DNA Replication

Biochemistry For Medics
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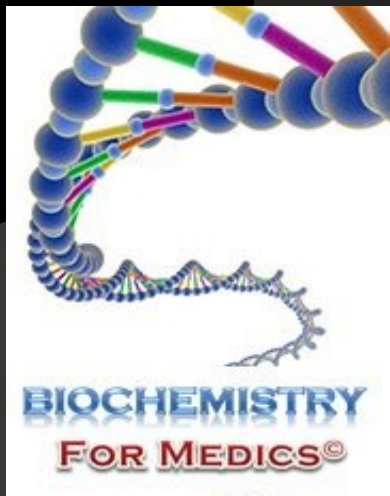
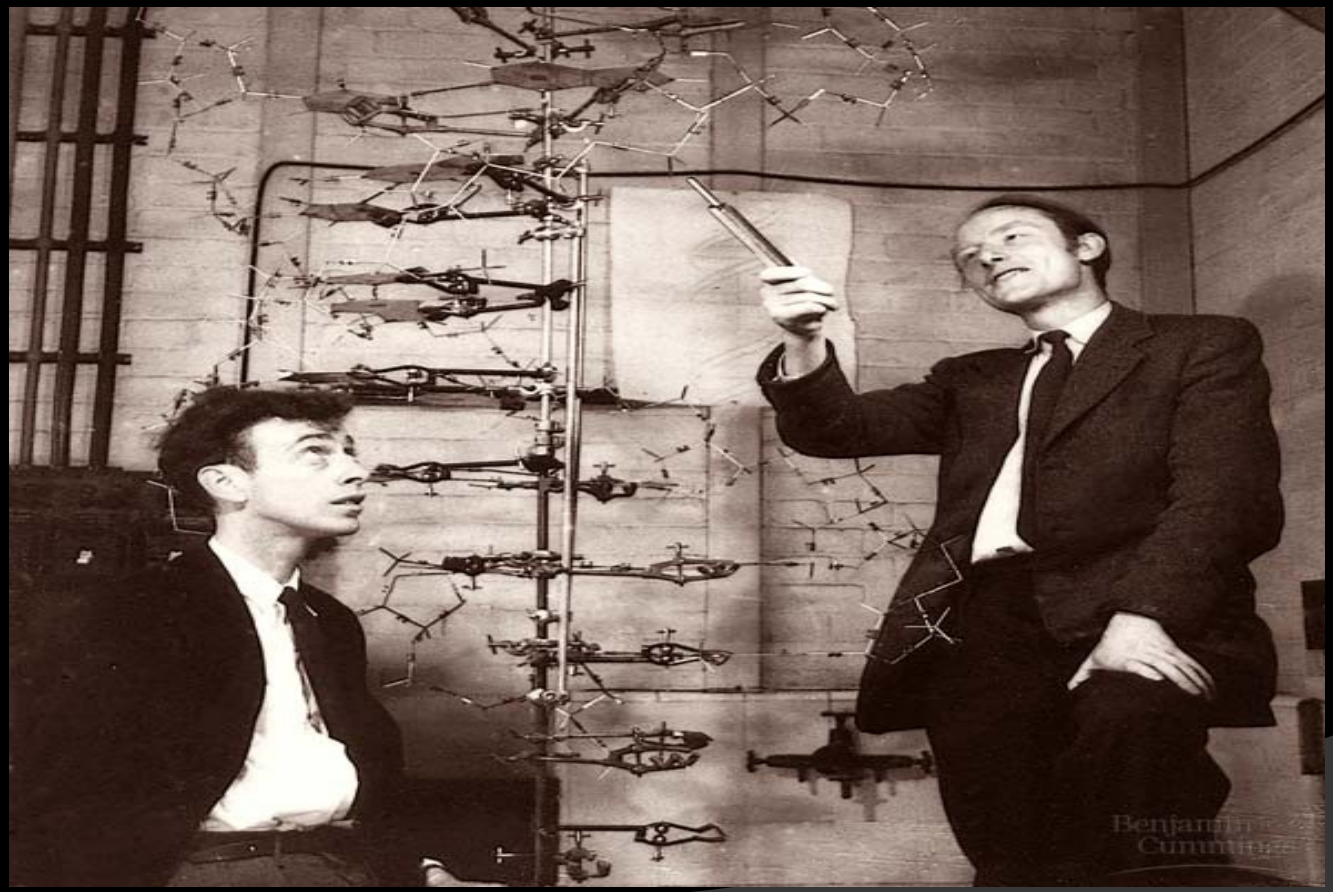
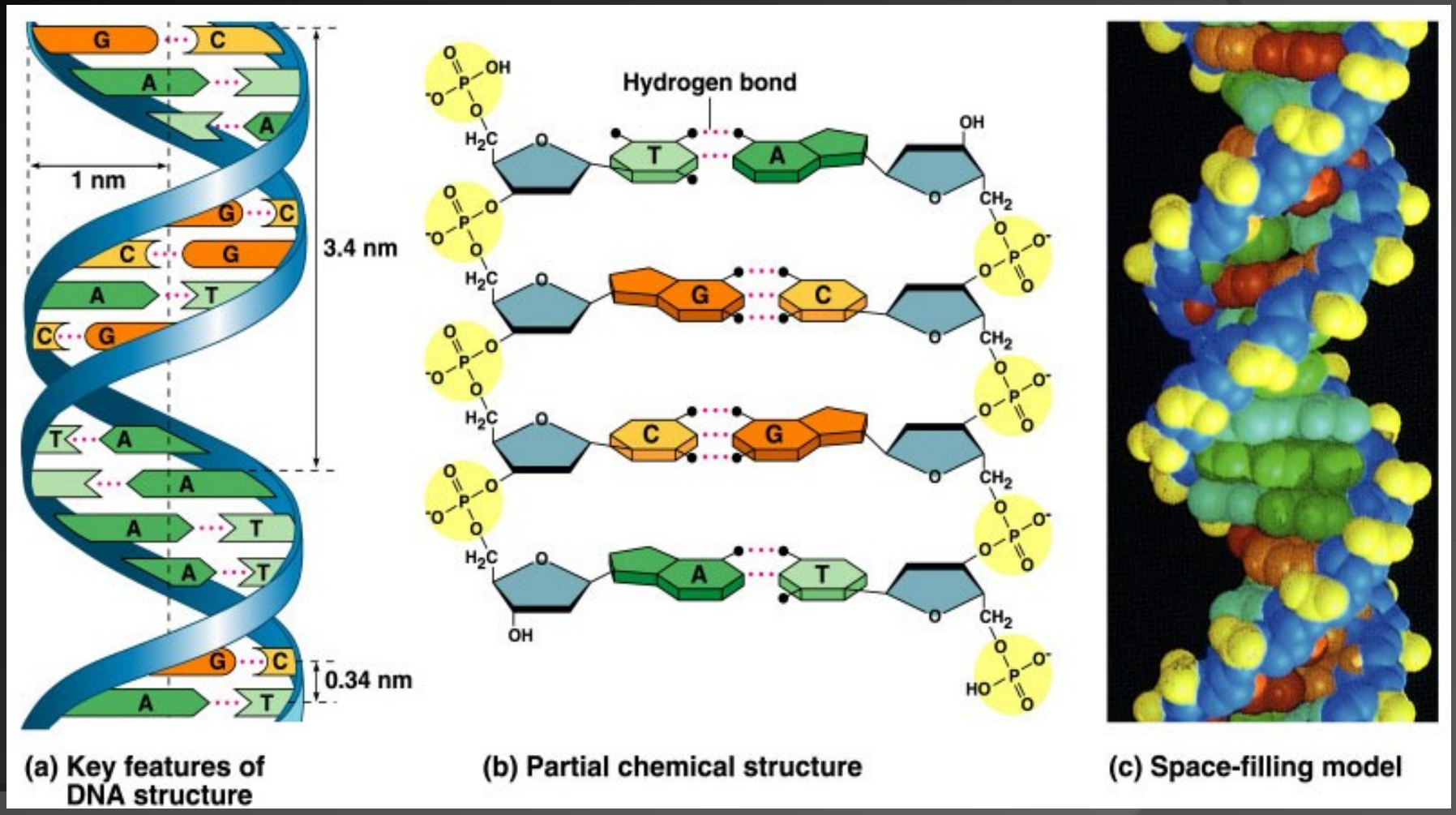


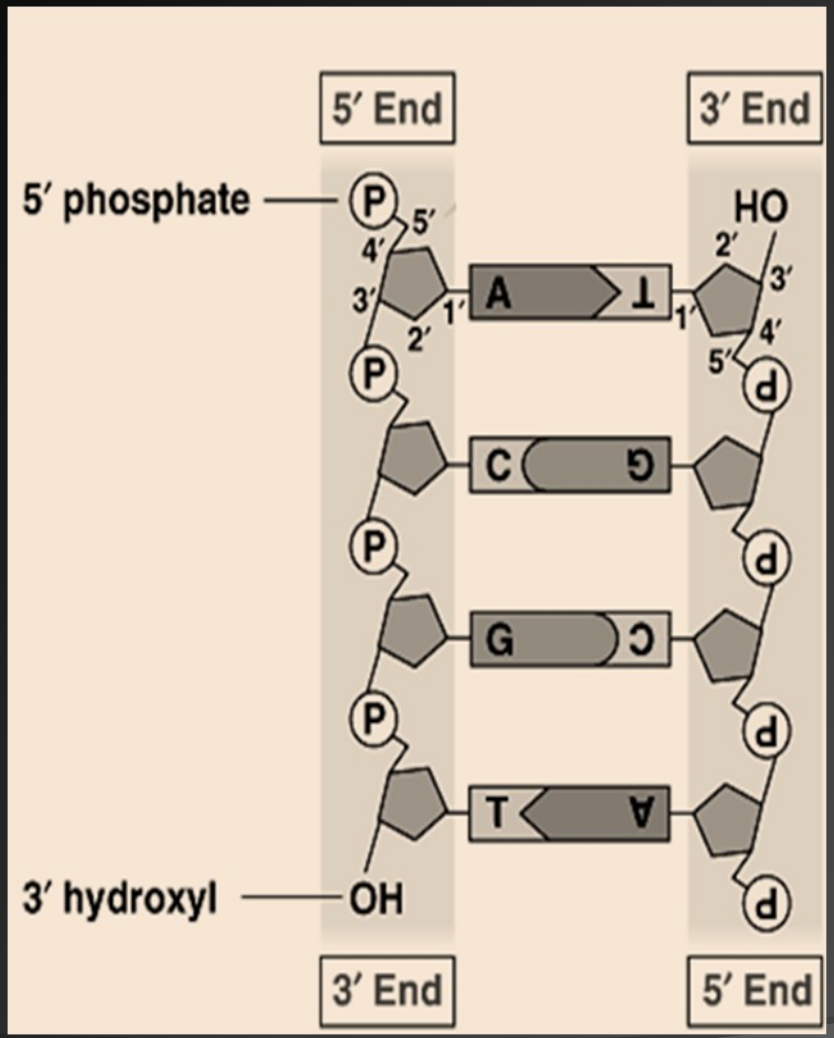
DNA – DOUBLE HELICALSTRUCTURE WATSON and CRICK- Model



DNA – DOUBLE HELICALSTRUCTURE



Directionality of DNA



Nucleotides in DNA backbone are bonded together by phosphodiester linkage between 3′ & 5′ carbons.

DNA molecule has “direction.“

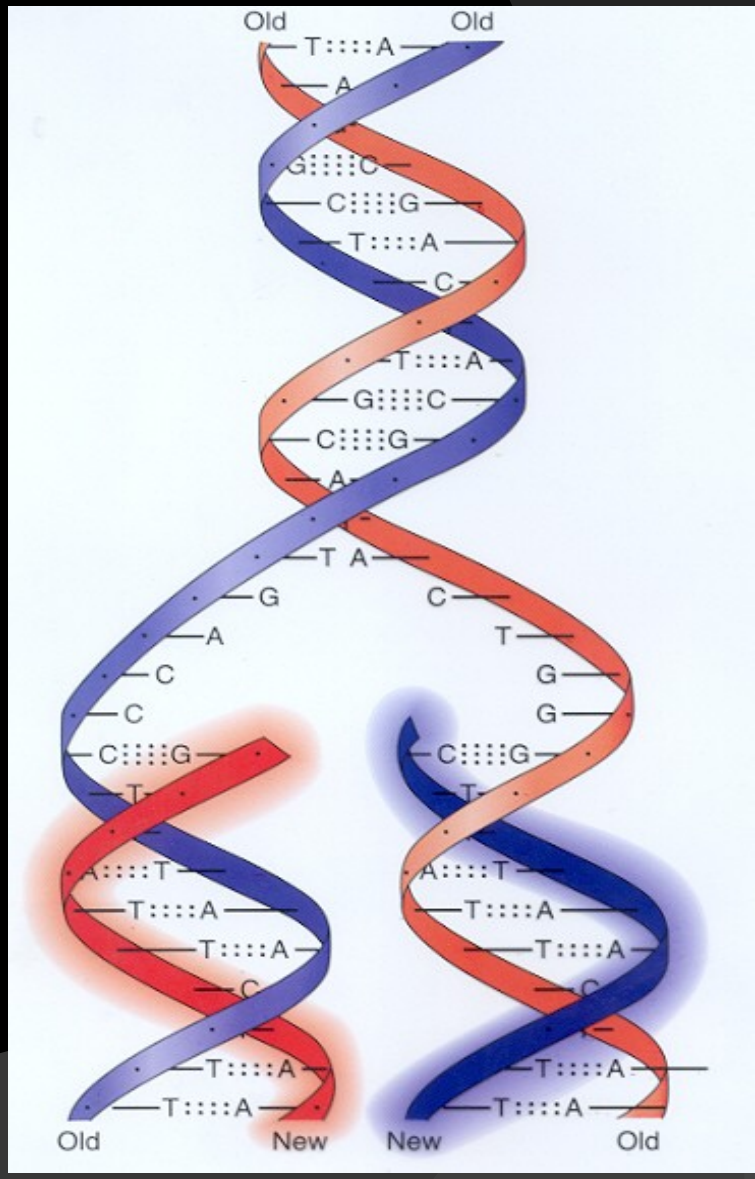
Complementary strands run in opposite directions.

DNA Replication- Introduction

- Basis for inheritance
- Fundamental process occurring in all cells for copying DNA to transfer the genetic information to daughter cells
- Each cell must replicate its DNA before division.

DNA Replication

- Semi conservative
- Parental strands are not degraded
  - Base pairing allows each strand to serve as a template for a new strand
  - New duplex is 1/2 parent template &
    1/2 new DNA



DNA Replication

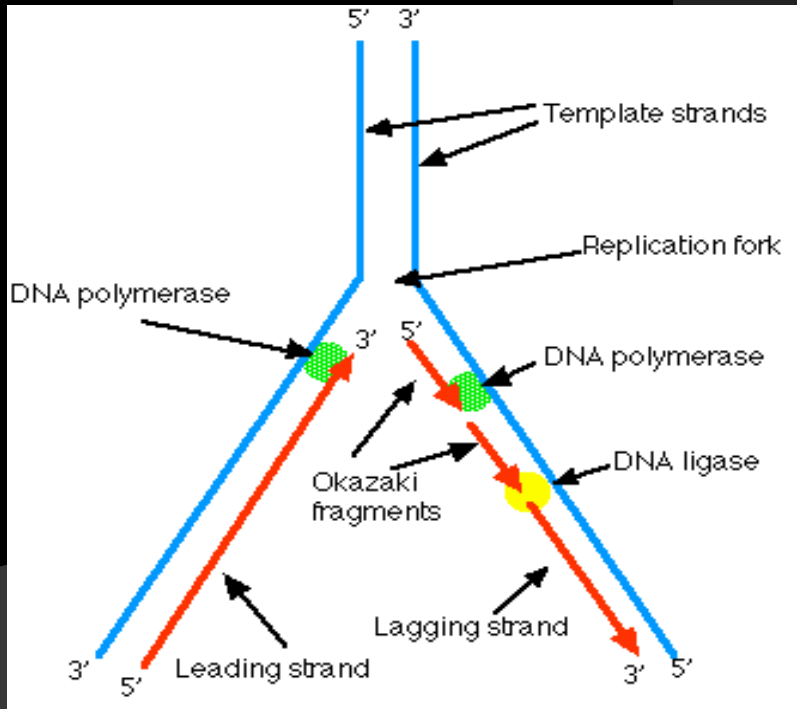
- Semi discontinuous
- Leading & Lagging strands

Leading strand

- **continuous synthesis**

Lagging strand

- **Okazaki fragments**
- **joined by ligases**



DNA Replication

- Energy of Replication
- The nucleotides arrive as
- nucleoside triphosphates
  - DNA base, sugar with PPP
    - P-P-P = energy for bonding
  - DNA bases arrive with their own energy source for bonding
  - bonded by enzyme: DNA polymerase III

DNA Replication

- Primer is needed
  - DNA polymerase can only add nucleotides to 3′ end of a growing DNA strand
    - need a “starter” nucleotide to make a bond
  - strand only grows 5′→3′.
  - Template is read in the 3′-5′ direction while polymerization takes place in the 5′→3′ direction

Primer

RNA primer

- Synthesized by Primase
- serves as a starter sequence for DNA polymerase III
- Only one RNA Primer-required for the leading strand
- RNA Primers for the lagging strand depend on the number of "OKAZAKI FRAGMENTS"
- RNA Primer has a free 3'OH group to which the first Nucleotide is bound.

DNA Replication-Steps

- Identification of the origins of replication
- Unwinding (denaturation) of dsDNA to provide an ssDNA template
- Formation of the replication fork
- Initiation of DNA synthesis and elongation
- Primer removal and ligation of the newly synthesized DNA segments
- Reconstitution of chromatin structure

Components of Replication

- DNA polymerases- Deoxynucleotide polymerization
- Helicase -Processive unwinding of DNA
- Topoisomerases Relieve torsional strain that results from helicase-induced unwinding
- RNA primase Initiates synthesis of RNA primers
- Single-strand binding proteins Prevent premature reannealing of dsDNA
- DNA ligase Seals the single strand nick between the nascent chain and Okazaki fragments on lagging strand

Origin of Replication- Prokaryotes

- At the origin of replication **(ori),** there is an association of sequence-specific dsDNA-binding proteins with a series of direct repeat DNA sequences.
- In *E coli,* the oriC is bound by the protein dnaA.
- a complex is formed consisting of 150–250 bp of DNA and multimers of the DNA-binding protein. This leads to the local denaturation and unwinding of an adjacent A+T-rich region of DNA.

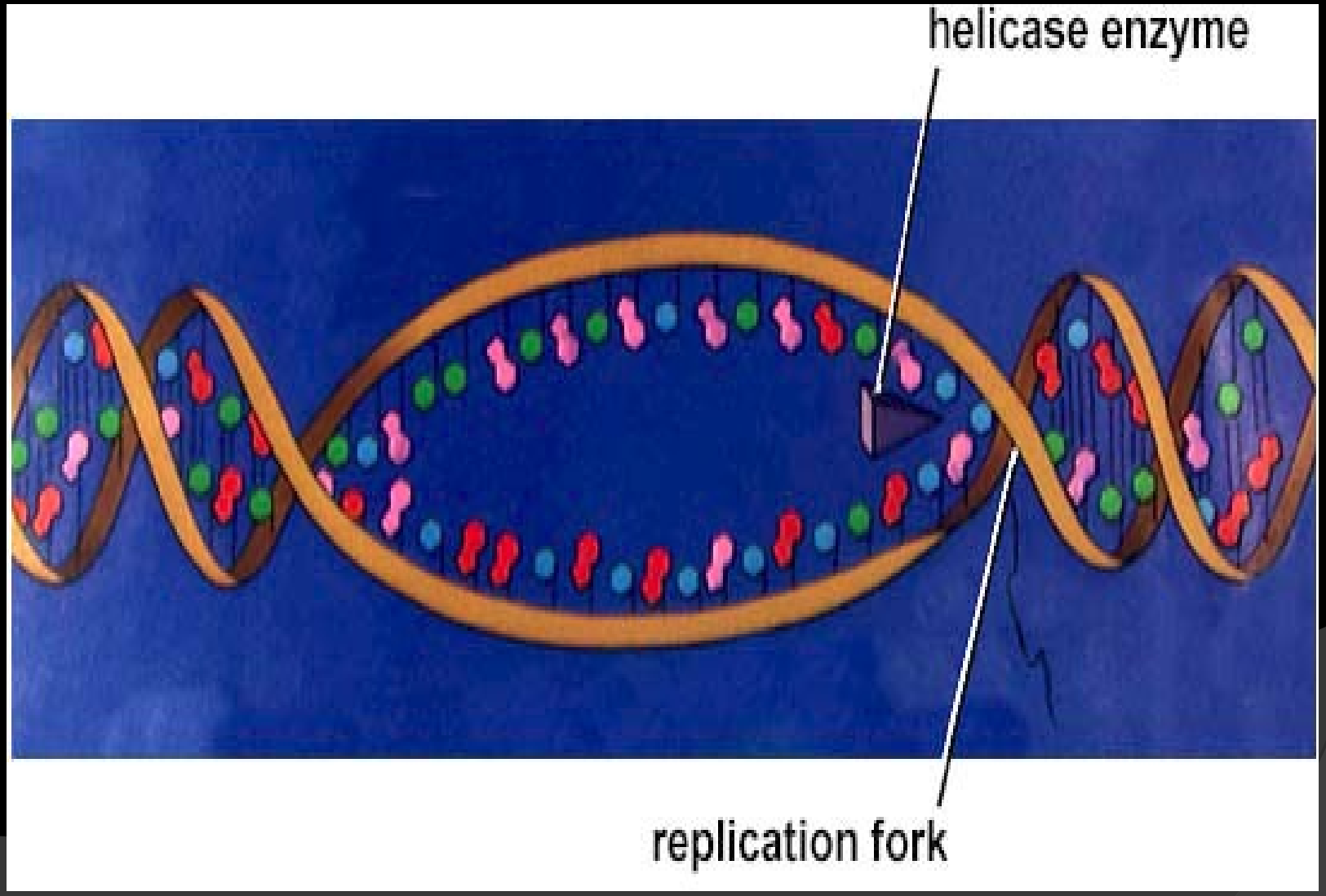
Origin of Replication -Eukaryotes

- Functionally similar **autonomously replicating sequences (ARS) or replicators** have been identified in yeast cells.
- The ARS contains a somewhat degenerate 11-bp sequence called the **origin replication element (ORE).**
- The ORE binds a set of proteins, analogous to the dnaA protein of *E coli,* which is collectively called the **origin recognition complex (ORC).**
- The ORE is located adjacent to an approximately 80-bp A+T-rich sequence that is easy to unwind. This is called the DNA unwinding element (DUE**).**

Unwinding of DNA

- The interaction of proteins with ori defines the start site of replication and provides a short region of ssDNA essential for initiation of synthesis of the nascent DNA strand.
- DNA Helicase allows for processive unwinding of DNA.
- Single-stranded DNA-binding proteins (SSBs) stabilize this complex.
- In cooperation with SSB, this leads to DNA unwinding and active replication.

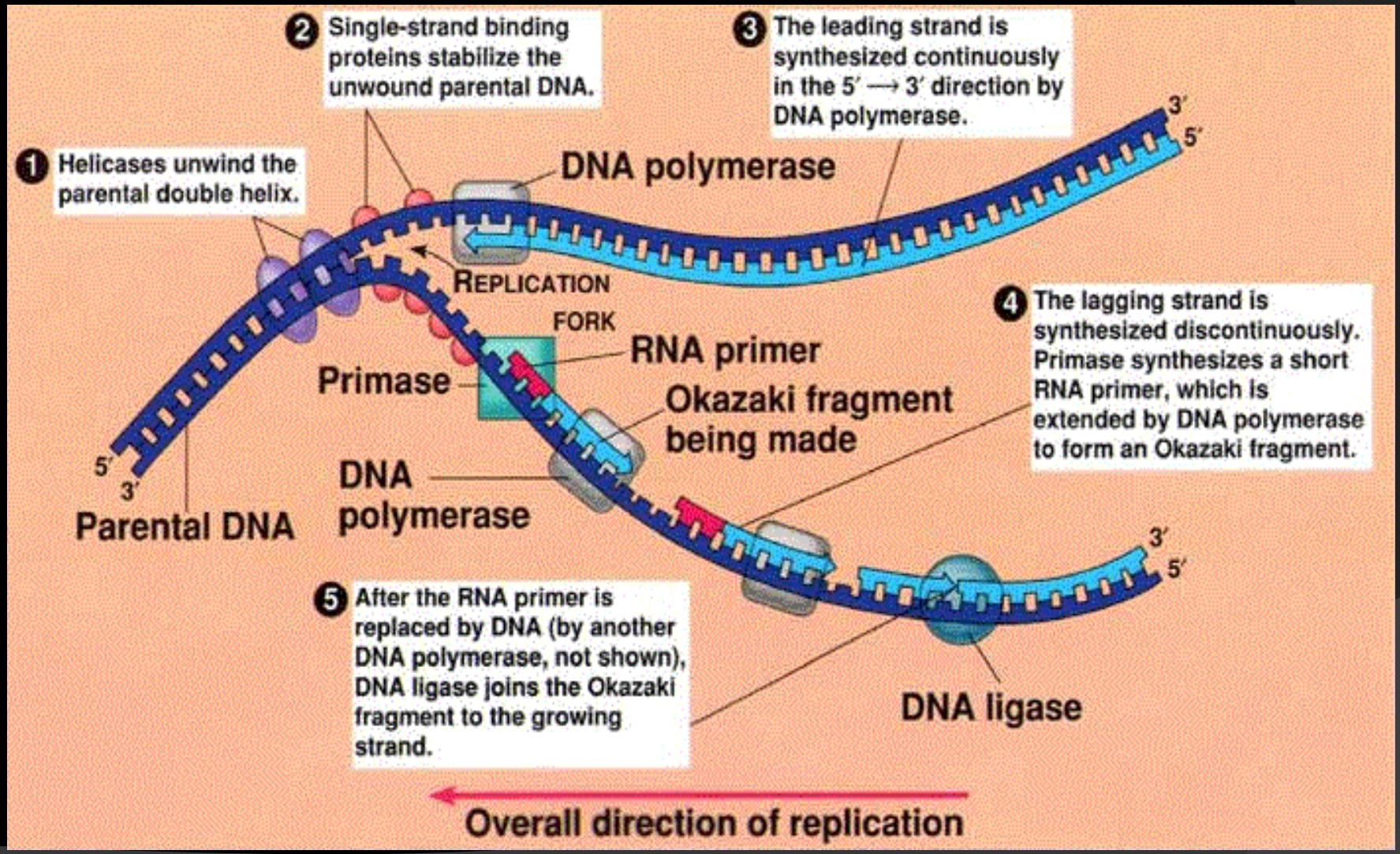
Unwinding of DNA



Formation of the Replication Fork

- The polymerase III holoenzyme binds to template DNA as part of a multiprotein complex
- DNA polymerases only synthesize DNA in the 5' to 3' direction,
- Because the DNA strands are antiparallel , the polymerase functions asymmetrically.
- On the leading (forward) strand, the DNA is synthesized continuously.
- On the lagging (retrograde) strand, the DNA is synthesized in short (1–5 kb)fragments, the so-called Okazaki fragments.

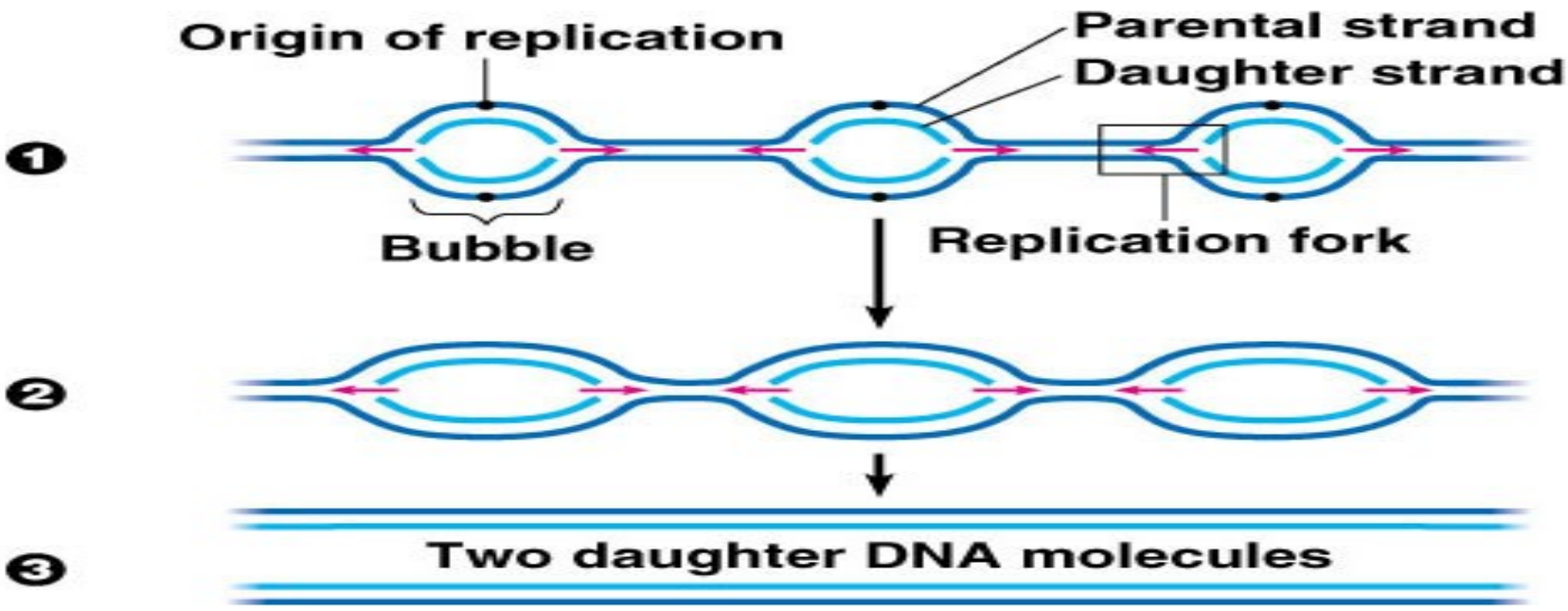
Replication Fork



Formation of Replication Bubbles

- Replication occurs in both directions along the length of DNA and both strands are replicated simultaneously.
- This replication process generates **"replication bubbles"**

Replication Bubbles



(a) In eukaryotes, DNA replication begins at many sites along the giant DNA molecule of each chromosome.

The DNA Polymerase Complex

- A number of different DNA polymerase molecules engage in DNA replication. These share three important properties: (1) **chain elongation,** (2) **Processivity,** and (3) **proofreading.**
- Chain elongation accounts for the rate (in nucleotides per second) at which polymerization occurs.
- Processivity is an expression of the number of nucleotides added to the nascent chain before the polymerase disengages from the template.
- The proofreading function identifies copying errors and corrects them

DNA Polymerase Complex

- In *E coli,* polymerase III (pol III) functions at the replication fork. Of all polymerases, it catalyzes the highest rate of chain elongation and is the most processive.
- Polymerase II (pol II) is mostly involved in proofreading and DNA repair.
- Polymerase I (pol I) completes chain synthesis between Okazaki fragments on the lagging strand.

Differences between DNA Polymerase I, II and III

	DNA pol I	DNA pol II	DNA pol III
Polymerization Rate	Low	Low	High
Processivity	Low	Low	High
Proof reading	3'-5' and 5'-3' Exonuclease activities	3'-5' Exonuclease activity	3'-5' Exonuclease activity
Primer removal	Best	Nil	Nil
Strand synthesis	Lagging strand	No role	Both strands
DNA repair	Active	Active	No role

Eukaryotic DNA polymerases

- Eukaryotic cells have counterparts for each of these enzymes plus some additional ones. A comparison is shown in Table-

E coli	Mammalian	Function
I	Alpha	Gap filling and synthesis of lagging strand
II	Epsilon	DNA proofreading and repair
	βeta	DNA repair
	Gamma	Mitochondrial DNA synthesis
III	delta	Processive , Leading strand synthesis

Initiation & Elongation of DNA Synthesis

- Primer-The priming process involves the nucleophilic attack by the 3'-hydroxyl group of the RNA primer on the phosphate of the first entering deoxynucleoside triphosphate with the splitting off of pyrophosphate.
- Mammalian DNA polymerase Alpha is mainly responsible for the synthesis of primer.

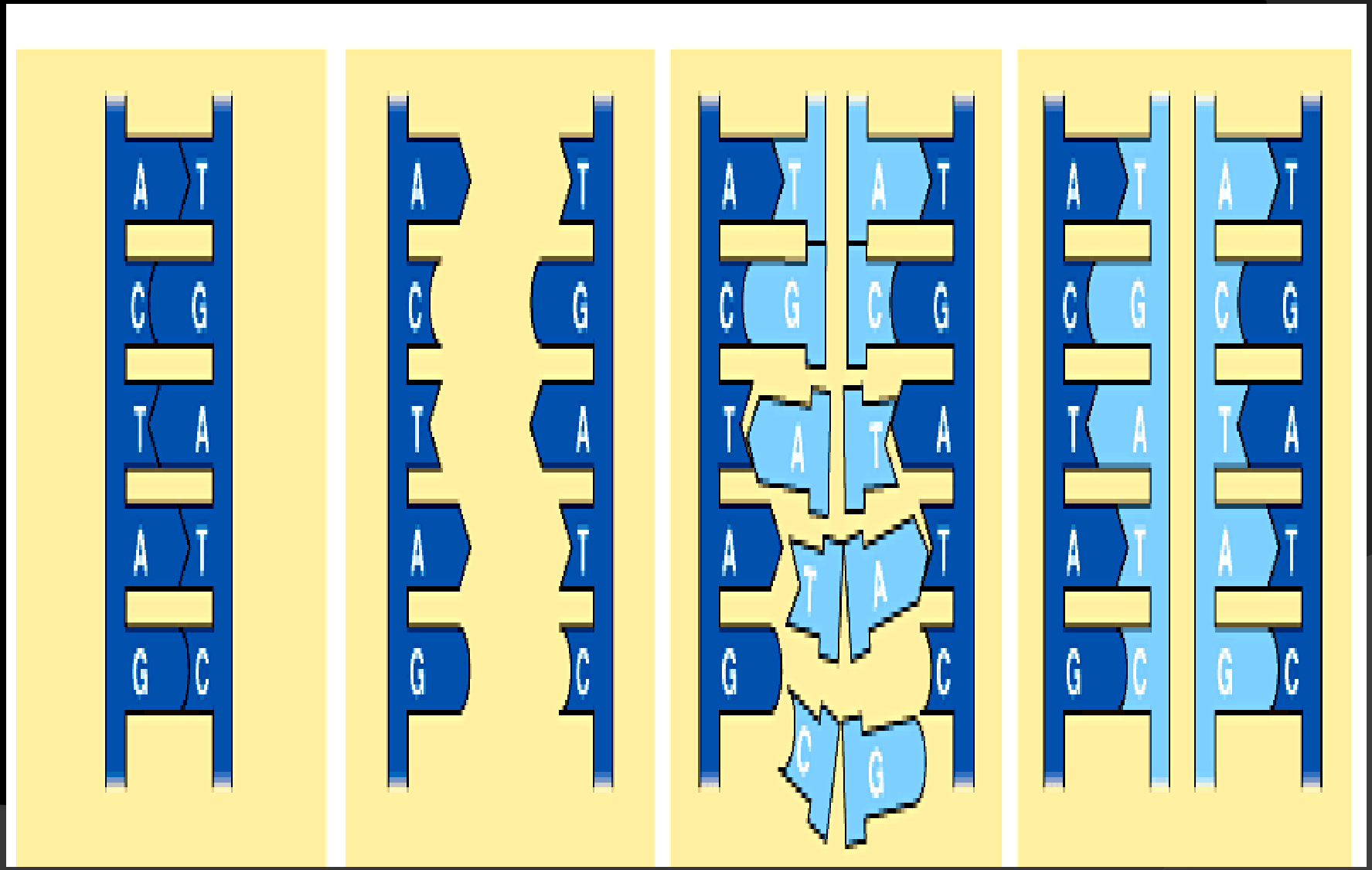
Initiation & Elongation of DNA Synthesis

- Selection of the proper deoxyribonucleotide whose terminal 3'-hydroxyl group is to be attacked is dependent upon **proper base pairing with the other strand** of the DNA molecule according to the rules proposed originally by Watson and Crick

Initiation & Elongation of DNA Synthesis

- When an adenine deoxyribonucleoside monophosphoryl moiety is in the template position, a thymidine triphosphate will enter and its phosphate will be attacked by the 3'-hydroxyl group of the deoxyribonucleoside monophosphoryl most recently added to the polymer.
- By this stepwise process, the template dictates which deoxyribonucleoside triphosphate is complementary and by hydrogen bonding holds it in place while the 3'-hydroxyl group of the growing strand attacks and incorporates the new nucleotide into the polymer.

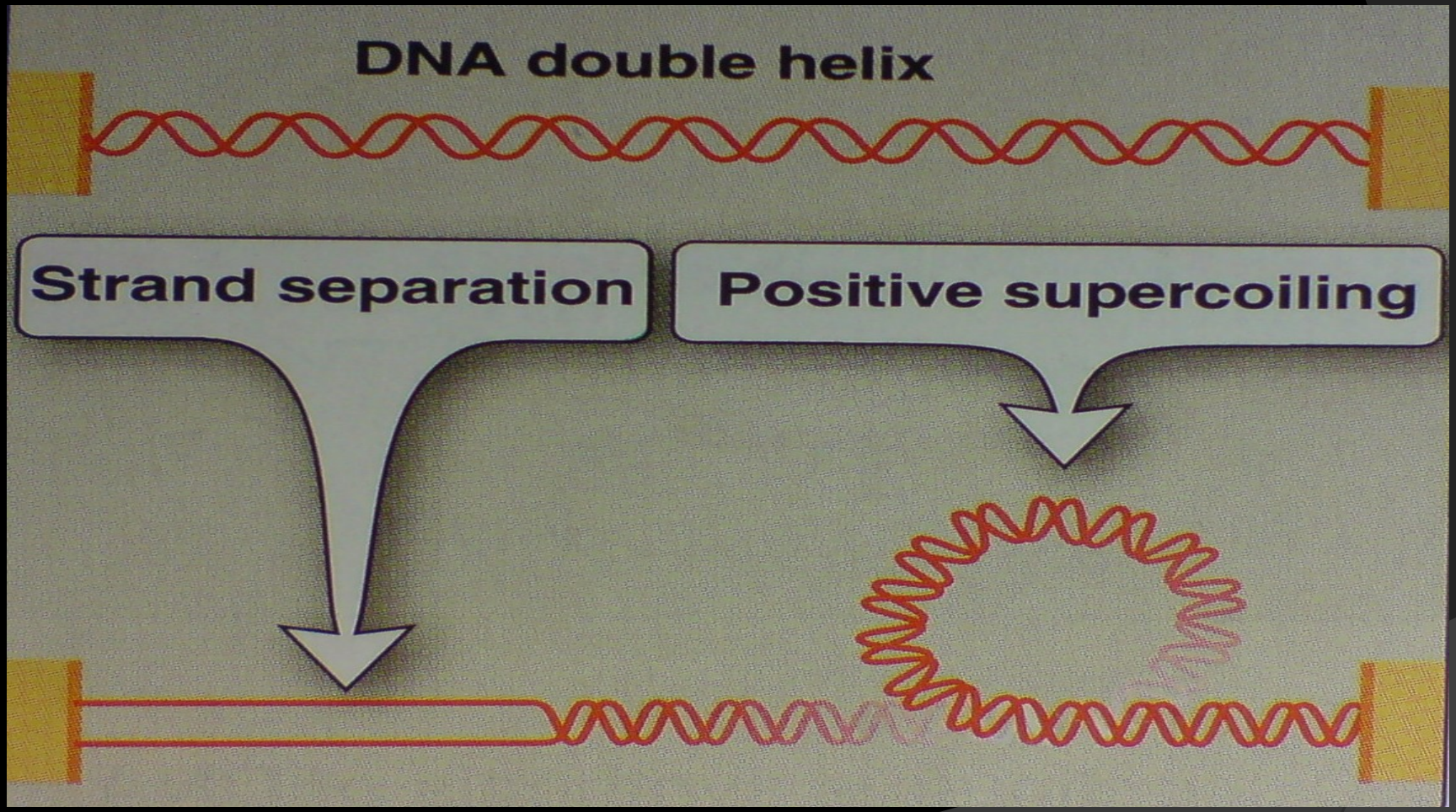
Base pairing in DNA Replication



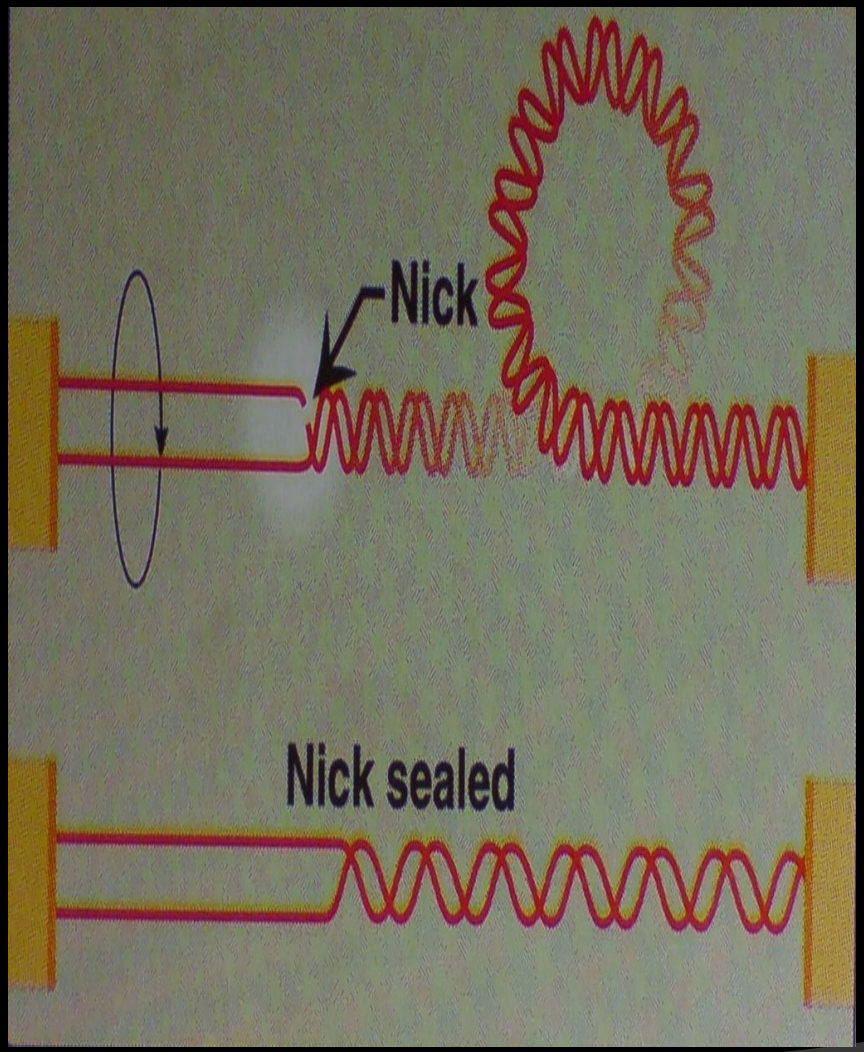
DNA Topo isomerases

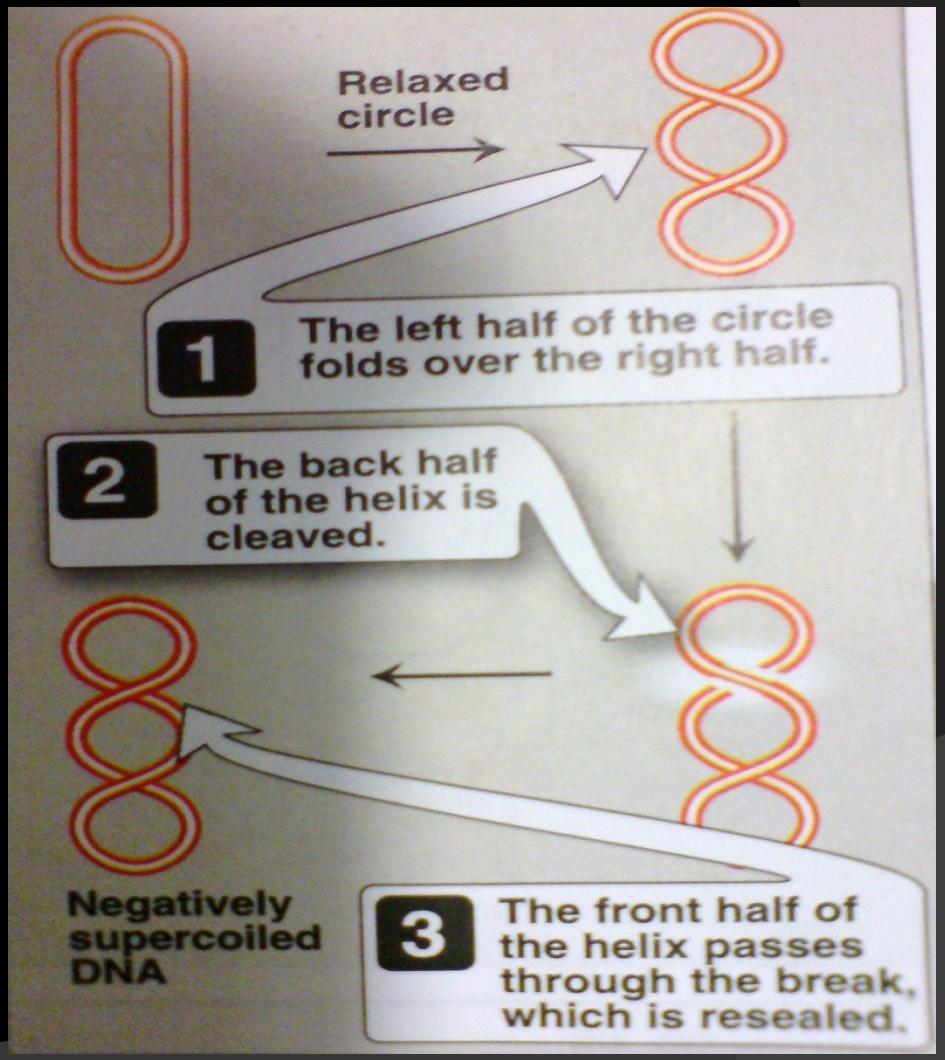
- Relief of super coils is dome by Topo isomerases
- Two types:
- **Topoisomerases I** : acts by making a transient single cut in the backbone of the DNA, enabling the strands to swivel around each other to remove the build-up of twists
- **Topoisomerase II** (DNA Gyrase) acts by introducing double standed breaks enabling one double-stranded DNA to pass through another, thereby removing knots and entanglements that can form within and between DNA molecules.

Formation of super coils



Mechanism of action of Topo isomerases(Type-I and Type-II)





Primer removal and Nick sealing

- Primers are removed by DNA polymerase I by replacing ribonucleotides with deoxy Ribonucleotides
- Nicks are sealed by DNA ligase
- Multiple primers on the Lagging strand while single primer on the leading strand.

Proof reading and Editing

- 1000 bases/second =
  lots of typos!
- DNA polymerase I
  - proofreads & corrects typos
  - repairs mismatched bases
  - removes abnormal bases
    - repairs damage
      throughout life
  - reduces error rate from
    1 in 10,000 to
    1 in 100 million bases

Termination of replication

- **In prokaryotes**:

  DNA replication terminates when replication forks reach specific "termination sites".

- the two replication forks meet each other on the opposite end of the parental circular DNA .

Termination of replication

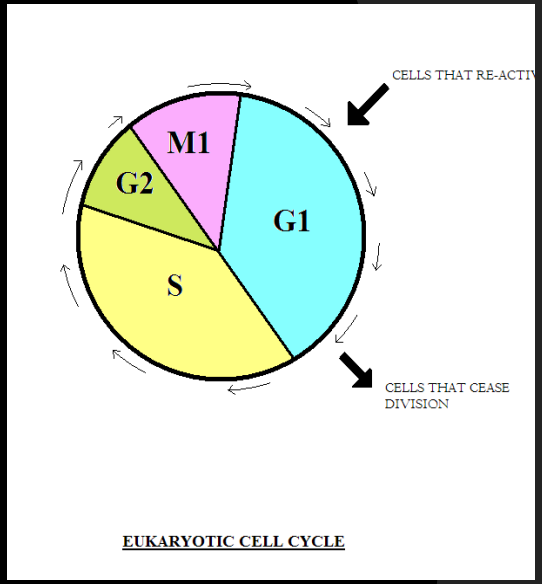
- This process is completed in about 30 minutes, a replication rate of 3 x 10^5 bp/min in prokaryotes
- The entire mammalian genome replicates in approximately 9 hours, the average period required for formation of a tetraploid genome from a diploid genome in a replicating cell.

Reconstitution of Chromatin Structure

- chromatin structure must be re-formed after replication. Newly replicated DNA is rapidly assembled into nucleosomes, and the preexisting and newly assembled histone octamers are randomly distributed to each arm of the replication fork.

DNA Synthesis and the Cell Cycle

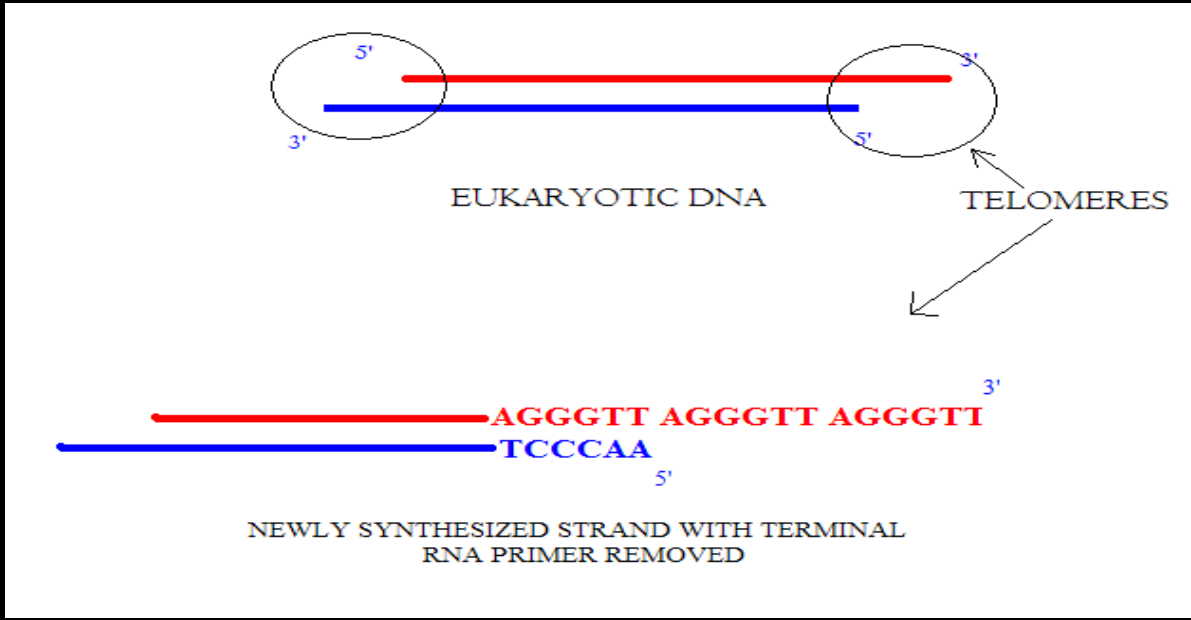
- In animal cells, including human cells, the replication of the DNA genome occurs only during the synthetic or S phase.
- This is usually temporally separated from the mitotic phase by non synthetic periods referred to as gap 1 (G1) and gap 2 (G2), occurring before and after the S phase, respectively
- The cell prepares for DNA synthesis in G1 and for mitosis in G2.



Telomeres

- In eukaryotic replication, following removal of RNA Primer from the 5'end of lagging strand; a gap is left.
- This gap exposes DNA strand to attack of 5' exonucleases.
- This problem is overcome by Telomerase.

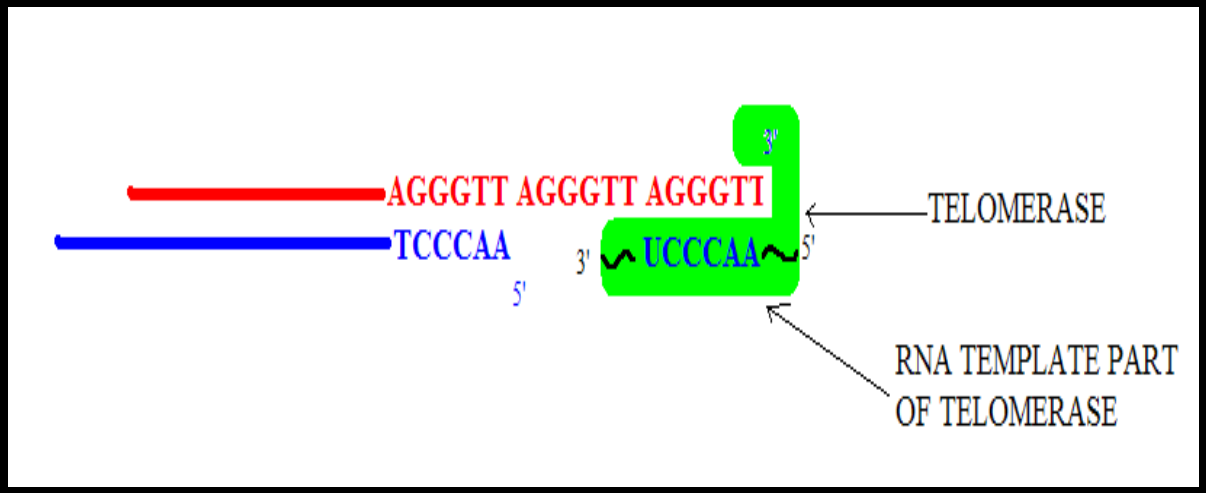
Telomeres



Repeating, non-coding sequences at the end of chromosomes = protective cap

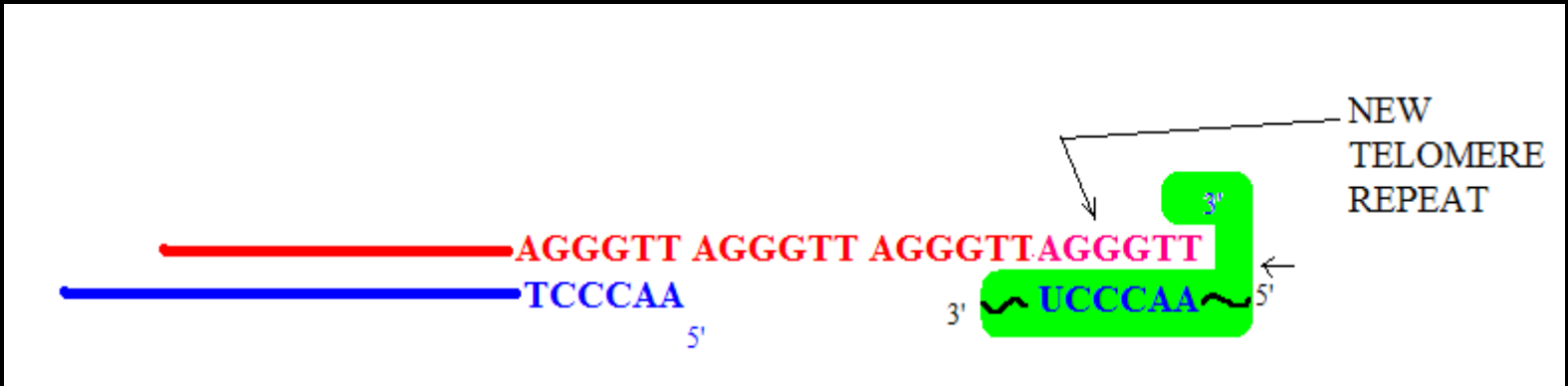
- **limit to ~50 cell divisions**

Mechanism of action of Telomerase



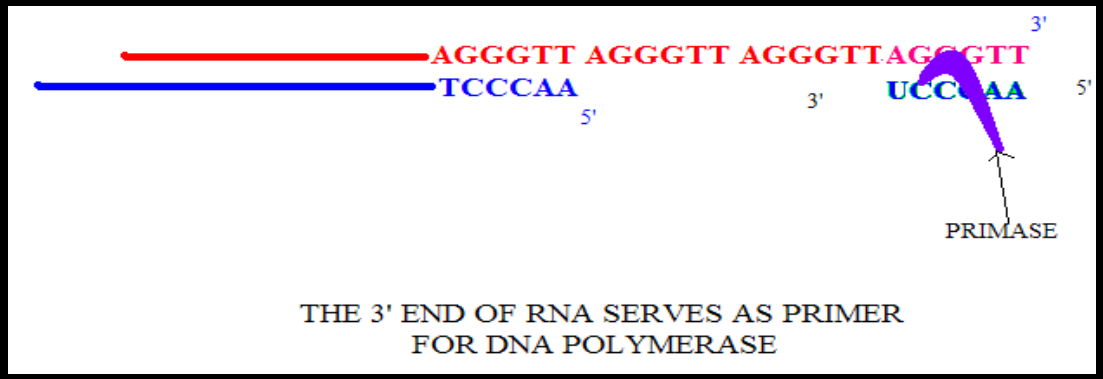
The enzyme synthesizes (TTAGGG)n repeats on to the Telomere sequences, using an internal RNA template.

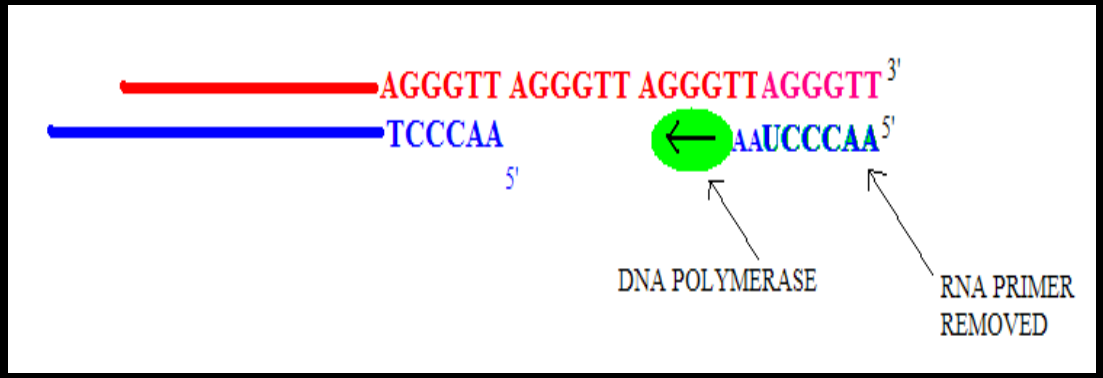
Mechanism of action of Telomerase(Contd.)



Telomerase acts like a reverse Transcriptase. It recognizes 3' end of telomere, based on the RNA component, a small DNA strand is synthesized

Mechanism of action of Telomerase(Contd.)





Telomerase

- different level of activity in different cells
  - High in stem cells & cancers
  - Activity lost in old age
  - Potential target for newer anticancer drugs

Inhibitors of DNA replication

- Bacterial DNA Gyrase(Type II Topoisomerase)- Inhibited by Novobiocin and Nalidixic acid.
- Ciprofloxacin interferes with DNA breakage and rejoining process
- Mammalian topoisomerases – inhibited by Etoposide and Adriamycin, used as anticancer drugs.
- Nucleoside analogues also inhibit replication and are used as anticancer drugs.

Summary of Replication

- Unwinding- forms replication fork
- Primase- Synthesizes RNA primer
- Continuous synthesis -Leading strand
- Discontinuous synthesis – Lagging strand (Okazaki fragments)
- Synthesis 5'-3' direction
- Primers removed, nick sealed
- Proof reading by DNA polymerases
- Organized in to chromatin structure