- RNA structure
- Ribsomes
- Types of RNA
- Denaturation
- Renaturation
- Hyprechromic&
- Hypochromic effect
- Tm
- Gene
- chromosone
- DNA-protein complexes
- Nucleosome
- Chromosome pairs
- Mitochondrial Chromosome
- info flow
- genetic code
- Genome

Nucleosides

functions:

- nucleoside 5' triphosphates are prime sources of chemical energy
- ATP - energy currency of cell
- CTP - phospholipid biosynthesis
- GTP - major energy source for protein synthesis
- UTP - essential for carbohydrate biosynthesis
- AZT - ~~used a~~ uses thymidine in the form of a retroviral drug used for the treatment of HIV/AIDS infection. Used as an analogue-nucleoside reverse transcriptase inhibitor

Acyclovir - a nucleoside analogue made using guanine; slows growth and spread of the herpes virus.



(nucleotide)

- nucleoside + 3 P'

Adenosine

- a local hormone or autocoid
- circulates in blood and affects blood vessel dilation
- smooth muscle contraction
- neurotransmitter release
- fat metabolism e.g when exercising muscles ~~it~~ release adenosine which increases blood flow and delivery of oxygen and nutrients to the muscles
- regulates heartbeat by slowing heart rate
- involved in sleep regulation

- malfunctioning nucleotides may cause cancer

Nucleotide

nucleoside + phosphate group.

Functions

- building blocks of nucleic acids
- derivatives used in metabolic pathways
  - UDP-glucose: glycogen synthesis
- Energy currency (energy stored in p⁻ linkages)
- Regulatory molecules
  - cAMP / G proteins

DNA Base Composition

- Analysis by Erwin Chargaff in 1940's

Established:

- four bases do not occur in equimolar amounts (varied from species to species)
- certain bases were always found in 1:1 ratio
- # pyrimidines always equaled # purines
- Chargaff's rules!

Chargaff's Rules

1. # purines = # pyrimidines
2. % amino bases (A & C) = % Keto bases (G & T)
3. Bond lenght shared by A+T = bond lenght shared by (C+G)
4. #A = #T , #C = #G
5. DNA from different cells in the same ~~organism~~ species is consistent with wide variance in the molar proportions of bases ⇒ same genes but different variants of the genes.

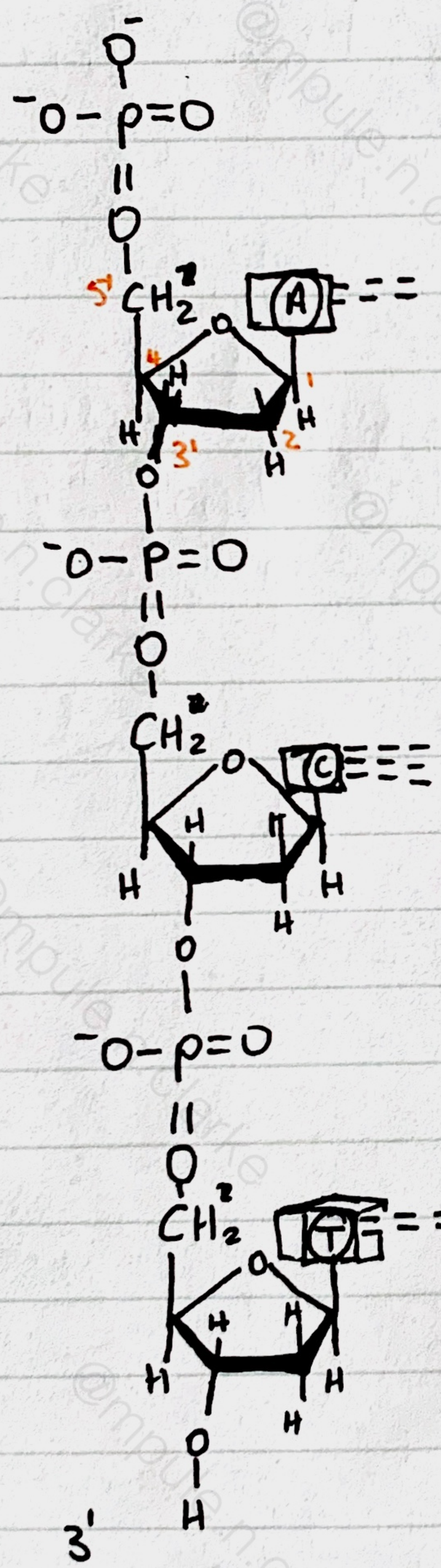
Base pair Rules

1. A ≡ T , C ≡ G

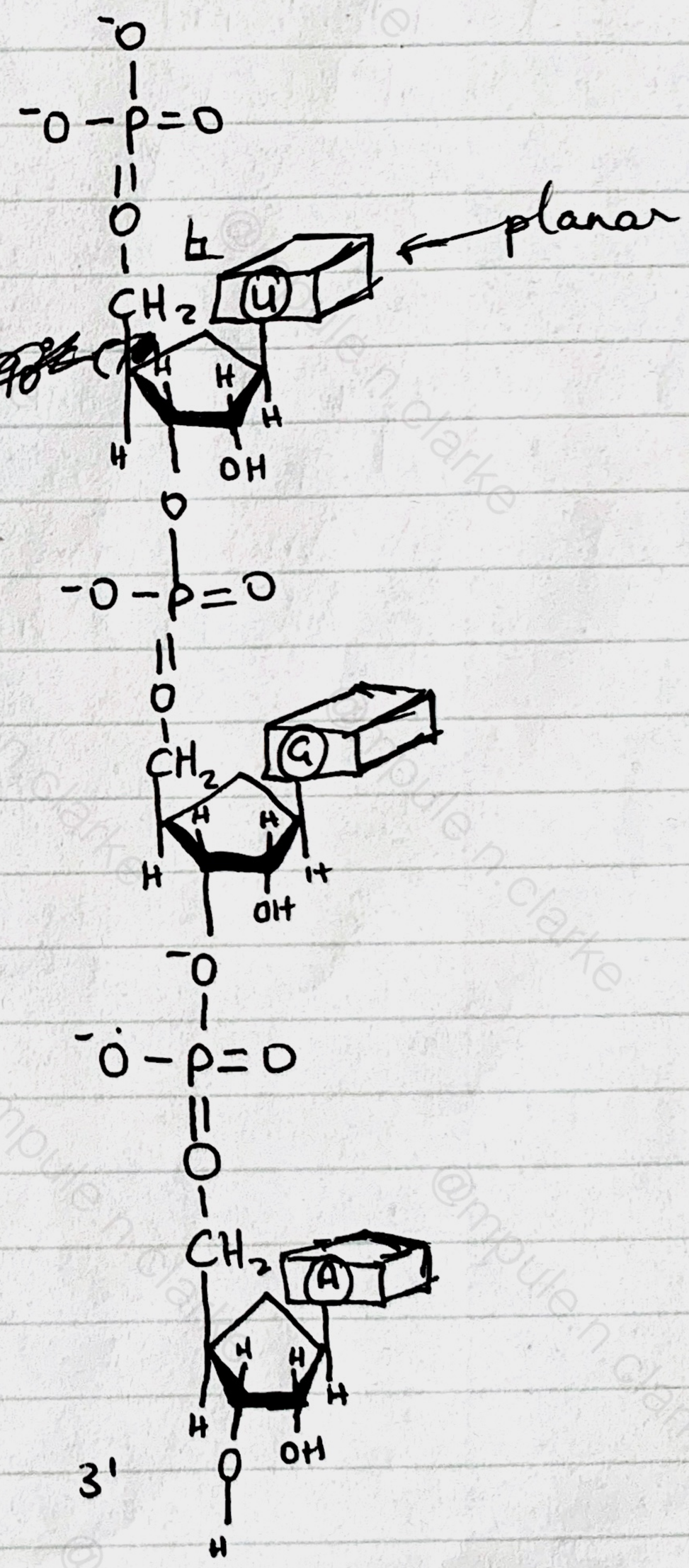
DNA & RNA

STRUCTURAL BACKBONE

DNA



RNA



DNA

Secondary Structure

Watson & Crick in 1953 proposed that the DNA molecule extended chain having a highly ordered structure & is composed of:

- two complementary polymeric chains forming a regular right handed double helix

- The two strands run in opposite directions (antiparallel alpha-helices) and are of opposite polarities
  => due to the charge composition of the molecule there is a net Ove charge on the 5' end of each strand; the strands run in opposite directions so the charges are of opposite polarity.

- The rails of the "ladder" run in opposite directions and alternate between the sugar & phosphate units.

- The sugar and phosphate groups are always linked by 3'-5' phosphodiester linkages

- Purines & Pyrimidines are planar & relatively water insoluble; stacked like a pile of plates

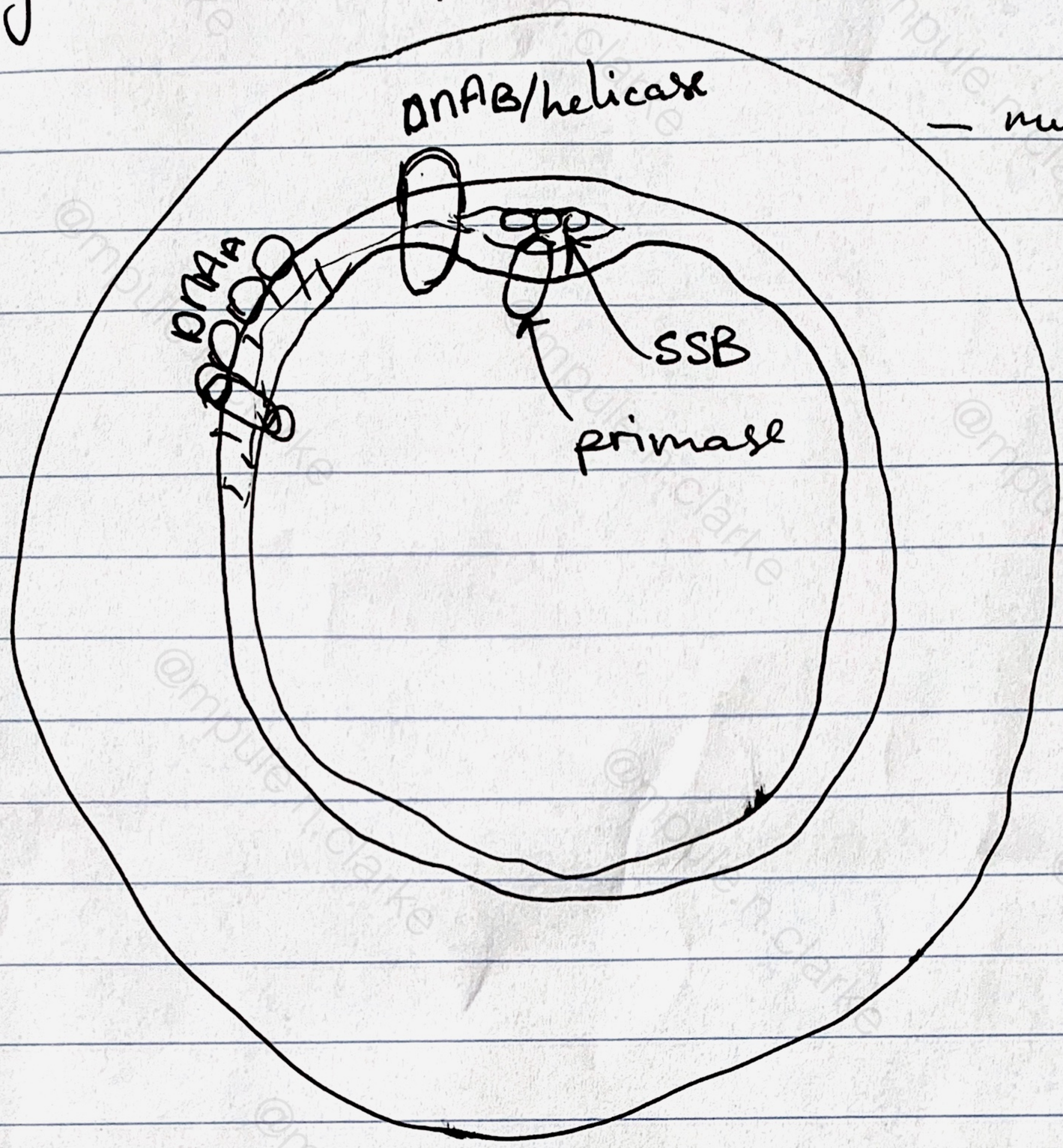
- bases arranged at right angles to the long axis of the polynucleotide chain.

- bases separated by 0.34 nm spacing
- helix has a width of 2nm

How does DNA fulfil its function of passing info from one generation to the next?

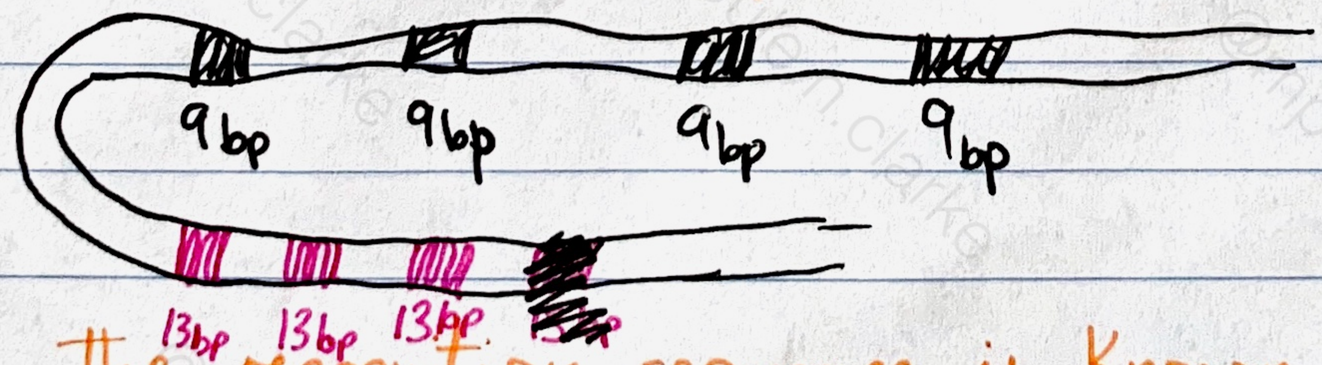
- The strands of the double helix separate and replicate simultaneously; two semi conservative progeny molecules are a result. These strands (new) are identical (exactly). ~~and. press~~ This semi conservative replication preserves the integrity of the information being transfered.

DNA ~~polymerization~~ Replication of Circular DNA



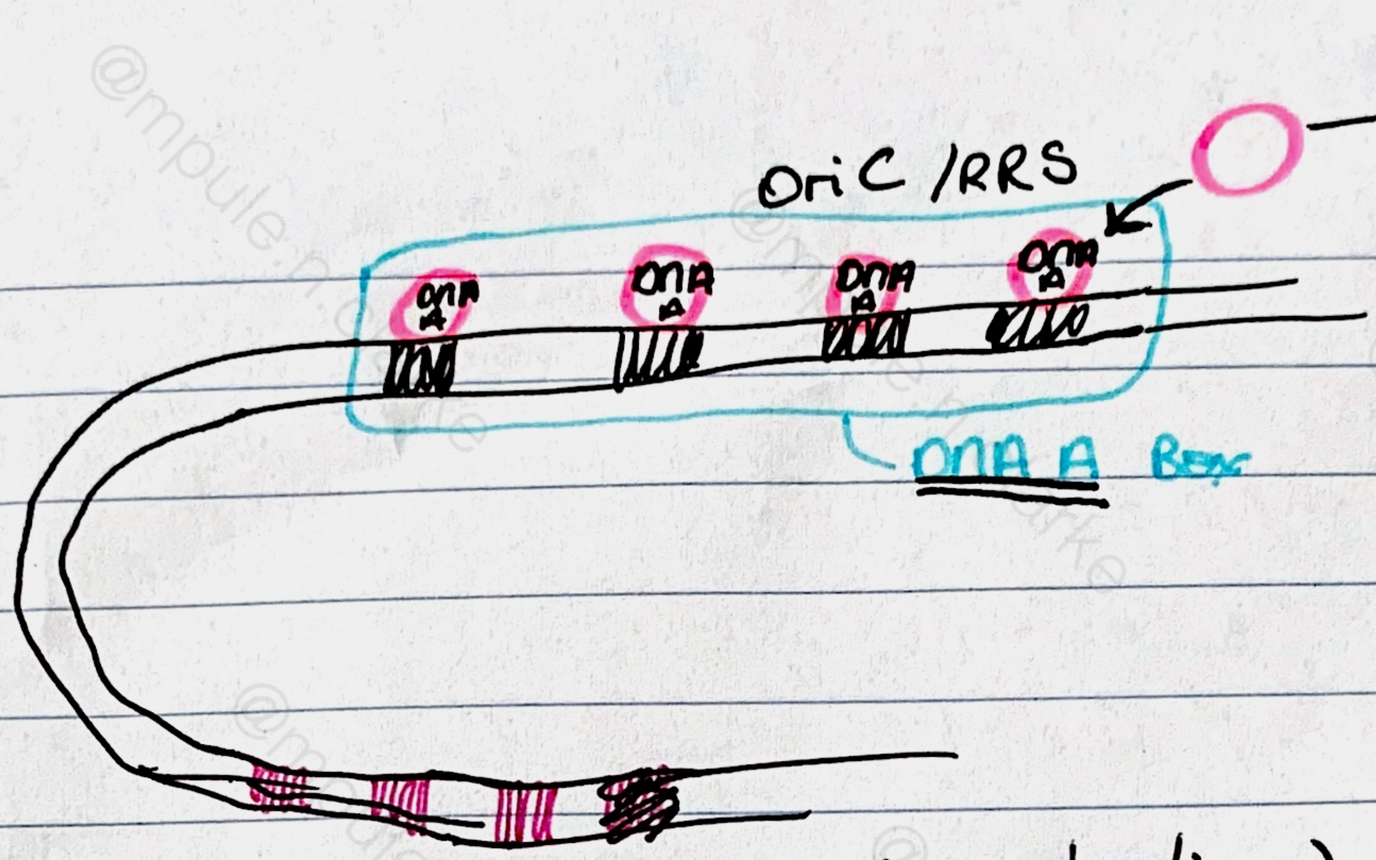
Initiation: on the level of the strands as macromolecular structures in the nucleus. (not to scale or literal)

recognition sequence / DiC / origin of replication



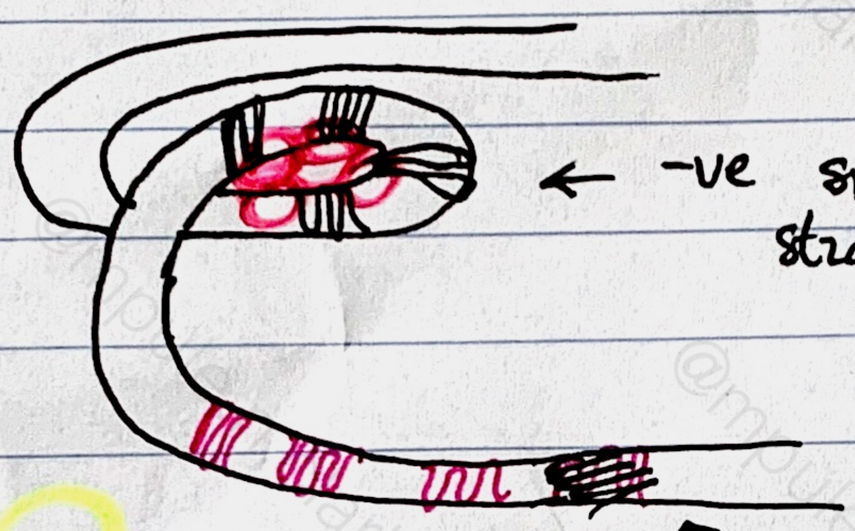
DNA_A - the recognition sequence is known as the DNA_A box

DNA_A gene is expressed when the cytosol to ~~nucleus~~ nucleic material ratio is out of balance; the cell is mature enough to replicate so the gene for DNA_A is expressed and the protein is now present to trigger the initiation of DNA replication.



free in ~~cytoplasm~~ cytosol
produced before cell division processes start

ORi (origin of replication)
↓ DNA melting site (A-T rich region)

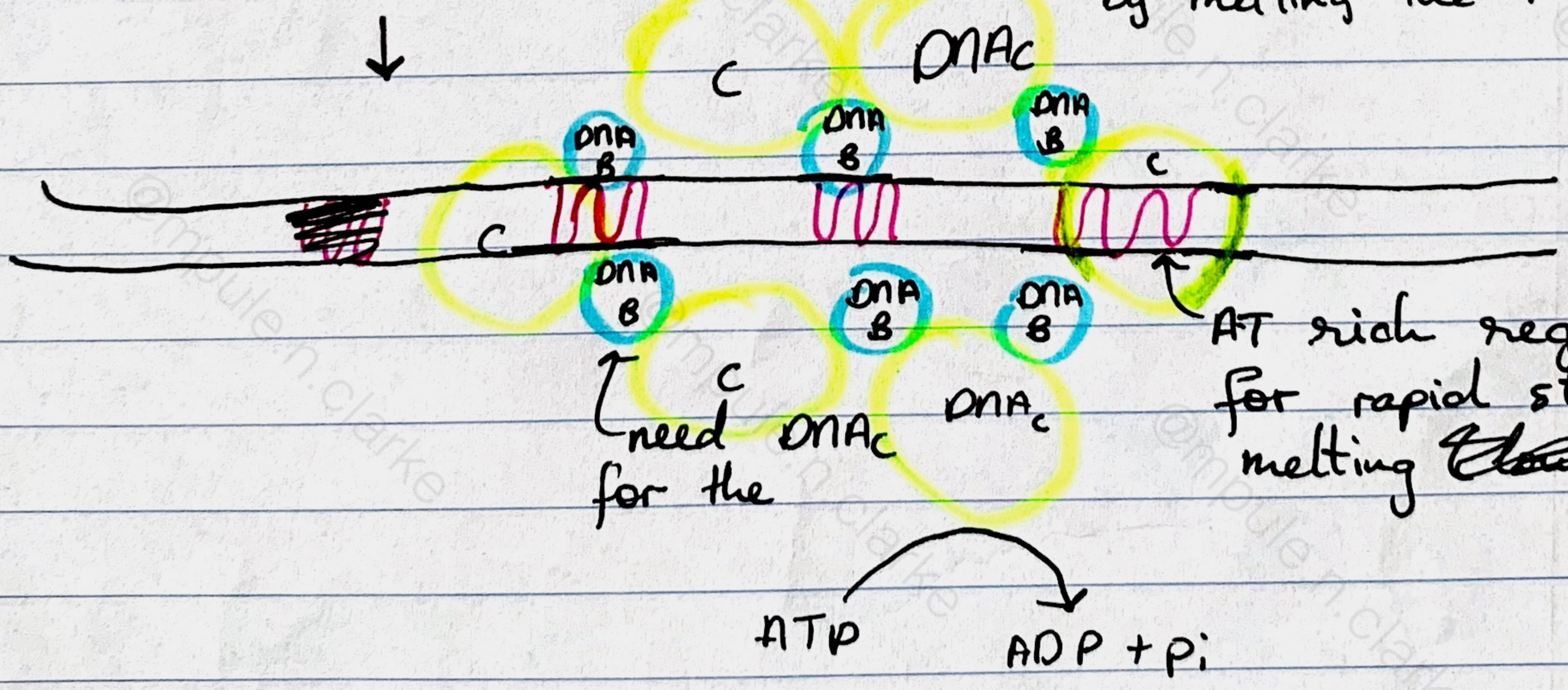


← -ve supercoiling caused by strain on circular strand

+ ATP

attach to origin of replication to melt the DNA & relieve the stress in the strand (undos -ve supercoiling) by melting the DNA

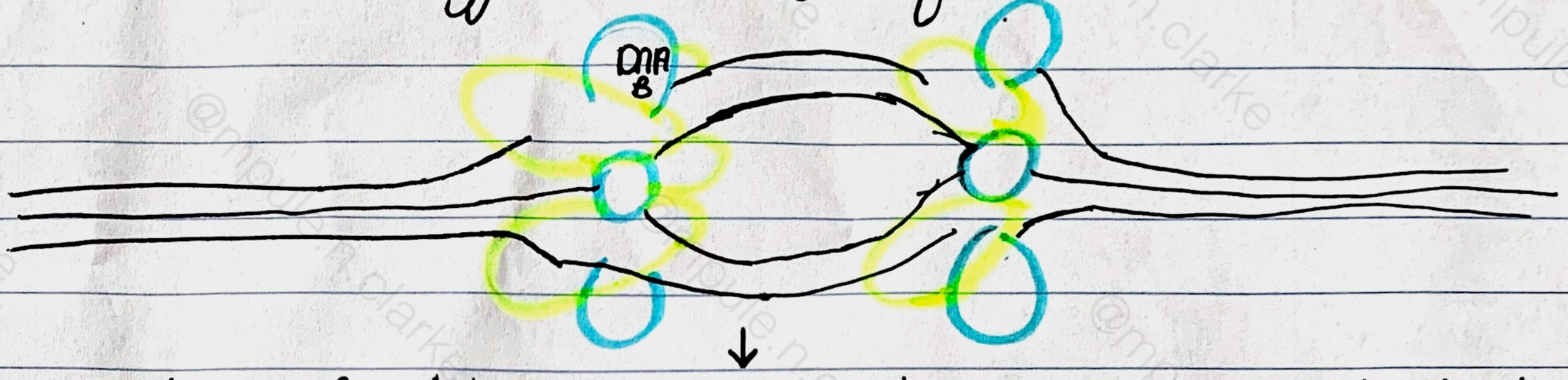
- $DnaA_C$ free in ~~cytoplasm~~ cytosol
- $DnaA_B$ free in ~~cytoplasm~~ cytosol enzyme to melt DNA by hydrolysis of ATP



AT rich regions for rapid strand melting ~~(bonds)~~; A ::: T

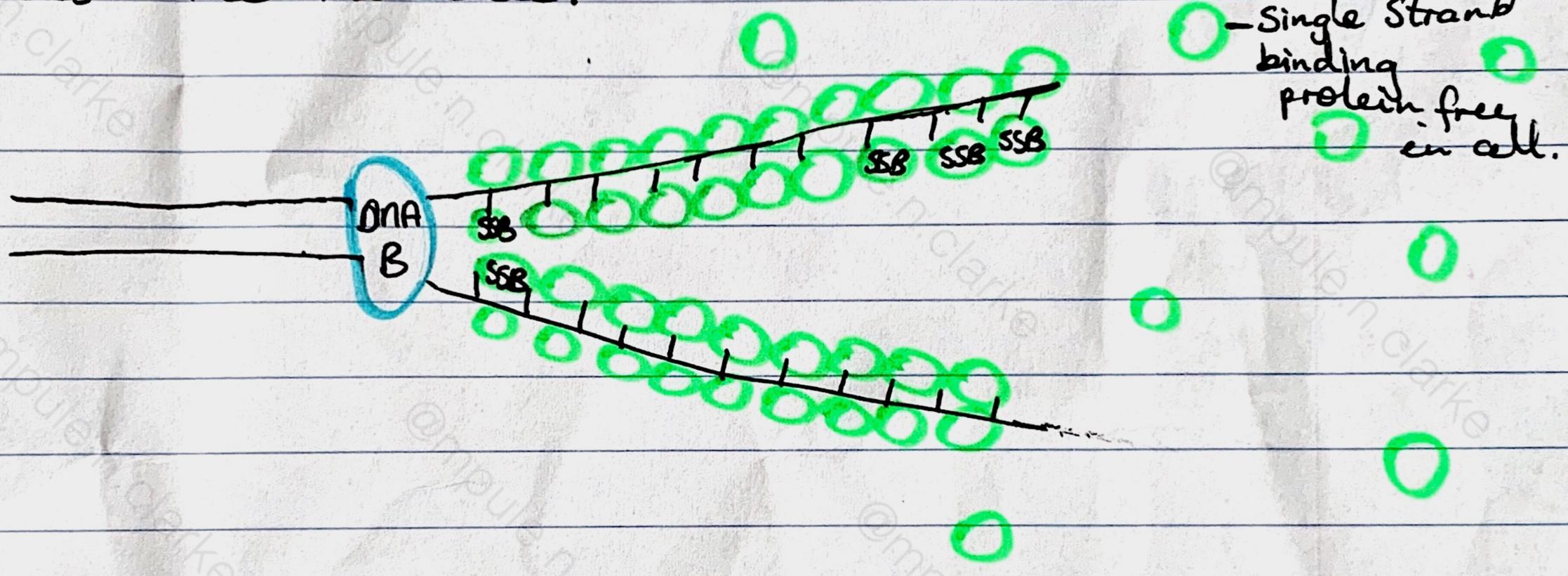
$DnaA_C$ loads/mounts $DnaA_B$ on to the strand at the AT rich origin of replication for DNA unwinding to be catalyzed by the DNAB at the core of the "helicase" hexamer. this happens in duplicate so bioectional replication

on the one chromosomal DNA circular strand occurs for optimal efficency of cell operations. ⇒ reactions tend to choose the most efficient route if resources allow.

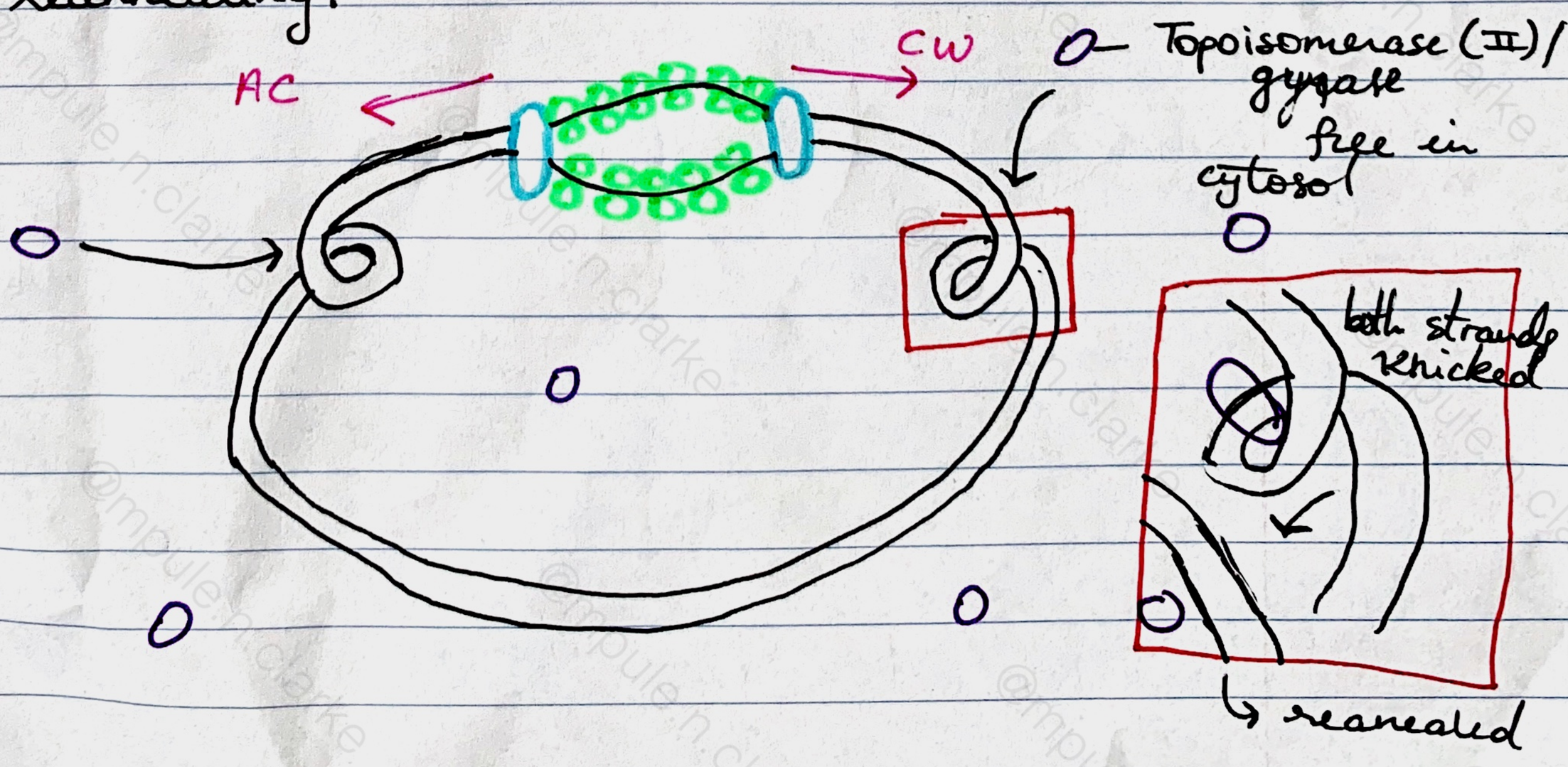


↓

A replication bubble occurs with two "forks" that move a long the strand as the DNA_{B+C} complex / helicase unwinds the molecule.



SSBs bind to the new single strands to prevent them from reannealing.



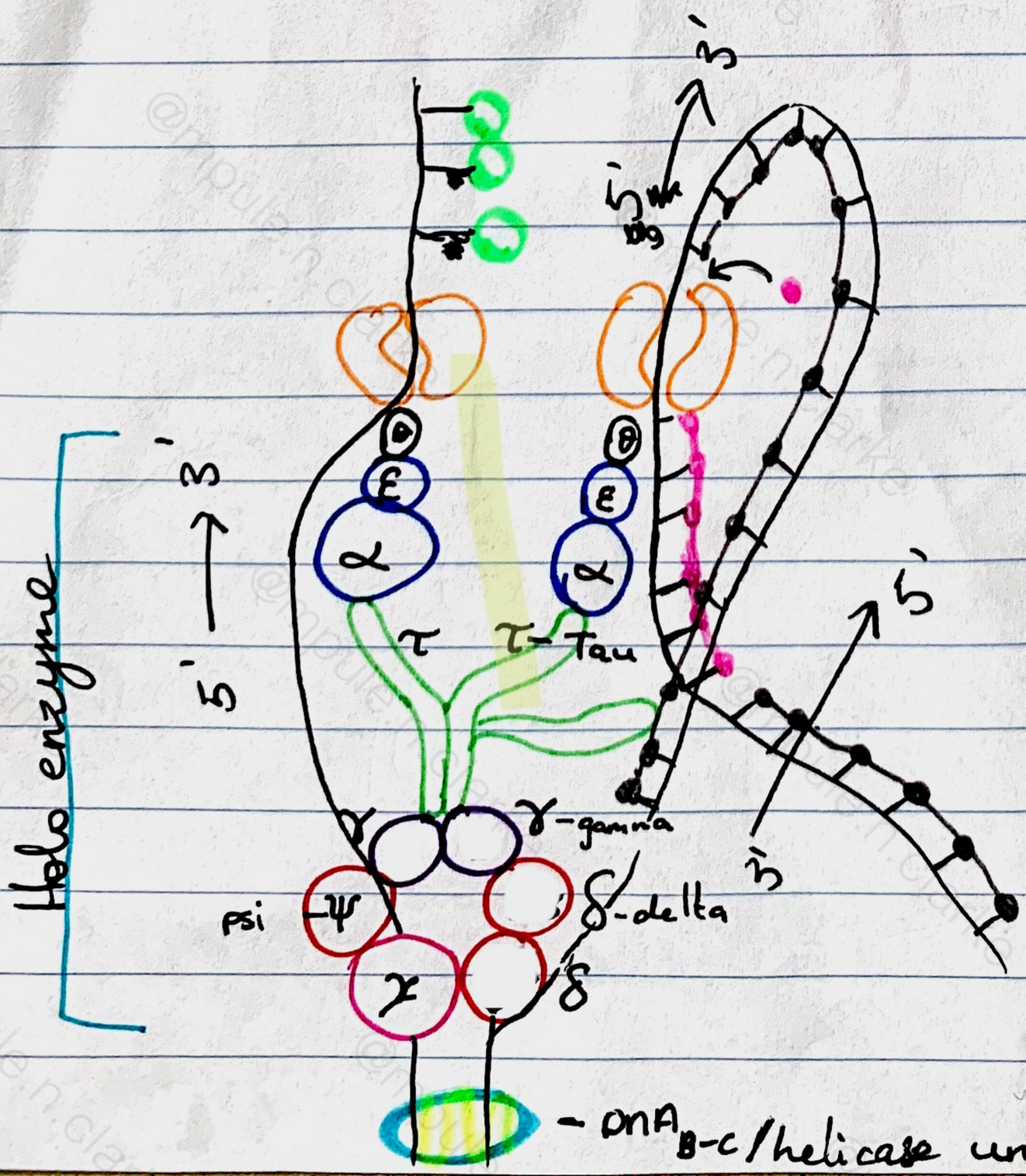
When the replication bubble is at an appropriate lenght RNA primers replace the SSB for DNA ~~&~~ polymerase III to attach and elongate the strand, By catalysis with DNAG

for reference :!!

DNA pol III has a higher processity than DNA pol I

recall : • processivity is the rate of addition of nucleotides before the polymer dissociates / polymerization ends.

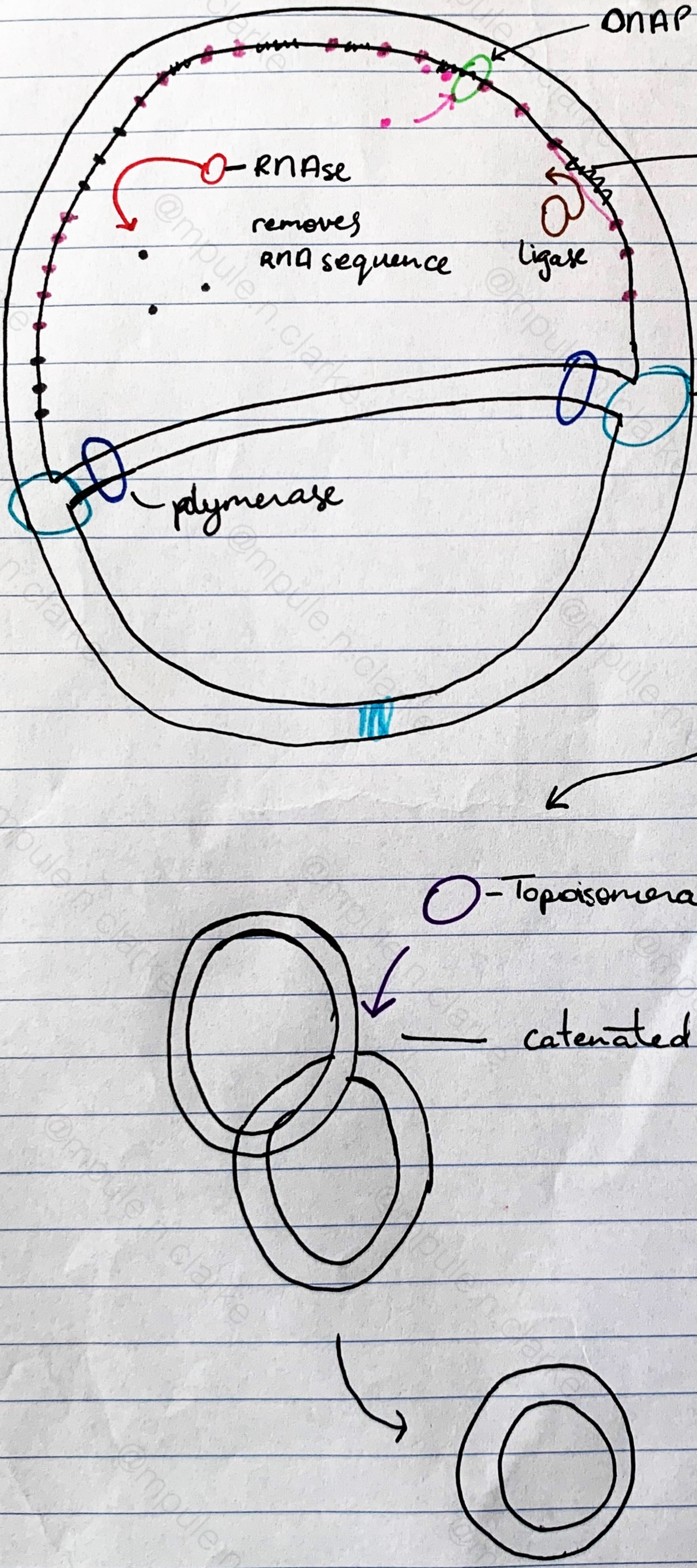
- cannot initiate base pairing (not a de novo process)
- polymerization occurs 5' → 3'
- requires RNA primers
- exonudease
- processivity is specific for the rate of H bonds per base => A on the template base paired with a C added in error will slow down the enzyme and intitiate proofreading ~~&~~; removal of the wrong base by the exonuclease 3' → 5' ~~activity~~ activity & replace with the correct nucleotide



- DNA B-C / helicase unwinding

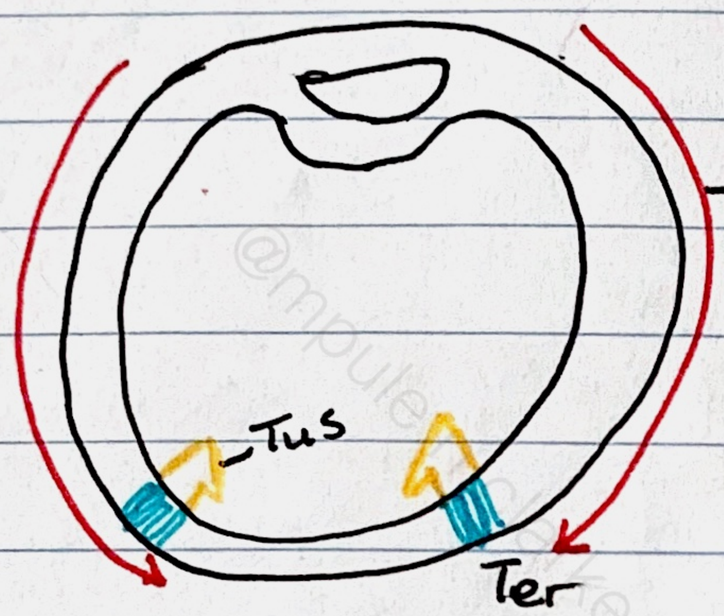
● - RNA primer RNAse removes RNA leaving Okazaki fragments

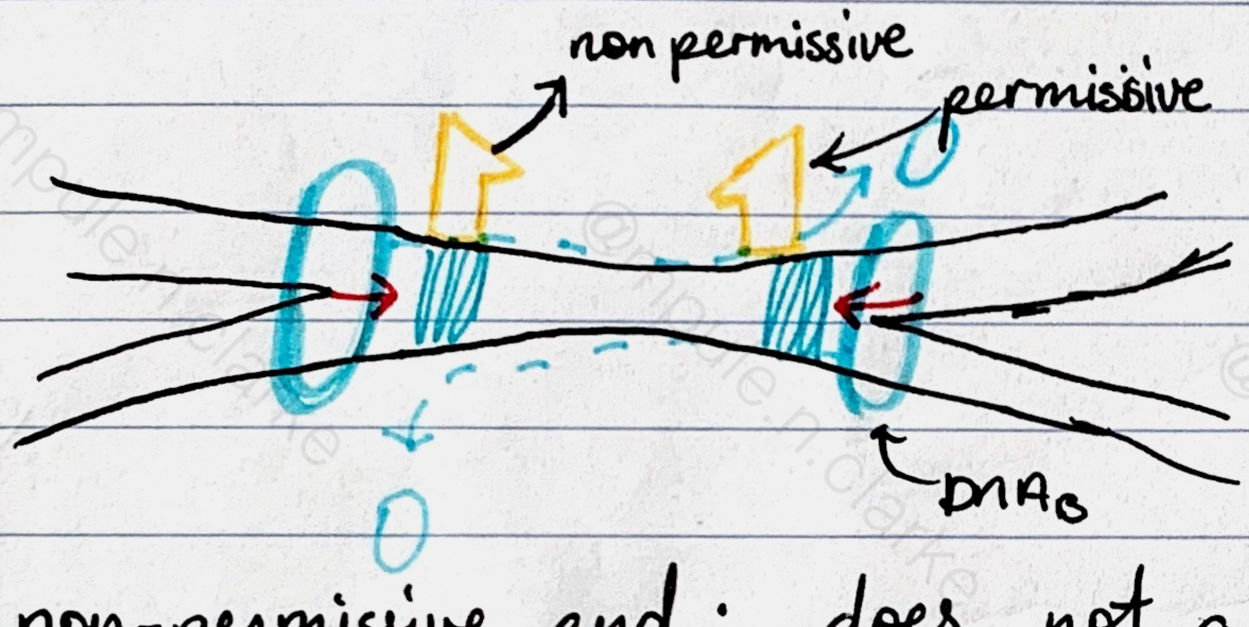
DNA polymerase I fills okazaki fragments



⇒ mitosis ~~ends~~ continues

Termination:





non-permissive end: does not allow replication fork to pass (DNA B does not pass) ⇒ dissasociates helicase from strands & catenated chromosomes occur.

DNA replication in E. coli

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- a summary

When the cell is ready to replicate its genetic material:

- factors for intiation, elongation and termination are transcribed

Initiation

- DNA A associates with the four 9mer ~~RKS~~ sequences in the OriC region. This causes a strain on the circular molecule ~~re~~ resulting in -ve supercoiling. DNA must be melted at this point to relieve the stress on the molecule.

- DNA_B binds to the ~~origin of~~ three 13mer sequences, A-T rich regions in the Origin of replication (Ori), assisted by DNA_C. DNA_B has helicase like properties which melt DNA.

- As the DNA melts a bubble is formed within the strand, this is called the replication bubble. The replication bubble forms two forks at each end known as replication forks.

- The exposed DNA strands attract single strand binding proteins (SSB) to prevent premature ~~ass~~ reannealing of the DNA.

+ve ~~+ve~~

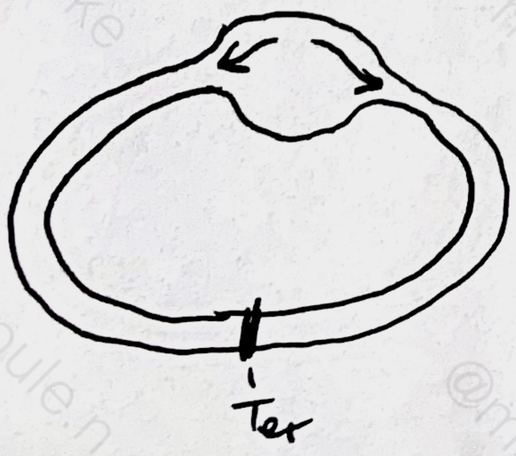
- Super coils are created in the strand & Topoisomerase II / gyrase relieves the stress by providive -ve supercoils.

- When the replication bubble is at an appropiate lenght DNA_G adds RNA primers as DNAP III cannot sythesize DNA de novo. Elongation begins when DNAP III is loaded to the replication forks (this happens on both ends of the replication bubble.

During the Elongation stage of DNA replication in E. coli: 17

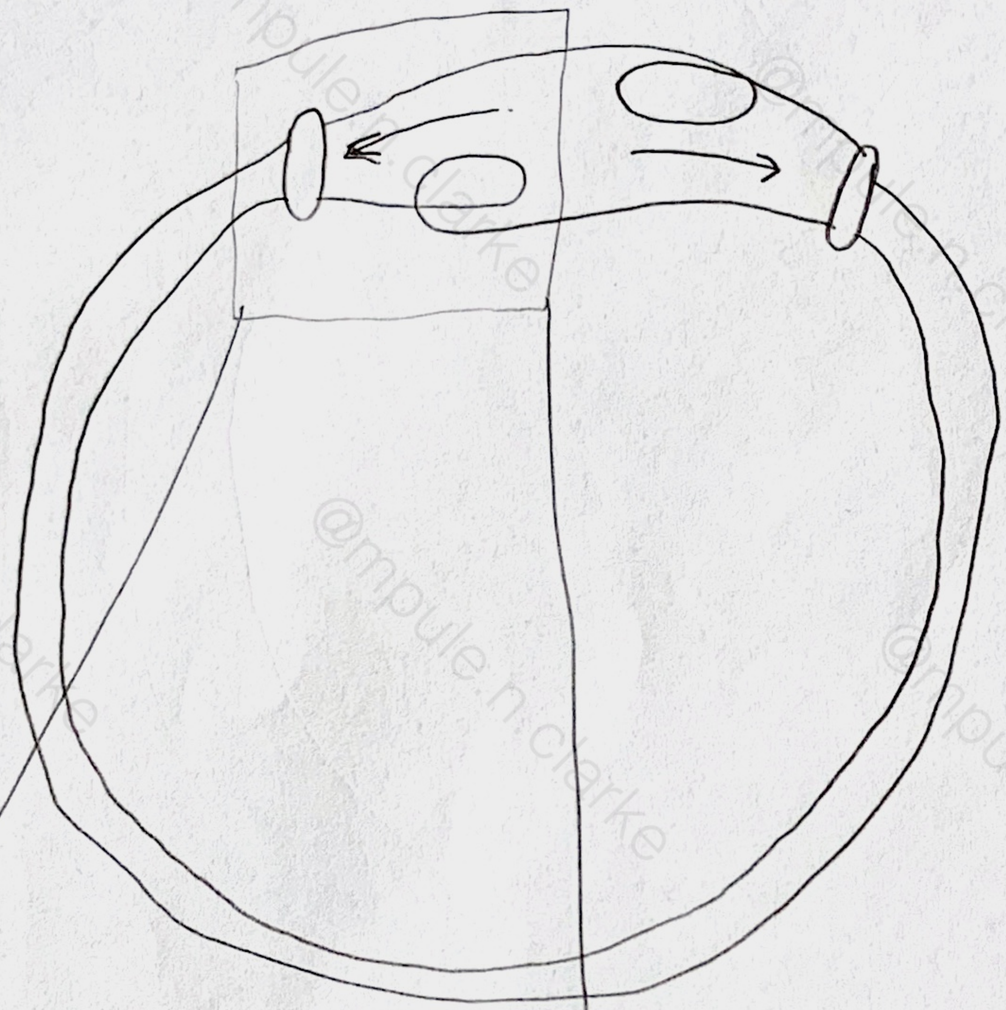
- DNAP III can only add dNTPs to the 3' end of the primer and as such one strand will have a mechanism of elongation that lags behind the other as its 5' end is what will be exposed moving away from replication fork. DNAP III continuously moves along the ~~strand~~ 3' to 5' strand as the 5' end of the nearest monomer in the elongating polymer is the 3' end.

- The lagging 5' to 3' strand has the 5' end of the nearest ~~###~~ nucleotide facing it so the enzyme uses a separate mechanism for polymerization from the lagging strand template to occur.

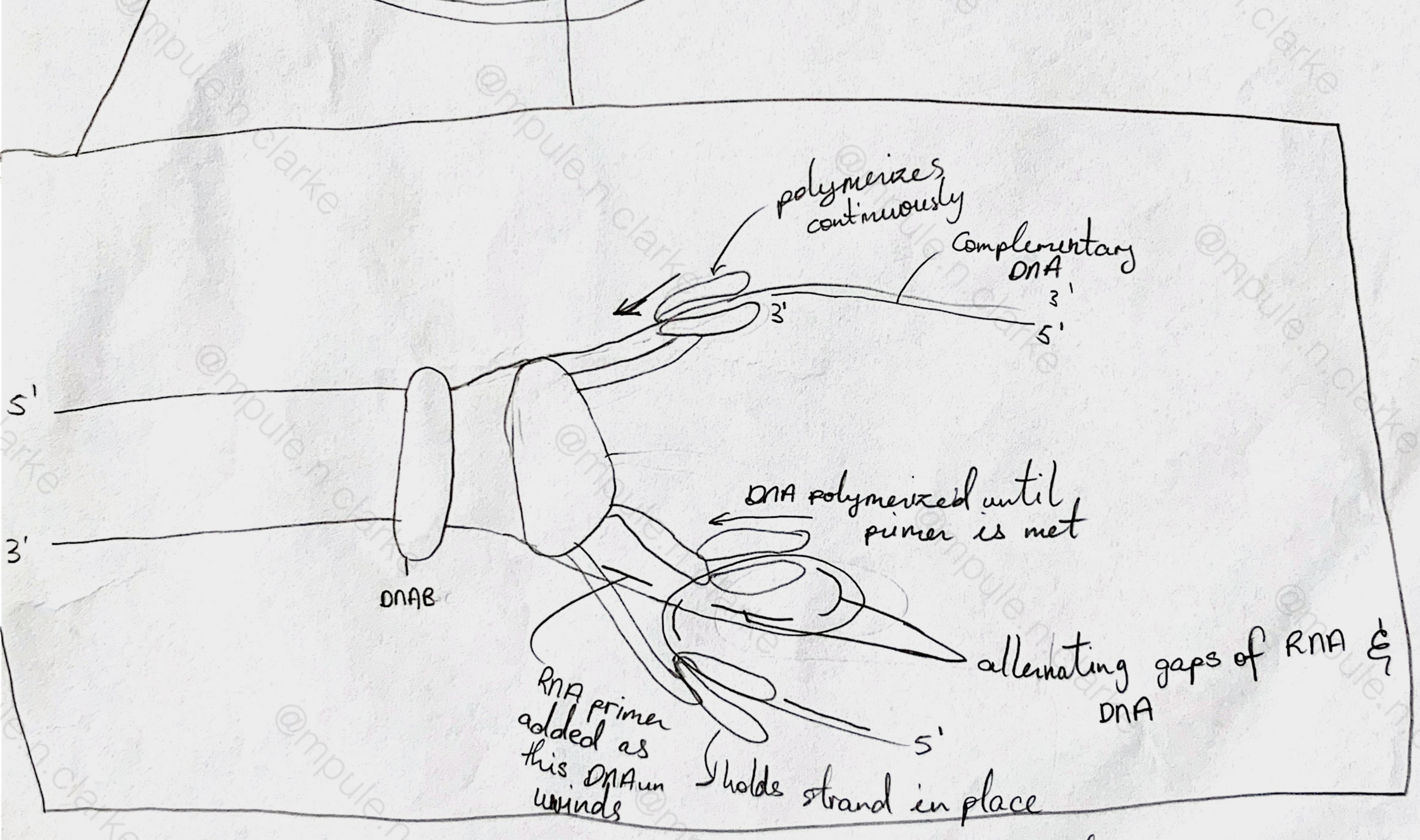


Tus proteins associate with termination sequences, dissociating both RNAP III structures from the DNA.

DNAP III Bidirectional elongation mechanism.



⇒ 5'→3' parent strands replicate continuously. 3'→5' strand lags until it is long enough for DNAP III to loop it.

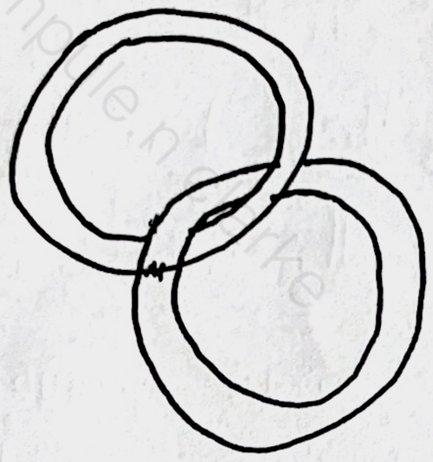


⇒ RNAse removes the RNA primers; gap present on the complement of the 3'→5' parent strand.

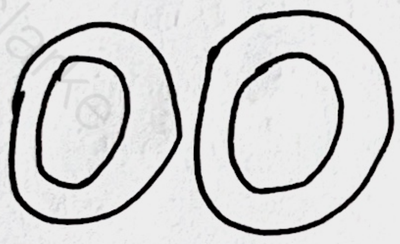
⇒ DNAP I fills the gaps with dNTP; Knicks are now present on the complement of the 3'→5' parent strand. These fragments of DNA are Okazaki fragments

⇒ Ligase ~~&~~ catalyses the bonding of the ~~Rim~~ Knicks in the back bone.

Terminated DNA molecules now exist in a catenated state



Topoisomerase I ~~cleaves~~ splits up the catenated molecules to result in two free ~~circles~~ circular DNA

 - two sister chromosomes present at the end of bidirectional replication with DNA pol III