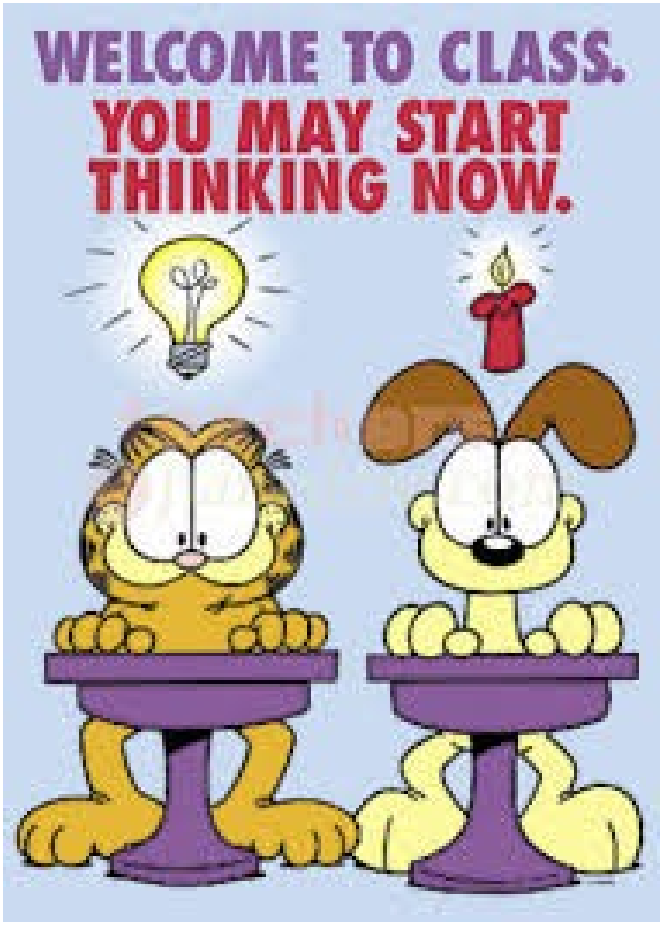
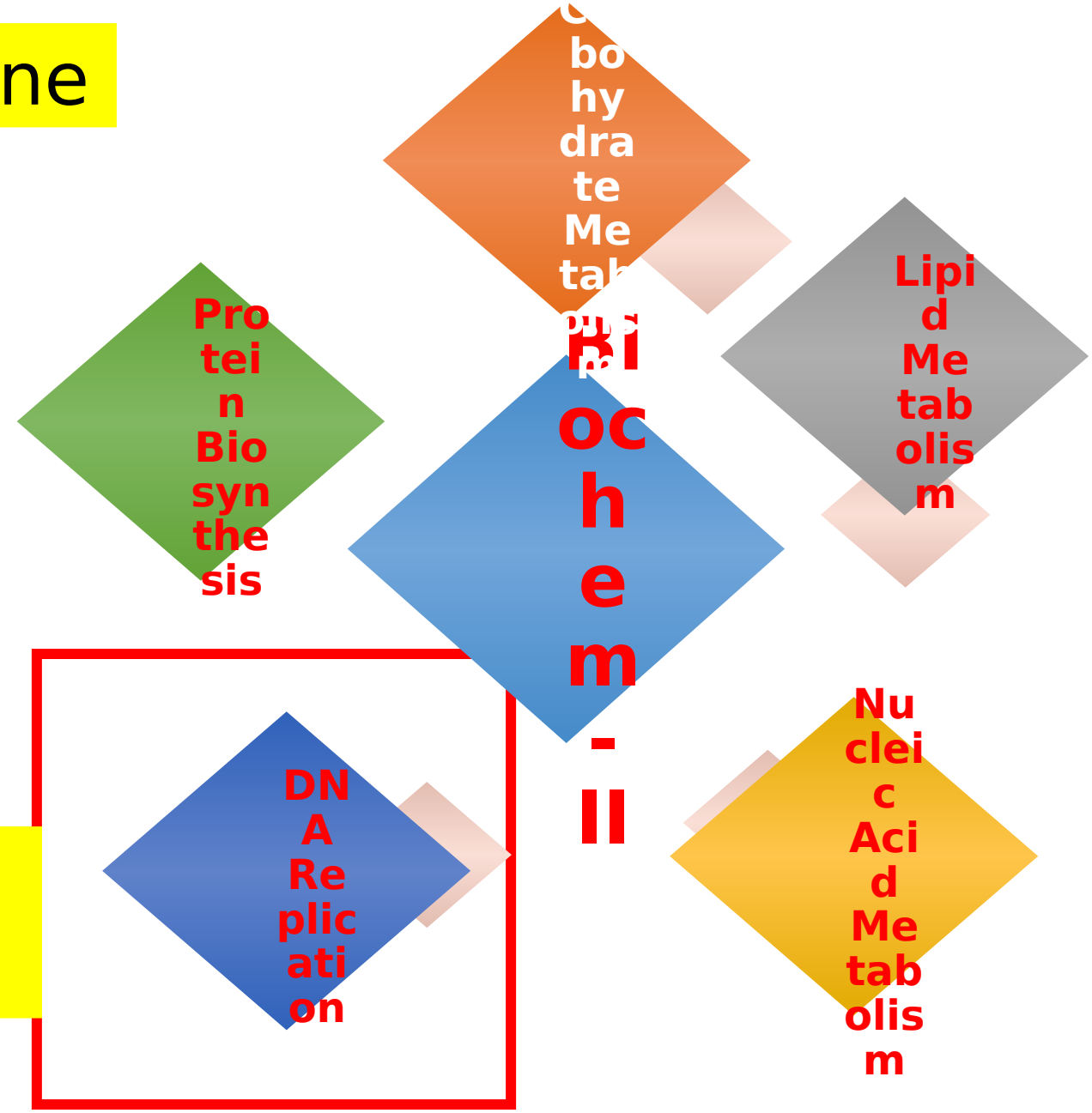
Yeah !! , it's the <u>Second year</u> !!!!!!

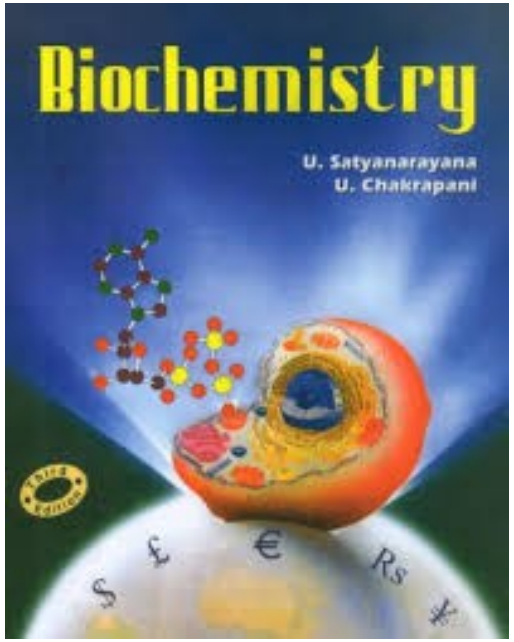


Course Outline



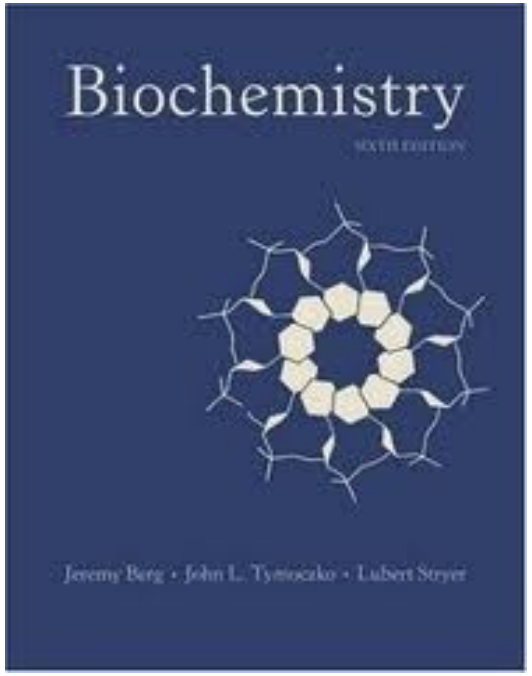
This is my portion

Books we will refer do

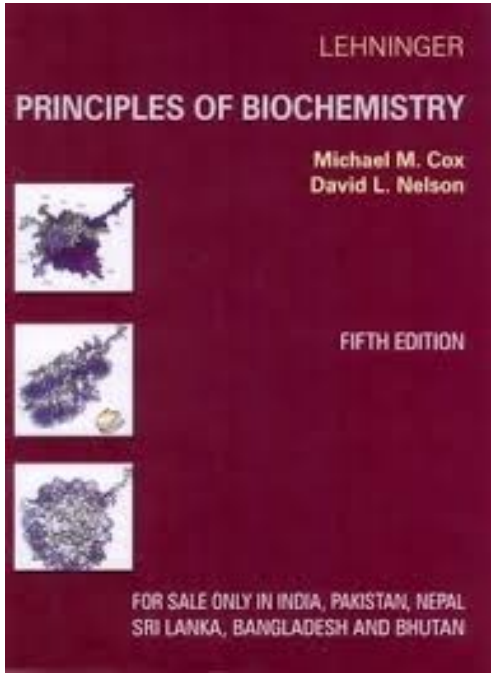


Satyanarayana

Primary Book



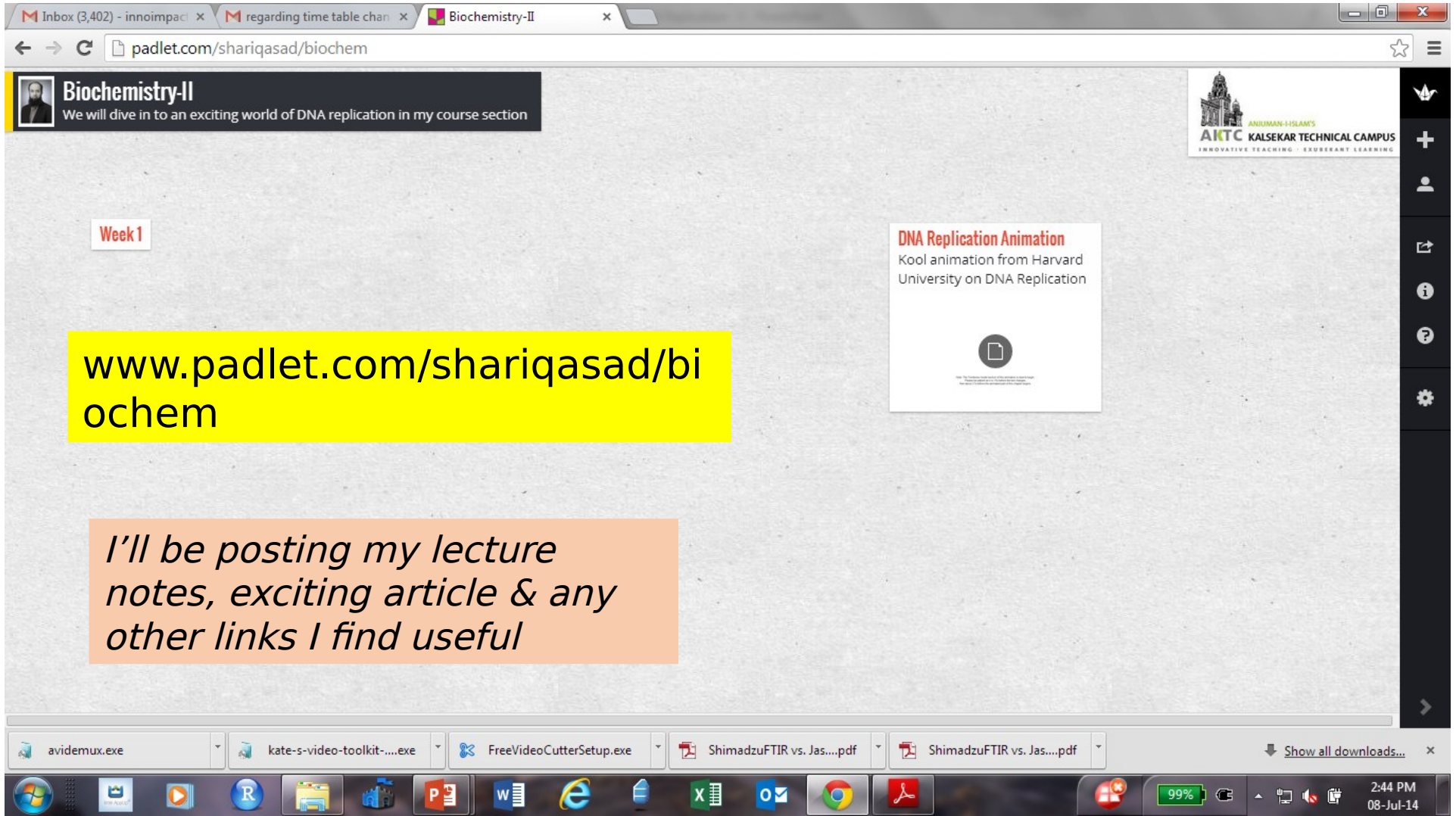
Stryer



Lehninger

Excellent Reference

We have a new "Kool" e-blackboard !!!



Biochemistry - II

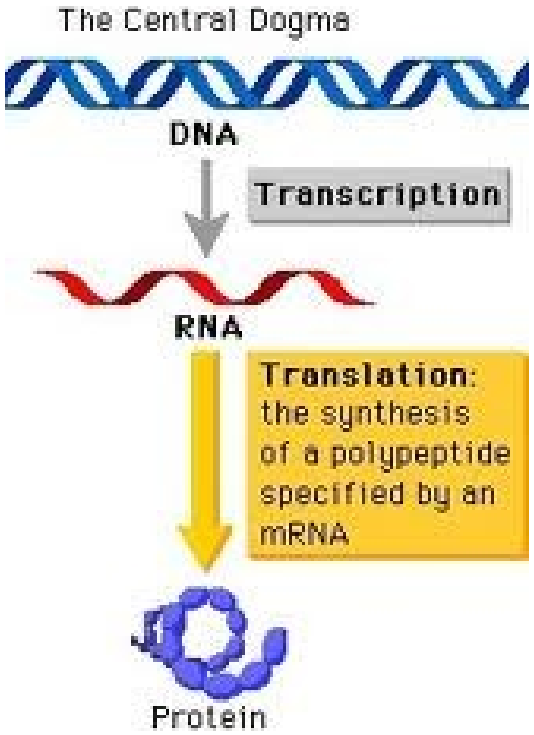
DNA Replication - 1

Dr. Shariq Syed

What are "Nucleic Acids" ??

TYPES

- Nucleic Acids
  - Deoxy-ribonucleic Acid (DNA)
  - Ribonucleic Acid (RNA)



What's job of nucleic acid ??

- **Storage**
- **Transmission of GENETIC**

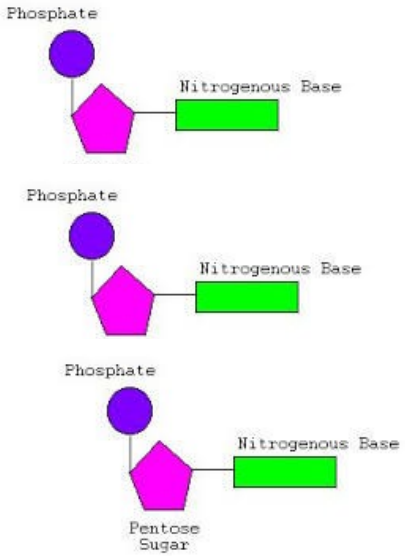
What's the composition/structure of Nucleic Acid ??

Both DNA, RNA are **polymers** of

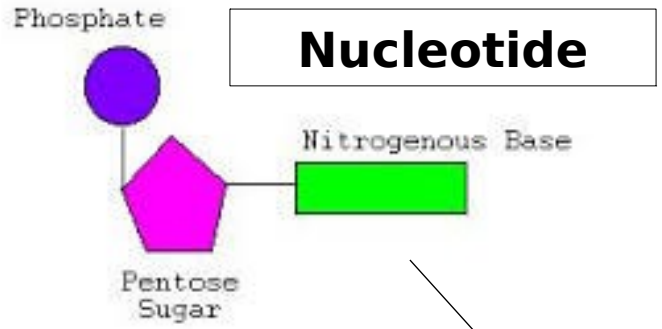
Nucleotide

- **Nitrogenous Base**
- **Pentose Sugar**
- **Phosphate**

Nucleoside



Nitrogenous Base

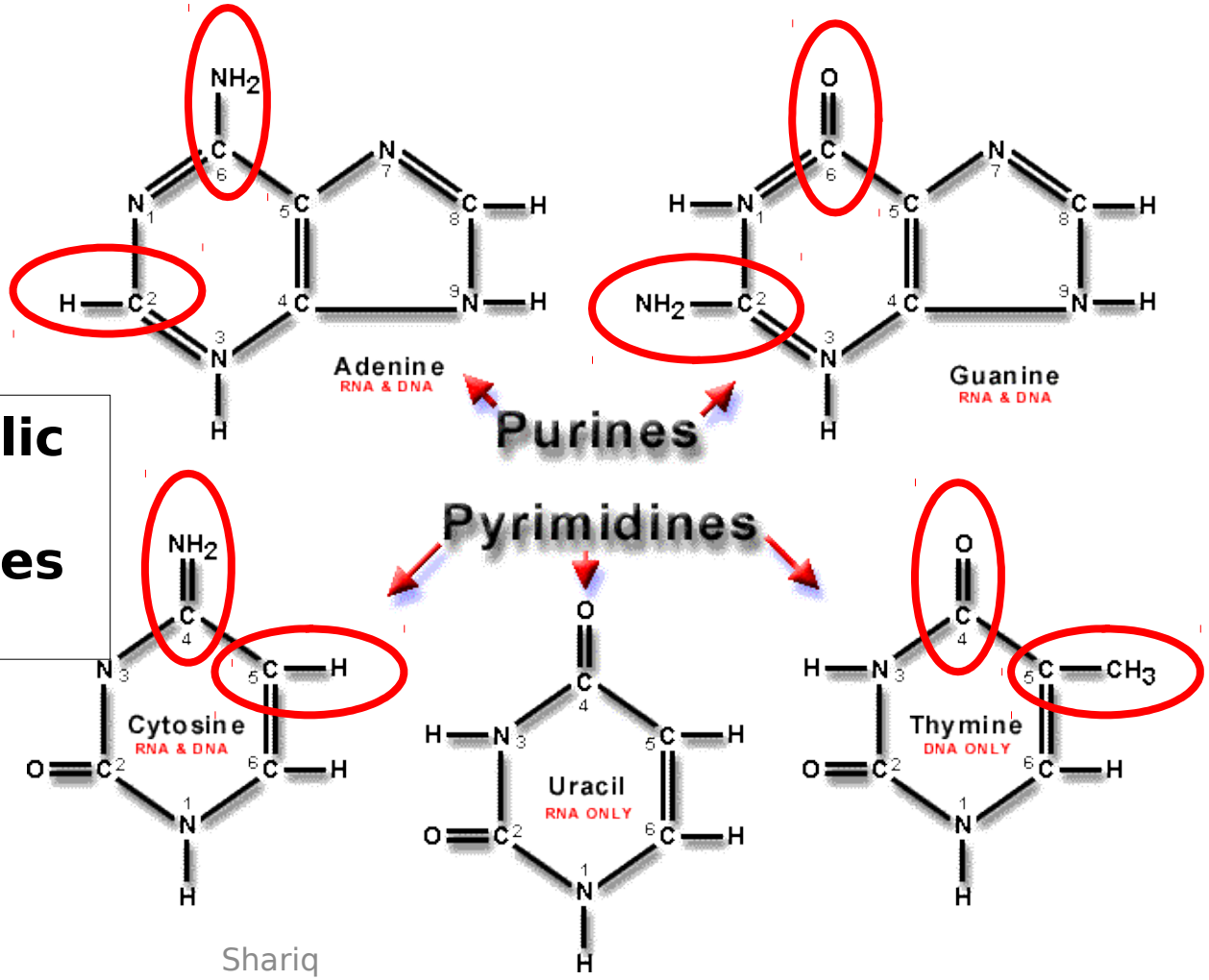


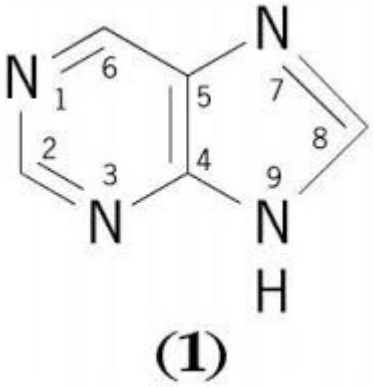
Nitrogenous base

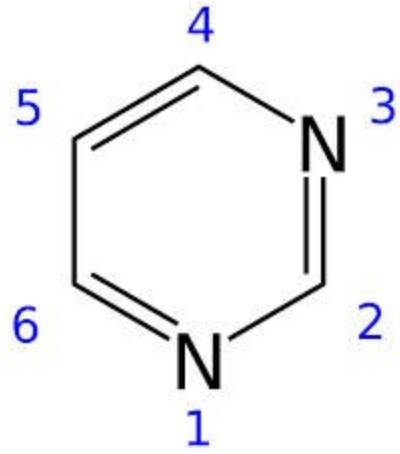
- Purines
  - Adenine
  - Guanine
  - **DNA or RNA**
- Pyrimidines
  - Thymine — **DNA or RNA**
  - Cytosine — **DNA**
  - Uracil — **RNA**

Nitrogenous Base

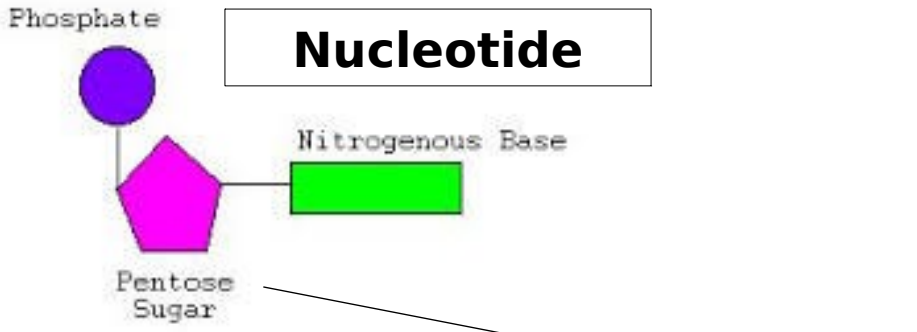
Aromatic Heterocyclic Compounds, derivatives of Purines & Pyrimidines

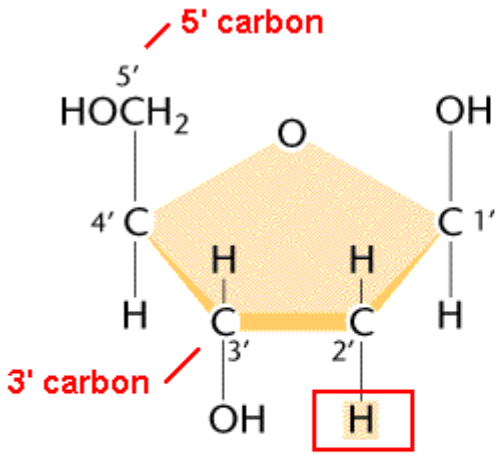




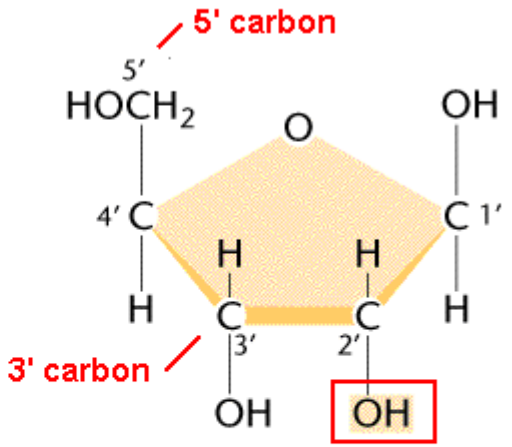


Pentose Sugars





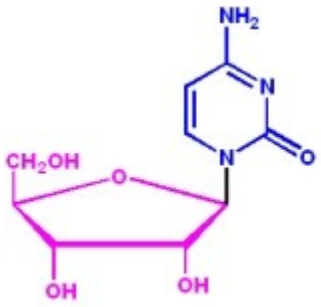
2-Deoxyribose



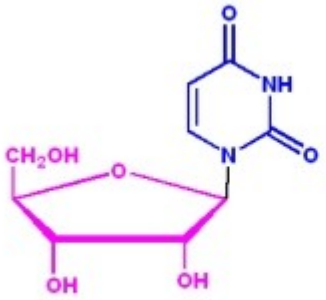
Ribose

(Klug & Cummings 1997)

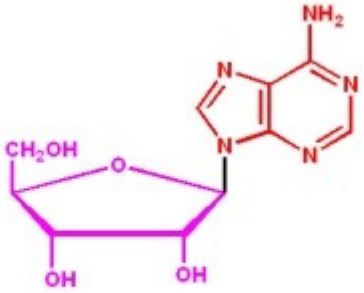
Nucleotide



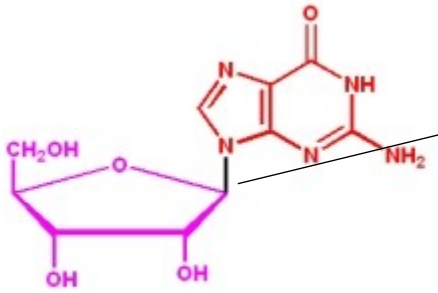
Cytidine



Uridine



Adenosine

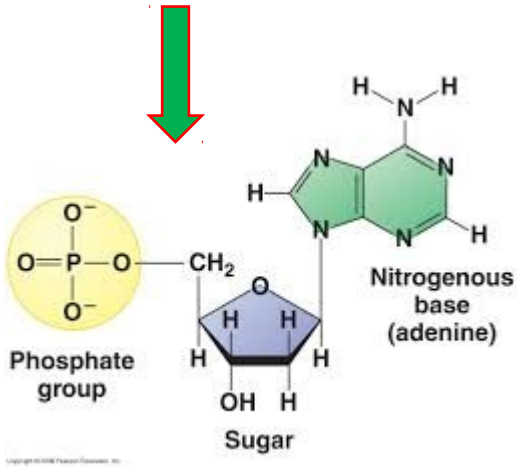


Guanosine

Base binds to C1 of sugars

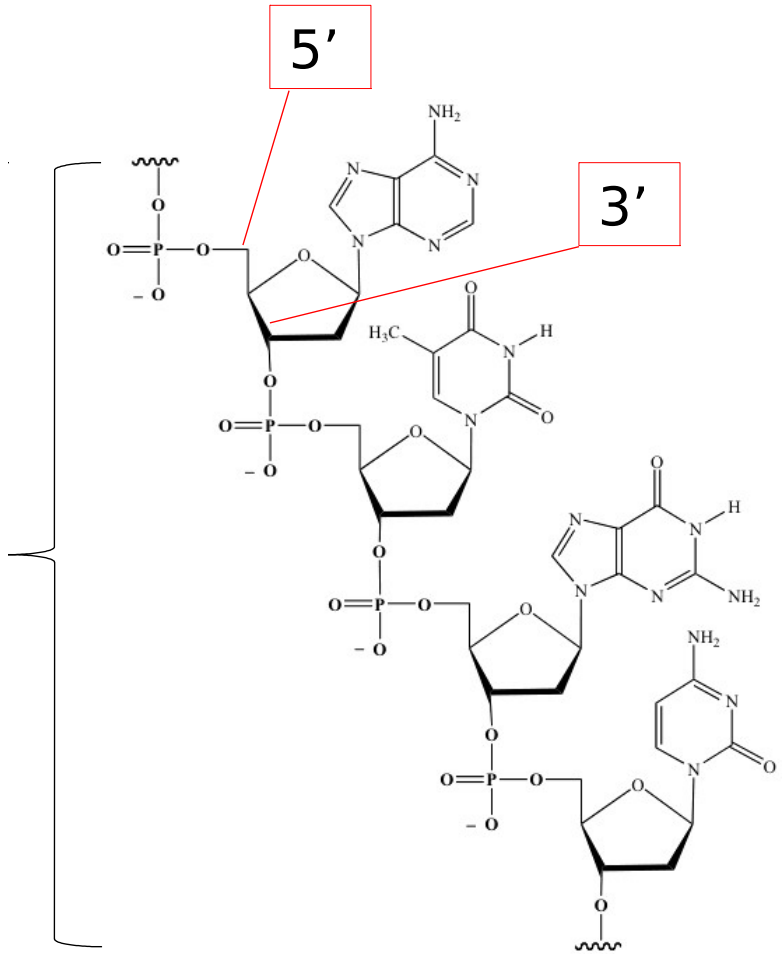
Nucleoside

-OH group of sugar esterified with phosphate group



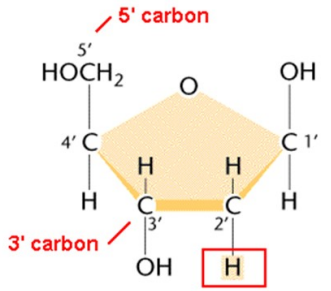
Nucleotide

Nucleotide

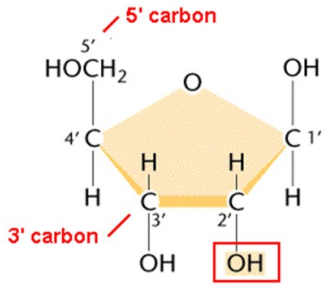


Esterification of –OH group happens at both 3’ & 5’ position of sugars

This “dual” esterification provides a phosphate backbone to nucleotide polymer



2-Deoxyribose



Ribose

(Klug & Cummings 1997)

Structure of DNA

What is DNA ??
It's a polymer of Nucleotides, held together by Sugar-phospho-diester backbone

Chargaff's Rule:

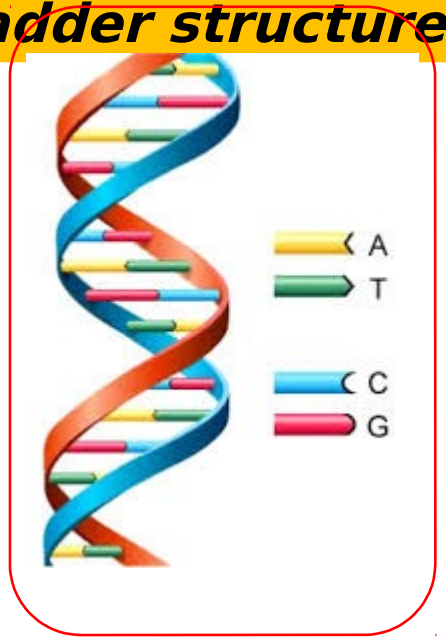
DNA ↓ *(Hydrolysis)*

DNA hydrolysate ↓

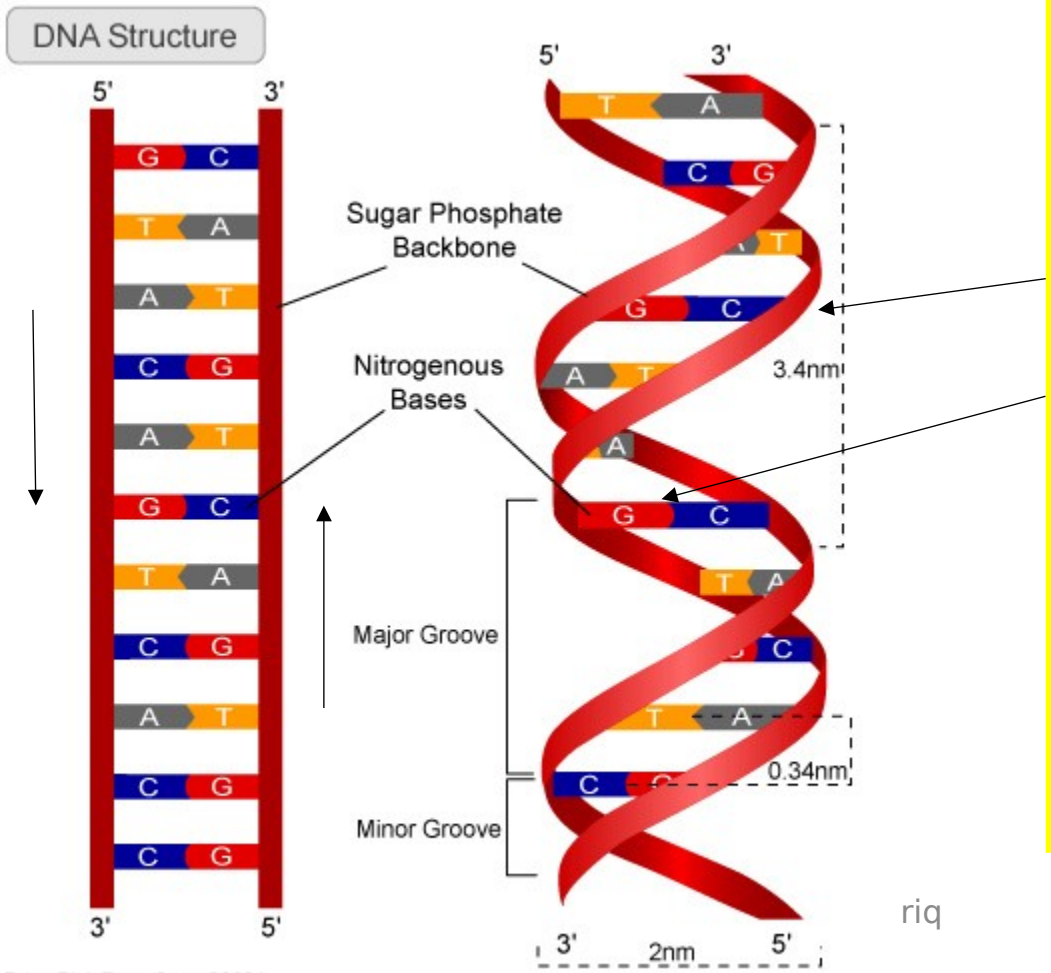
A=T, G=C residues
Purines = Pyrimidines
molar equivalent

<u>Watson & Crick</u> proposed a Double-Helix structure for DNA

Twisted Ladder structure



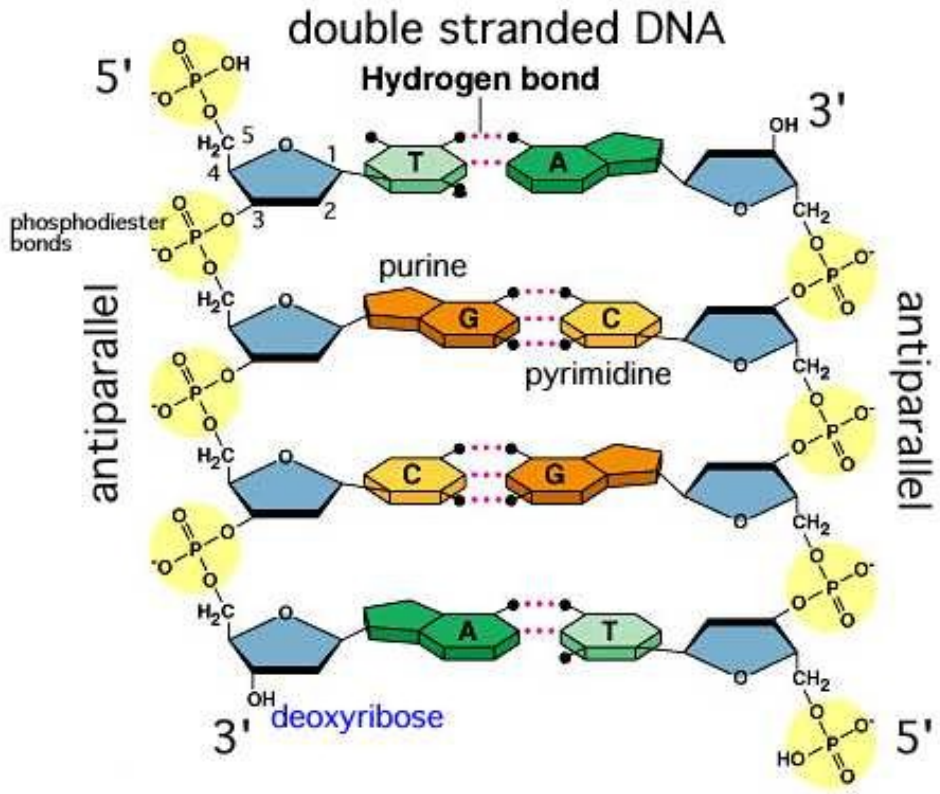
Double Helix Structure of DNA



- **<u>Two</u>** polynucleotide chains twisted around each other on common axis
- The Two strands run **<u>anti-parallel</u>**
- **Hydrophilic** phosphate chain outside
- **Hydrophobic** bases form inside core
- Two chains held together by H-bonds
- A=T, G C
- Proposed structure agrees to Chargiff's rule

riq

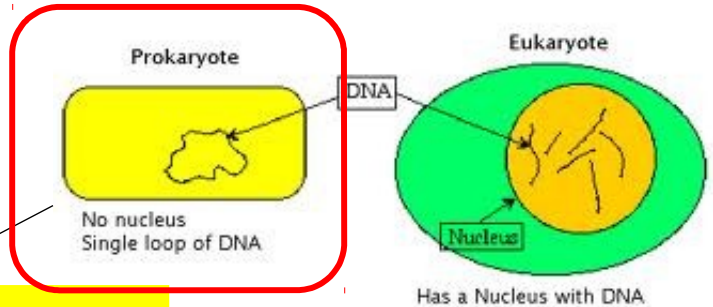
Double Helix Structure of DNA



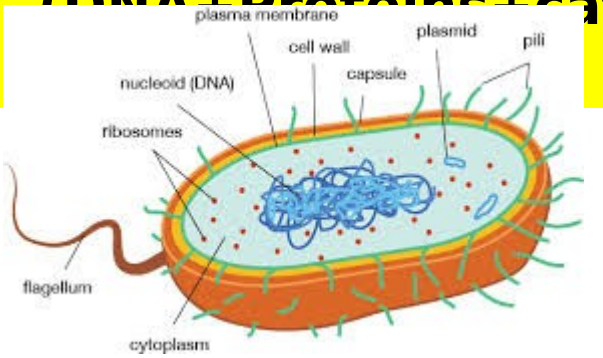
- Two chains run anti-parallel, 5’ end, 3’ end
- Space orientation **only** permits purine=pyrimidine H-bonds
- Purine=purine would not fit in structure while
- Pyrimidine=pyrimidine would be too far to form H-bonds

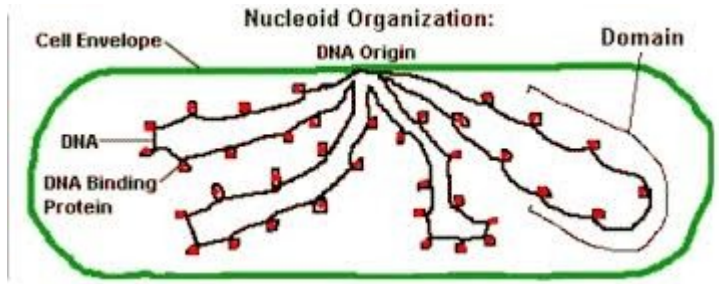
How is DNA organized in a CELL

PROKARYOTES



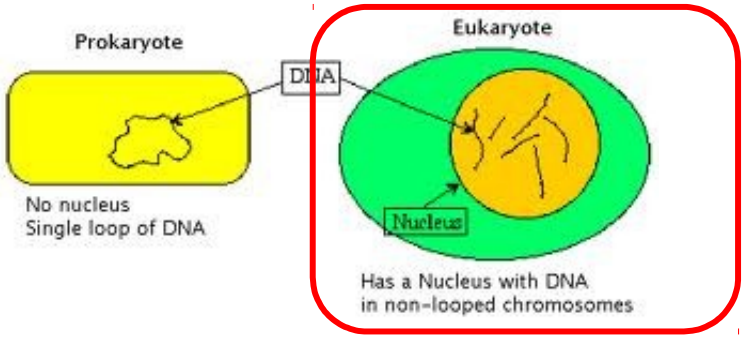
- **Single chromosome**
- **Double stranded circle**
- **Chromosome are packed in form of nucleoids (DNA+Proteins+cation**



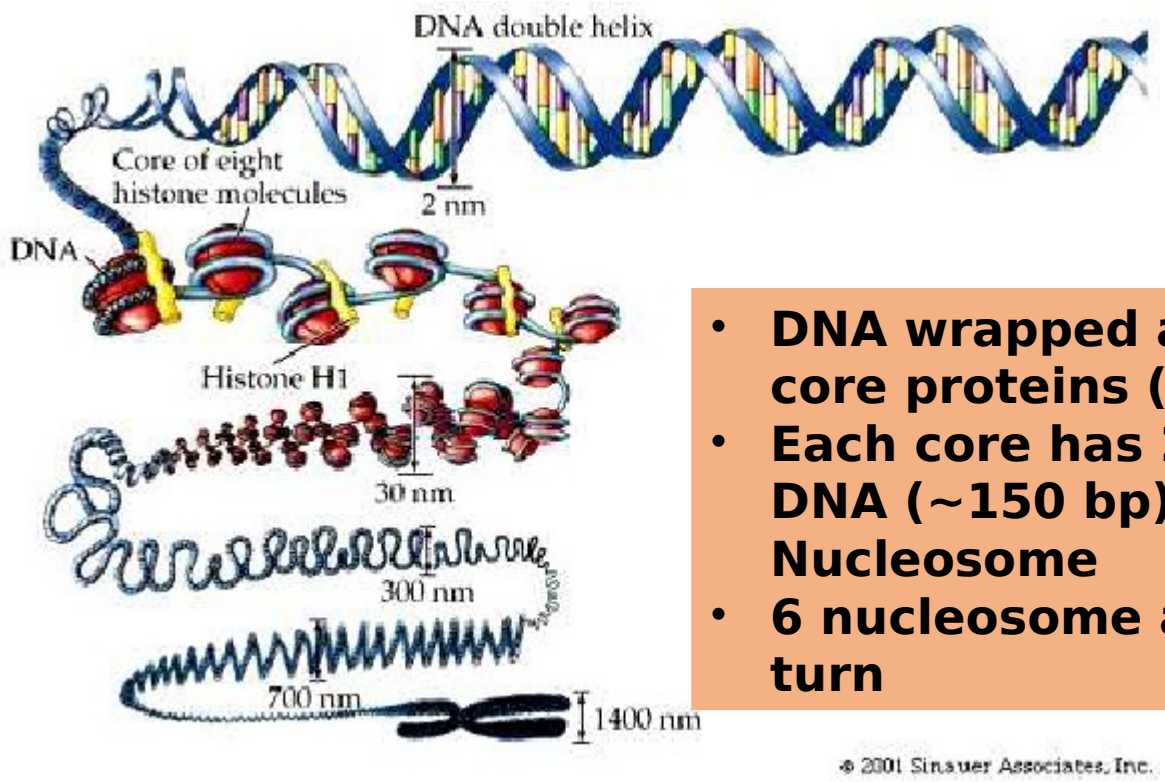


- **The NUCLEOID is an irregularly-shaped region within the cell of a prokaryote that contains all or most of the genetic material.**

How is DNA organized in a CELL

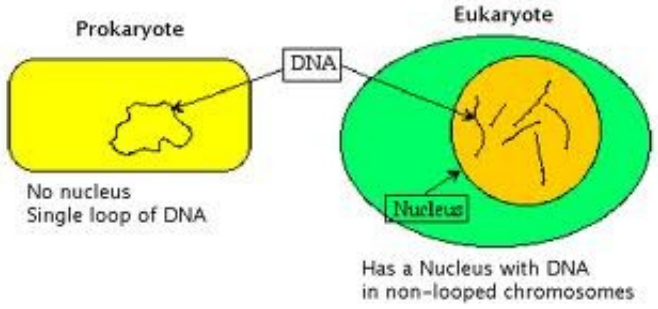


- **Eukaryote = DNA in clearly defined nucleus**
- **DNA + Proteins = chromatin which gets organized as chromosome**



- **DNA wrapped around core proteins (Histones)**
- **Each core has 2 turns of DNA (~150 bp) = Nucleosome**
- **6 nucleosome at every turn**

How is DNA organized in a CELL



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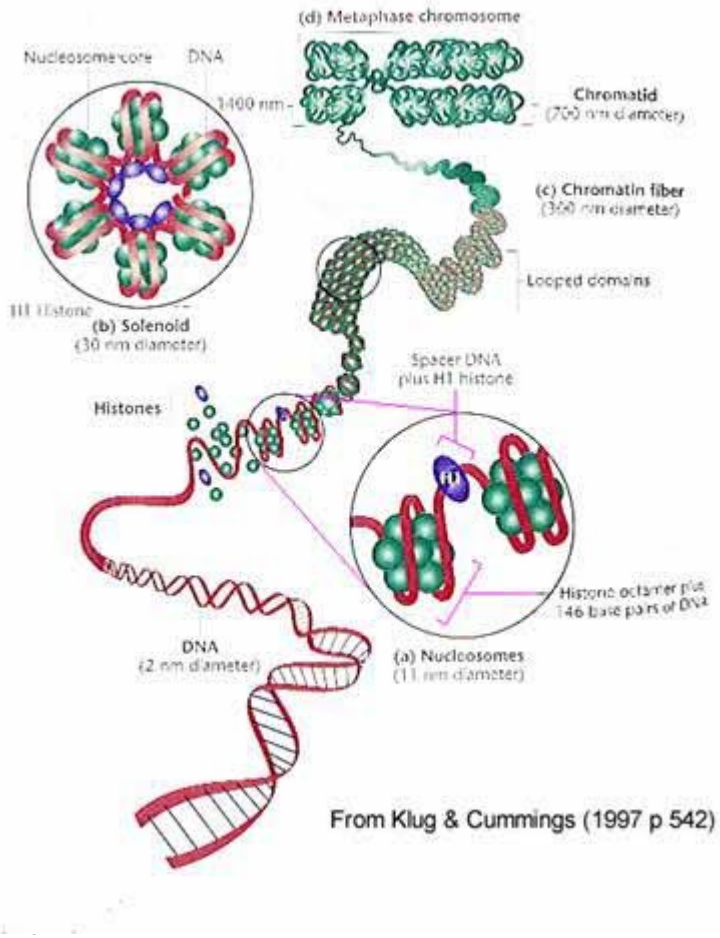
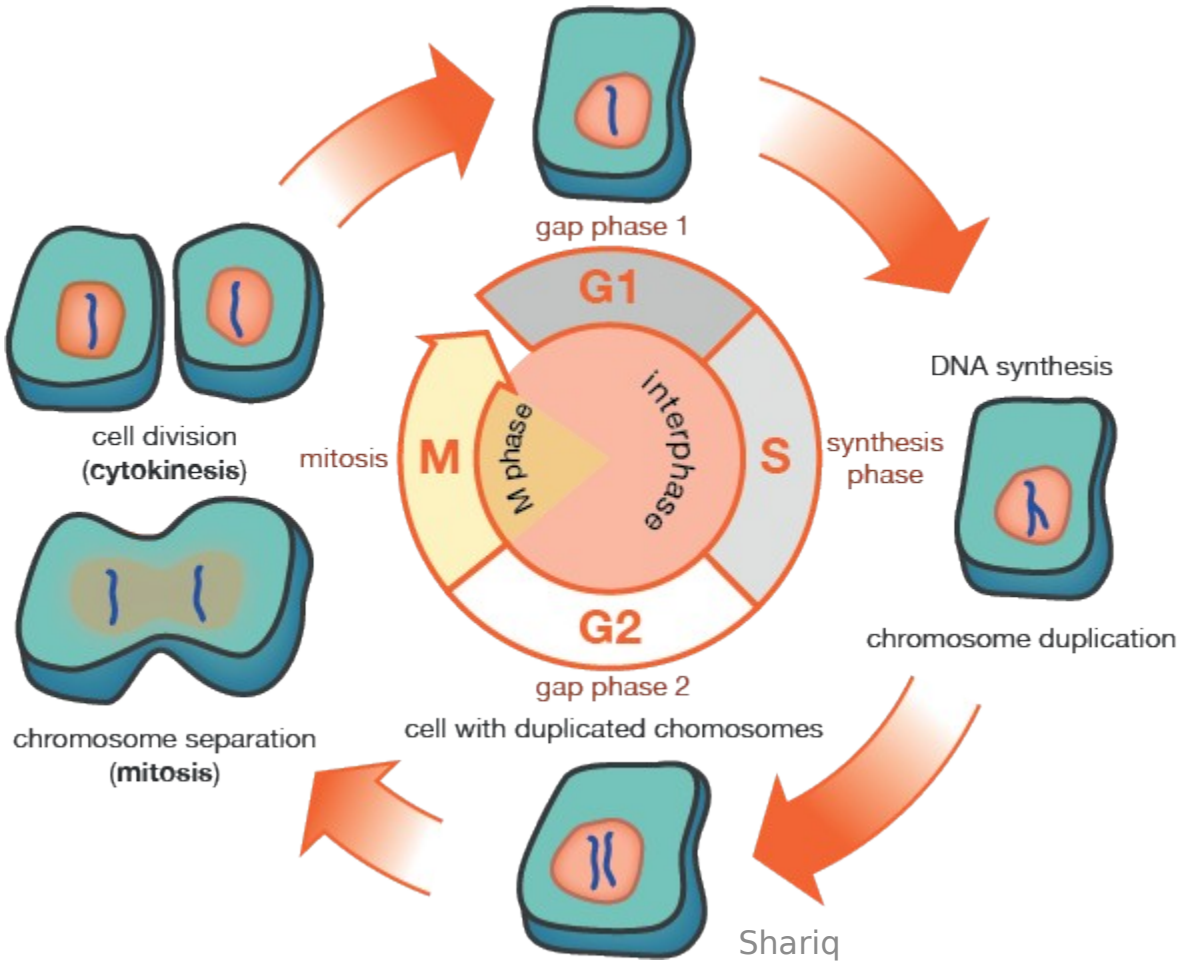


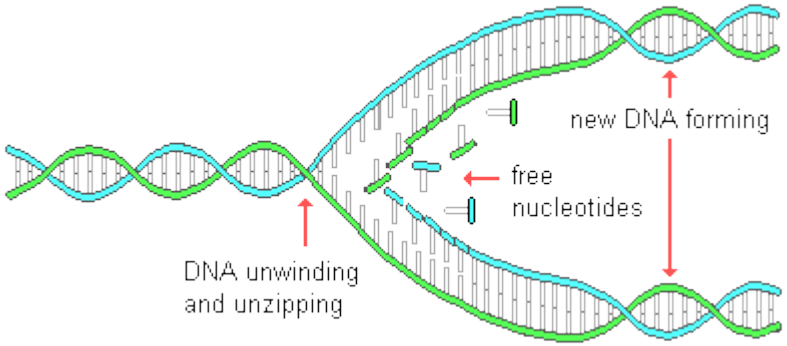
- DNA wrapped around core proteins (Histones)
- Each core has 2 turns of DNA (~150 bp) = Nucleosome
- 6 nucleosome at every turn

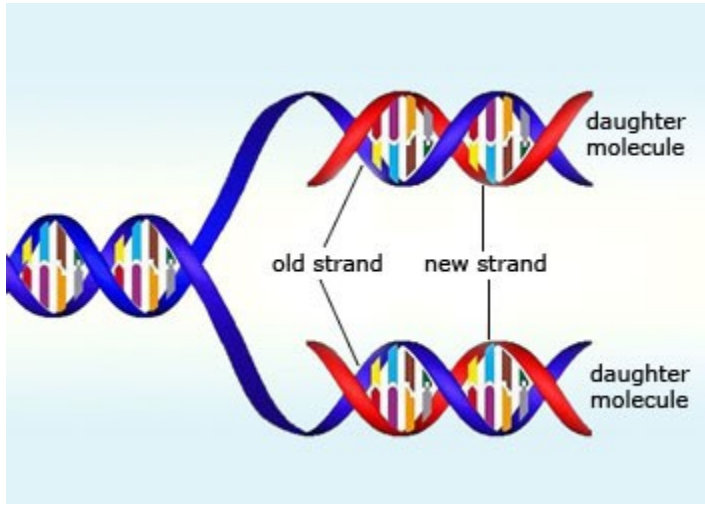
When does DNA Replication occur during Cell Cycle ??



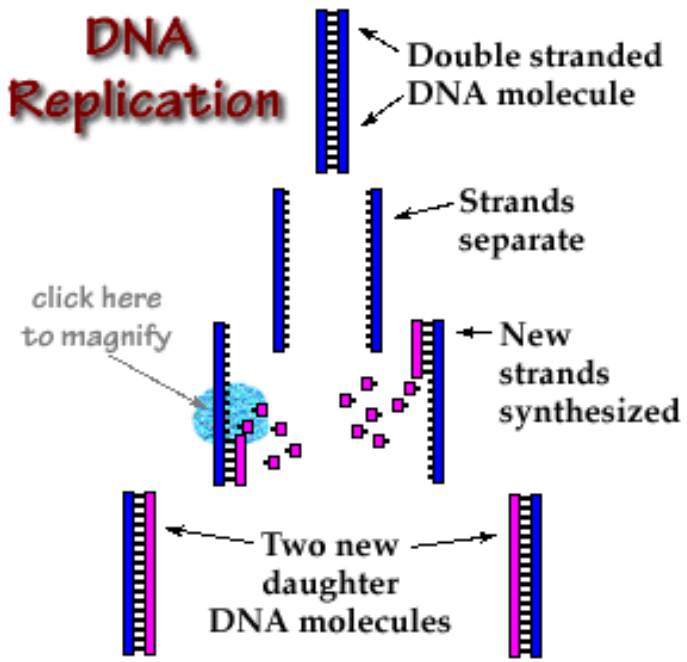
- DNA synthesis takes place in <u>S-phase</u>
- Entire process take 8-10 hrs
- Large number of DNA Synthesizing enzymes (500-1000) are involved

DNA Replication; Short story





DNA Replication is *Semi-conservative*



What happens during replication ??

- Parent strand is separated
- Creating two separate strand
- These single strand act as template for new strand
- “Semi-conservative” because half of parent DNA retained in daughter DNA

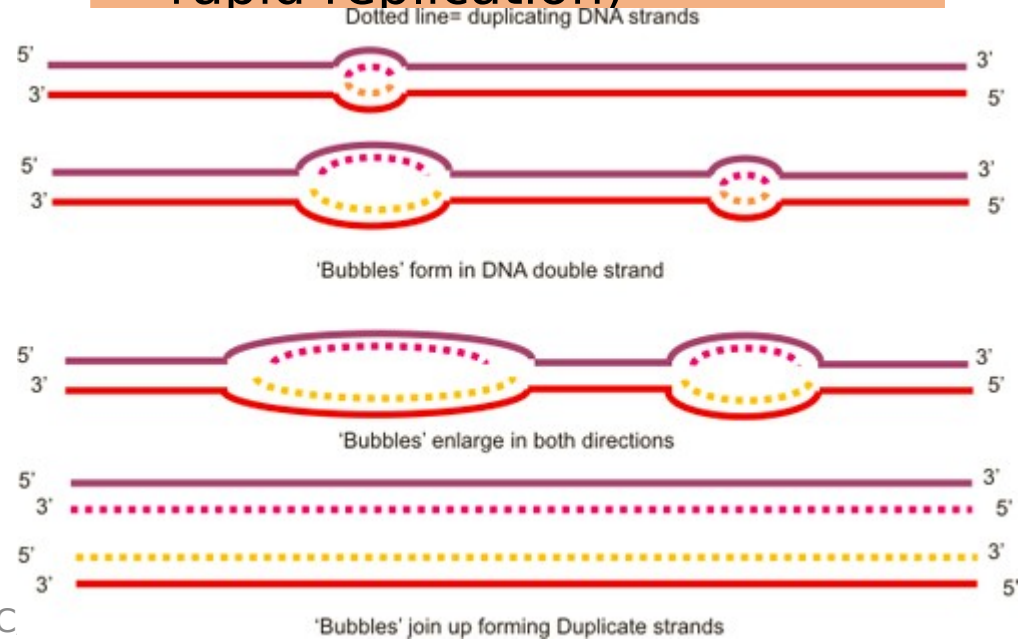
Site of DNA Replication

Site of Replication:

Location where the DNA “unzips”
Single location for prokaryotes, multiple for eukaryotes
Region rich in A=T sequence since easy to break A=T bond

Specific protein “dna A” opens up DNA

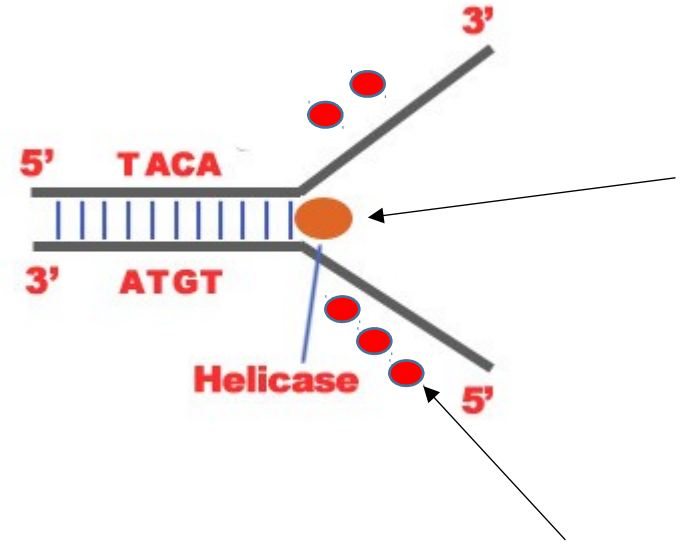
- Opening of DNA leads to formation of **replication bubbles**
- Multiple in case of eukaryotes (Important for rapid replication)



DNA Replication; *Let's get to details*

Site of Replication:

- Separation of DNA strands results in formation of **replication fork**
- Replication fork moves forward along parent DNA, daughter DNA is synthesized

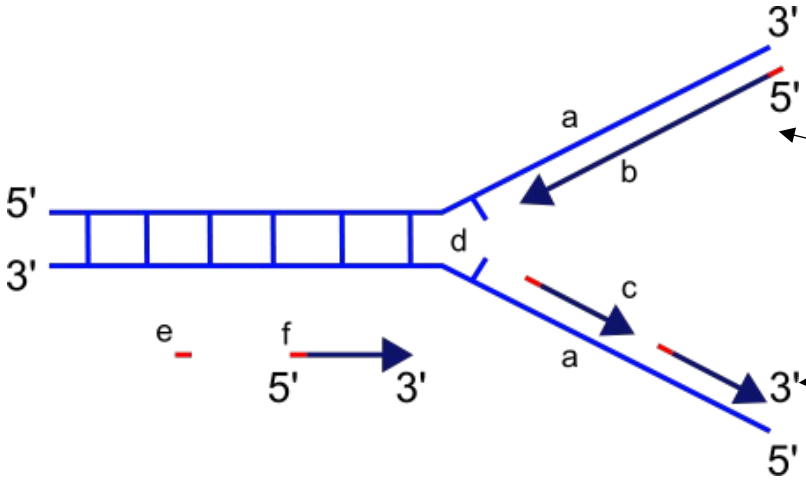


Helicase: Enzyme that binds to DNA and separates two strands

SSB Proteins:

- Keeps the two strands separate
- Prevent degradation by nucleases
- No enzymatic activity

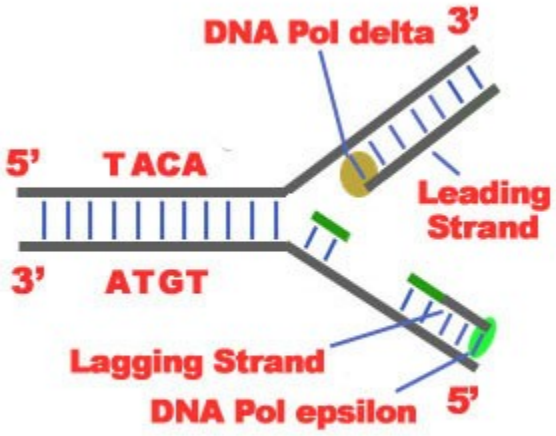
DNA Replication; *Let's get to details*



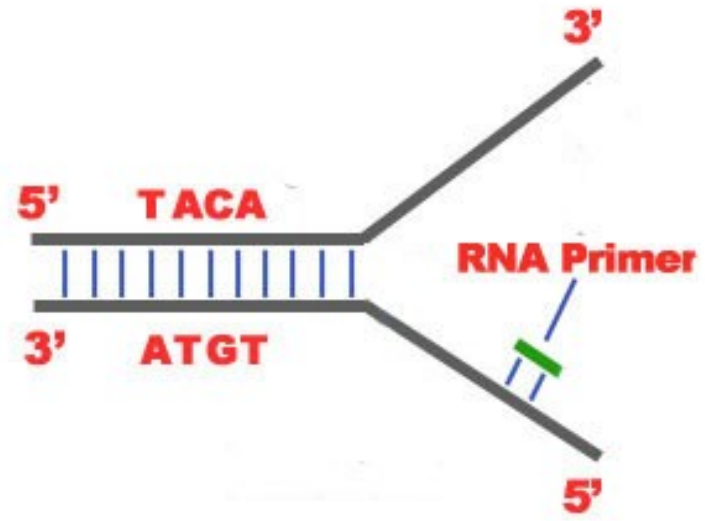
- **DNA replication occurs only in one direction 5'-3'**

At one strand there is Continuous synthesis of DNA (5'-3' direction)

At another strand there is Discontinuous synthesis



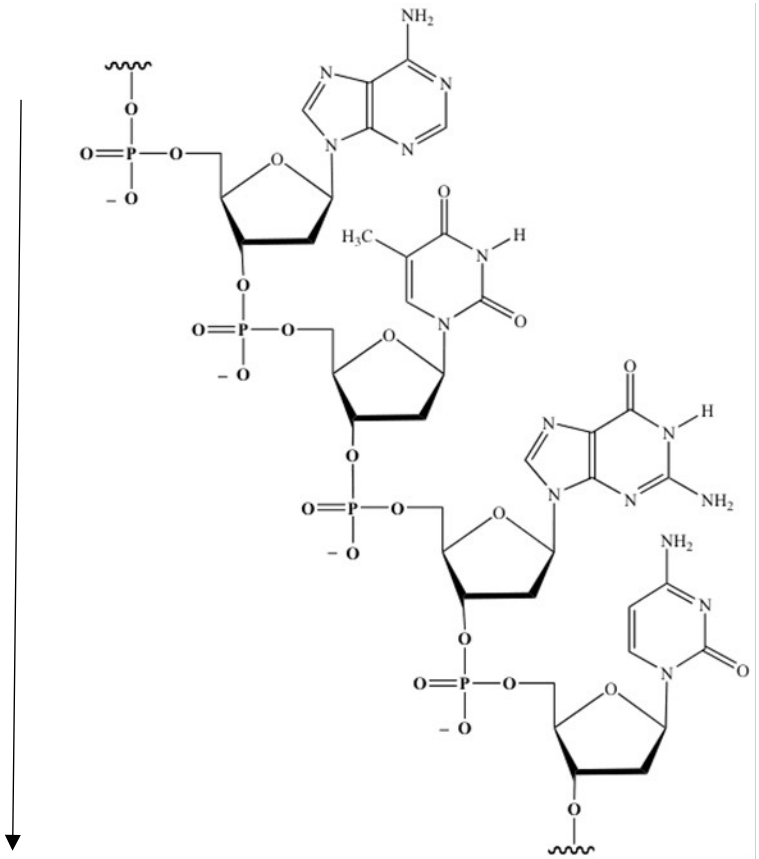
DNA Replication; *Let's get to details*

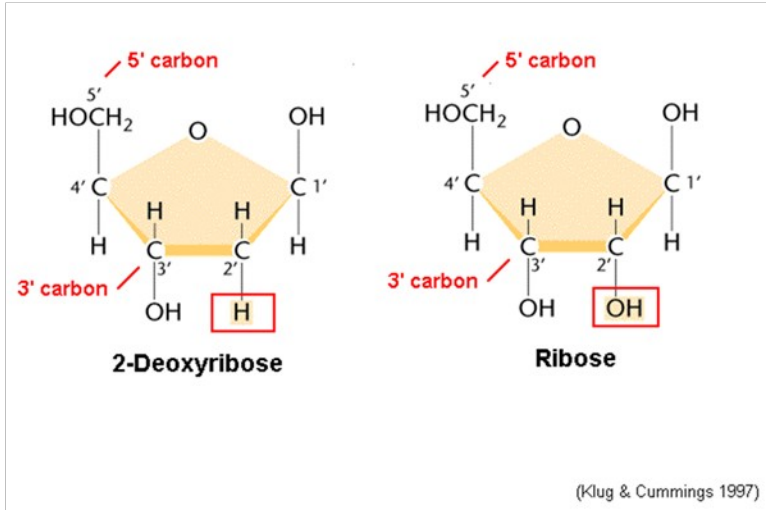


- **RNA Primase** binds to initiation point of the 3'-5' parent chain
- **RNA Primase** synthesis short RNA (~ 5nucleotides) which bind to the DNA nucleotides of the 3'-5' strand
- **RNA nucleotides are the primers (starters)** for the binding of DNA nucleotides
- These RNA primers are later removed by hydrolysis & replaced by DNA

DNA Replication; *Let's get to details*

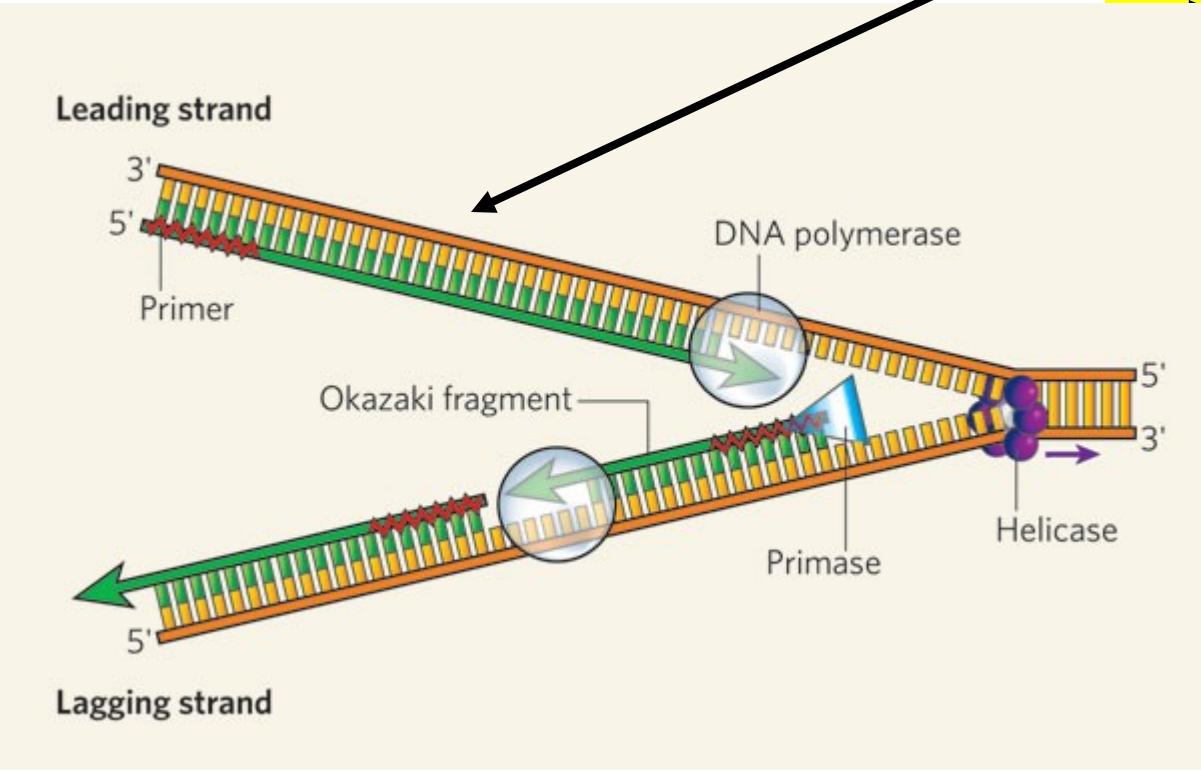
DNA Polymerase can add nucleotide ONLY to 3' end





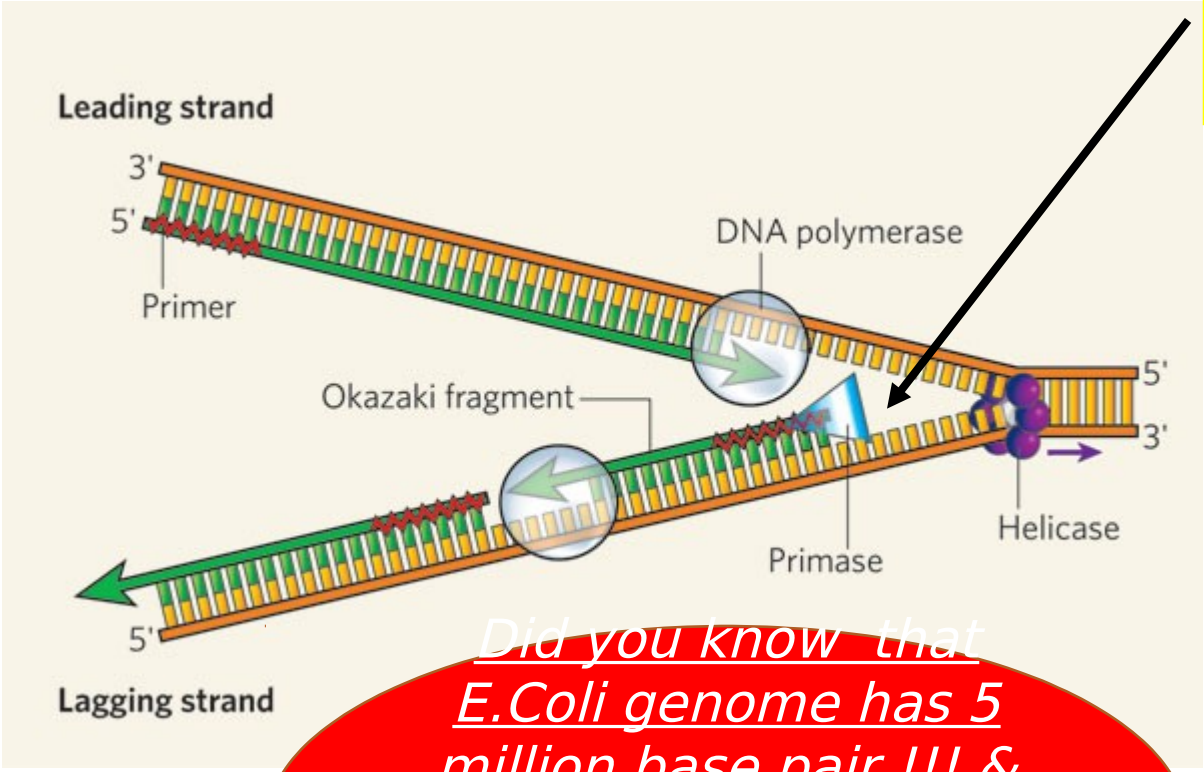
DNA Replication; *Let's get to details*

- **DNA Replication at "Leading" Strand**



- **<u>DNA Polymerase</u>** catalyses synthesis of new DNA **ONLY** in 5'-3' direction
- Synthesis occurs continuously at leading strand
- Incoming nucleotides are appropriately added by DNA polymerase to 3' end of growing chain
- Nucleotide in triphosphate form, when added to chain, single Ppi is removed

DNA Replication; *Let's get to details*



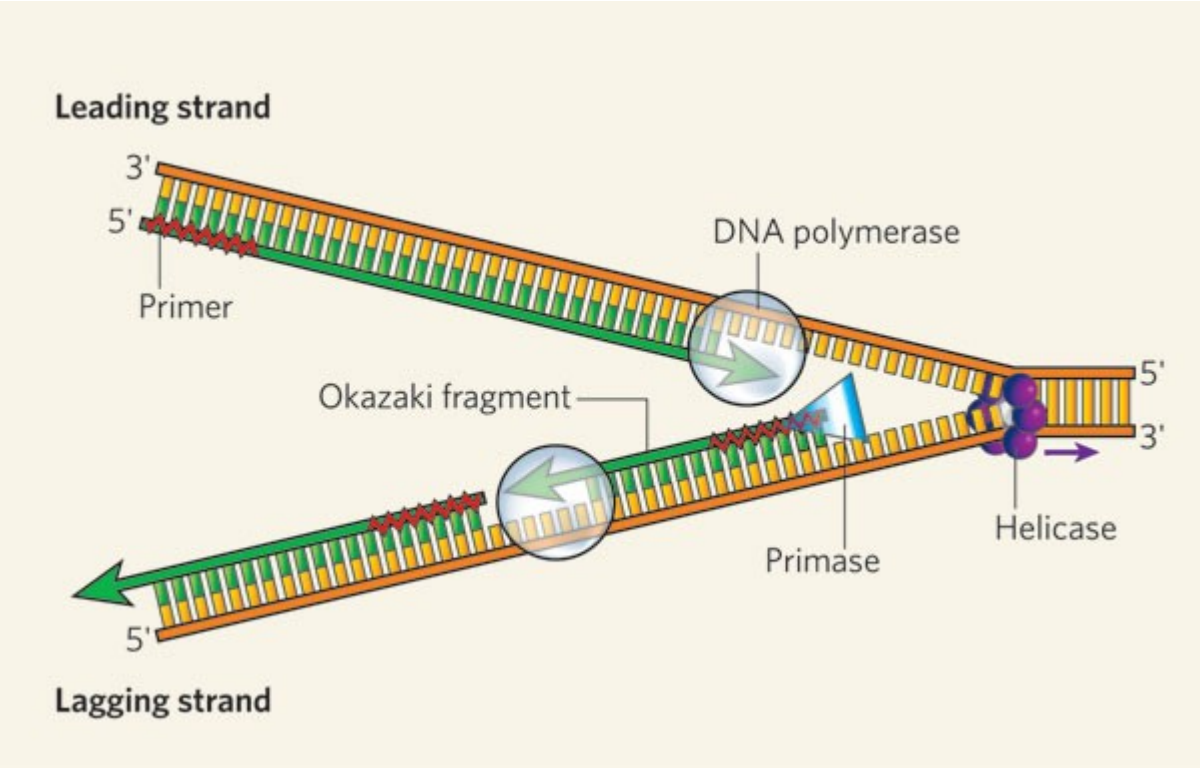
- **DNA Replication at "Lagging" Strand**

DNA Polymerase-III catalyses synthesis of new DNA **ONLY** in 5'-3' direction

- For lagging strand, several RNA primers by Primase enzymes formed
- Short DNA sequences synthesized around these primers
- These short DNA fragments are called "Okazaki" fragments

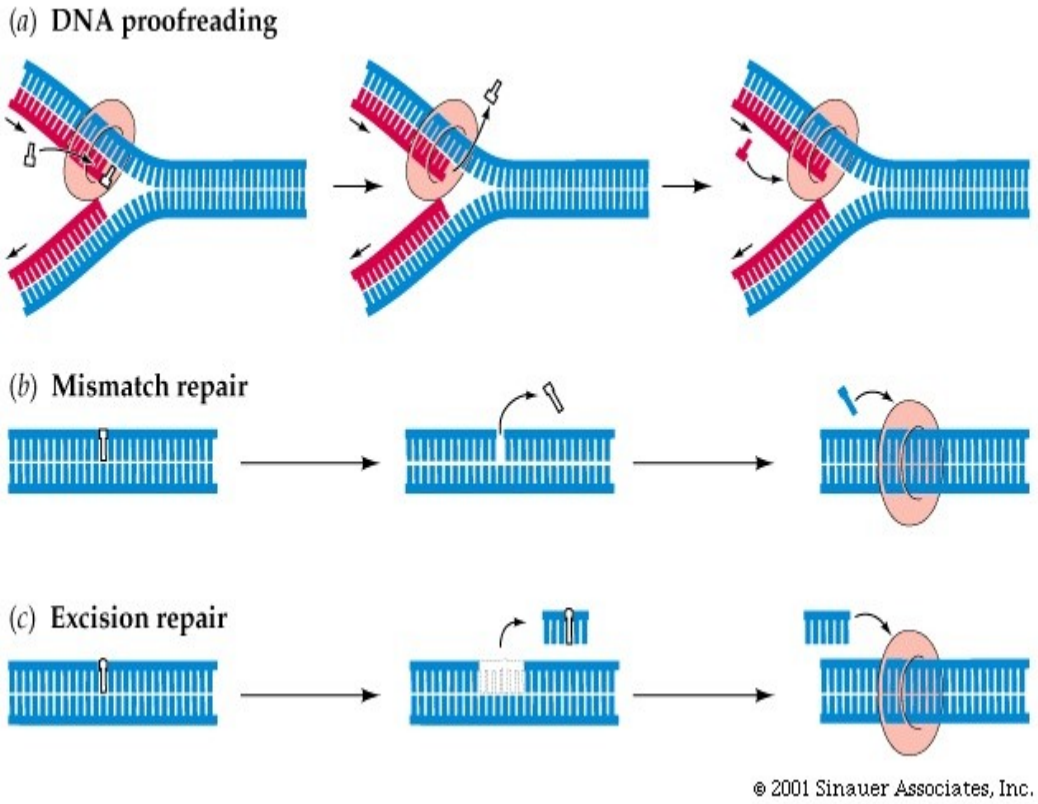
Did you know that E.Coli genome has 5 million base pair !!! & is copied in <40 minutes , ie 2000 bases/second

DNA Replication; *Let's get to details*



- **There are multiple RNA primers on lagging strand & one on leading strand**
- **DNA synthesis continues till the strand is near to primers**
- ***DNA polymerase-I* removes primer, synthesizes fresh DNA portion**
- **DNA ligase enzyme seals the two DNA ends**

Proof reading DNA !



- As they add new bases to a growing strand, DNA polymerases-III make a proofreading check
- When a DNA polymerase recognizes an error, it removes the wrong nucleotide and tries again.
- The error rate of DNA polymerase on each attempt is only about 1 in 10,000, so the second attempt at matching the template is very likely to be successful.
- This proofreading function reduces the overall error rate to about one base in a billion (one in 10^{9})

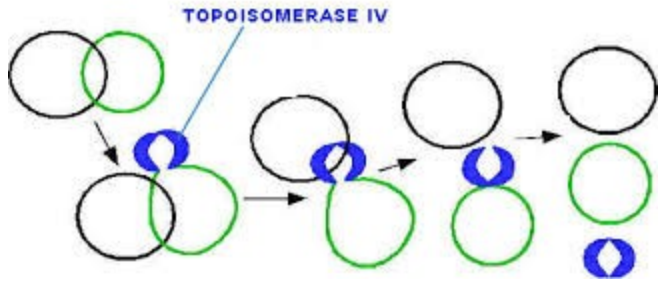
Termination of replication

- DNA has a certain base pair region called “Ter”
- When replication fork enters, it cannot leave (it’s a trap !)

- These supercoiled chromosomes are called “catenated”

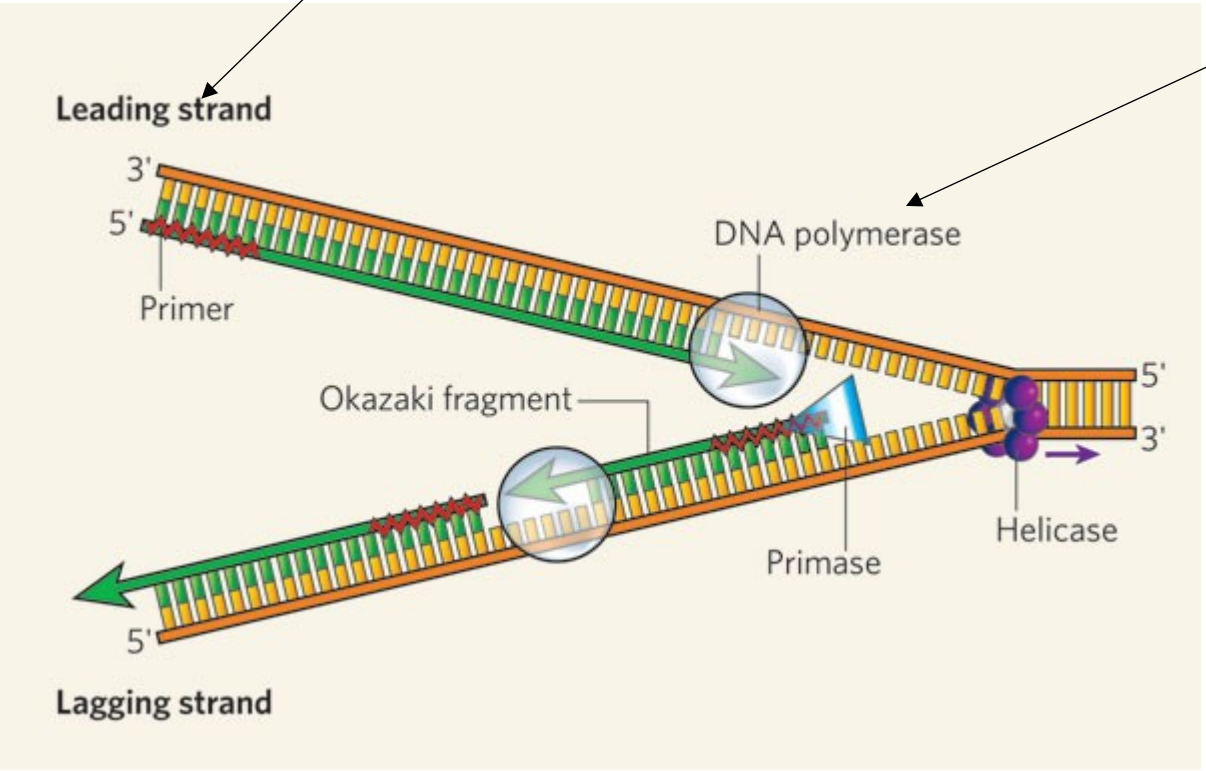
Role of Topoisomerase

- Topoisomerases are enzymes that regulate the overwinding or underwinding of DNA
- During DNA replication, DNA becomes overwound ahead of a replication fork
- Topoisomerases bind to either single-stranded or double-stranded DNA and cut the phosphate backbone of the DNA
- This intermediate break allows the DNA to be untangled or unwound, and, at the end of these processes, the DNA backbone is resealed again



Eukaryote DNA Replication is *Different*

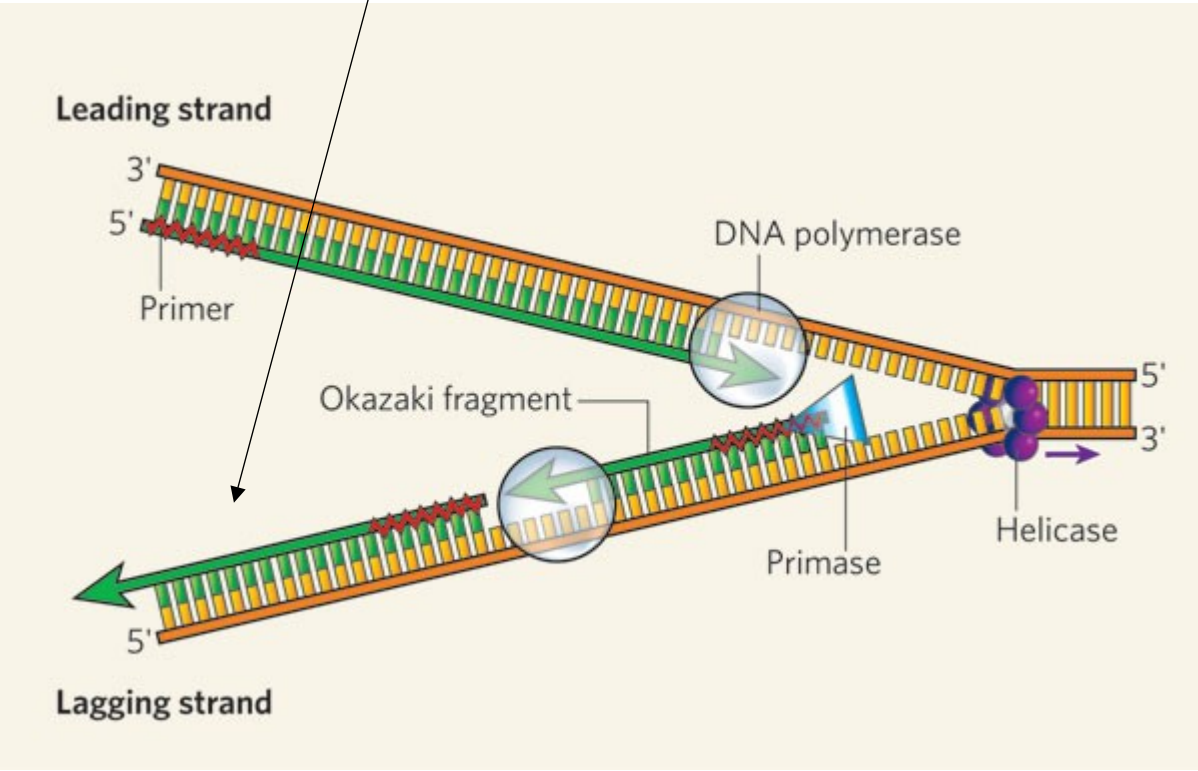
Replication at Leading Strand



- DNA polymerase- δ along with clamp slider PCNA (proliferating cell nuclear antigen)
- PCNA forms a ring around DNA to which polymerase binds

Eukaryote DNA Replication is *Different*

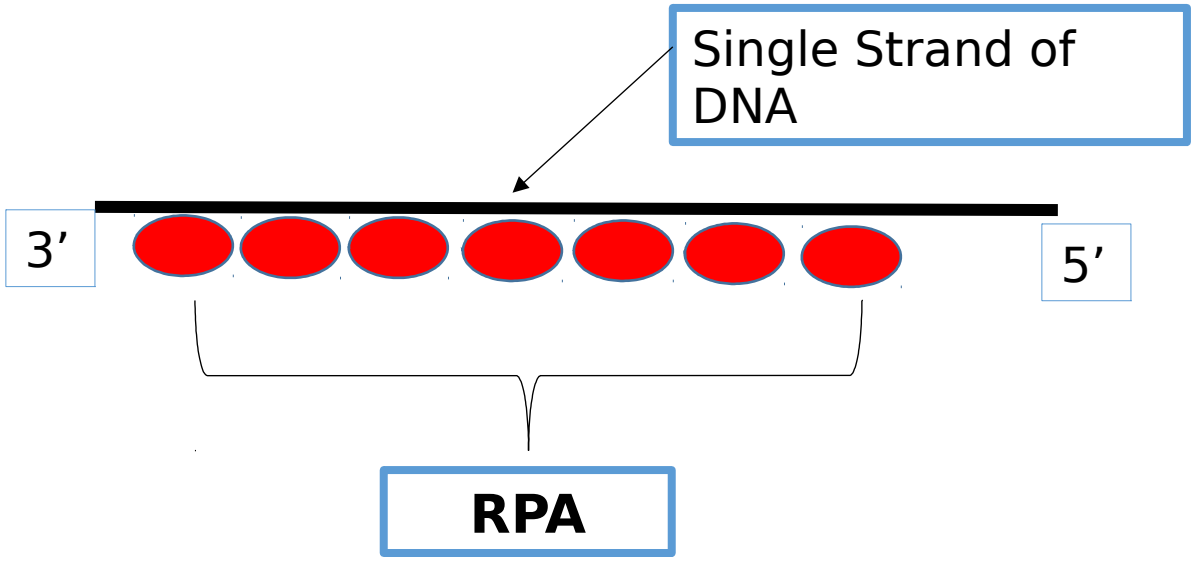
Replication at Lagging Strand



- DNA synthesis at lagging strand is slightly a <u>COMPLEX (multi-step)</u> compared to prokaryotes

Eukaryote DNA Replication is *Different*

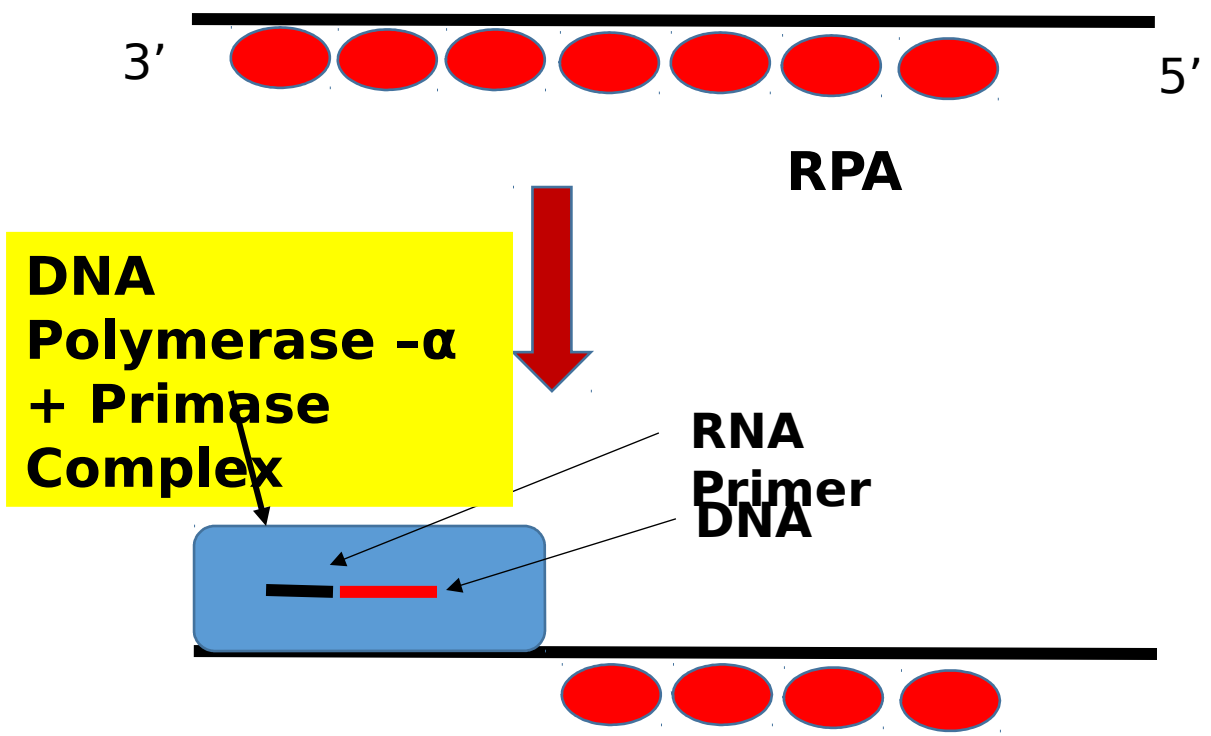
Replication at Lagging Strand (STEP 1)



- Parental DNA separated by *Helicase*
- Exposed single strand is stabilized by *Replication Binding Protein* (RPA)

Eukaryote DNA Replication is *Different*

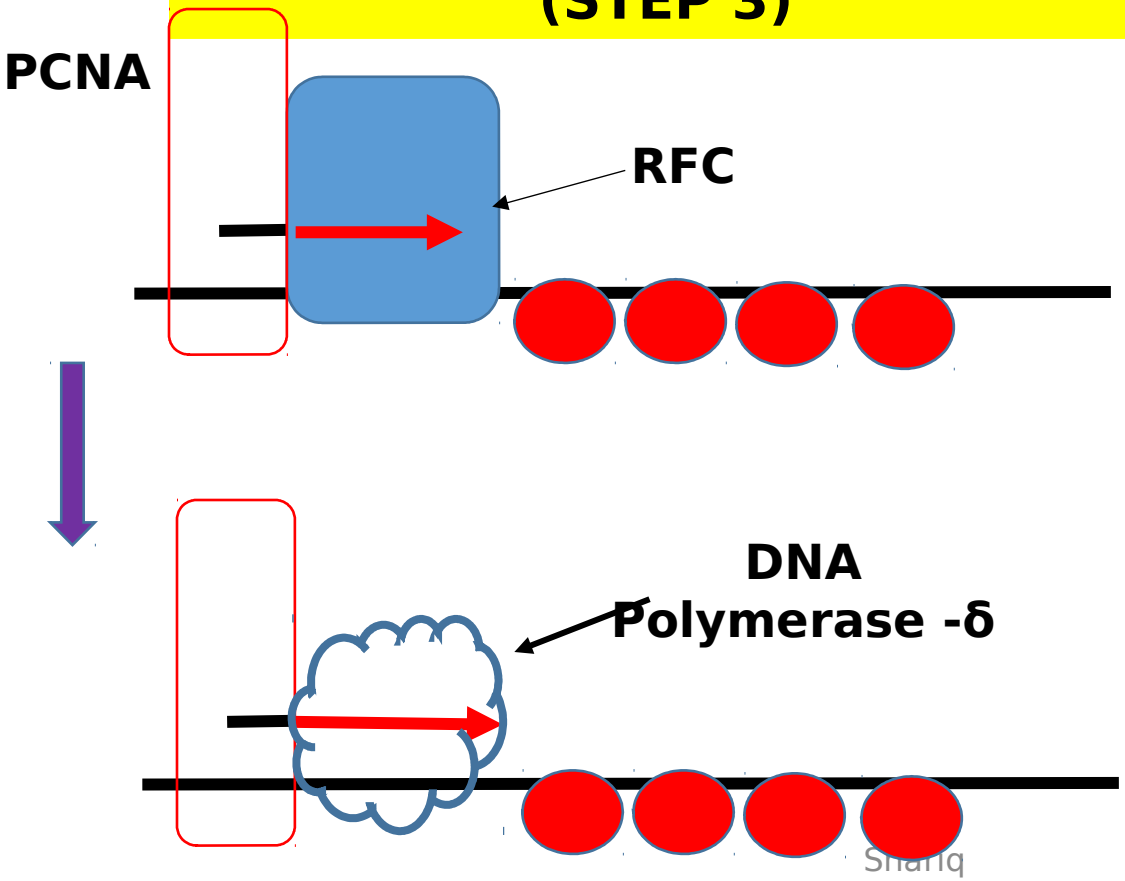
Replication at Lagging Strand (STEP 2)



- Primase forms complex with DNA *Polymerase-α*
- Primase produces RNA primer (10 nucleotide)
- DNA Polymerase-α synthesizes short DNA (20-30 nucleotide)
- Primase+Poly complex dissociates

Eukaryote DNA Replication is *Different*

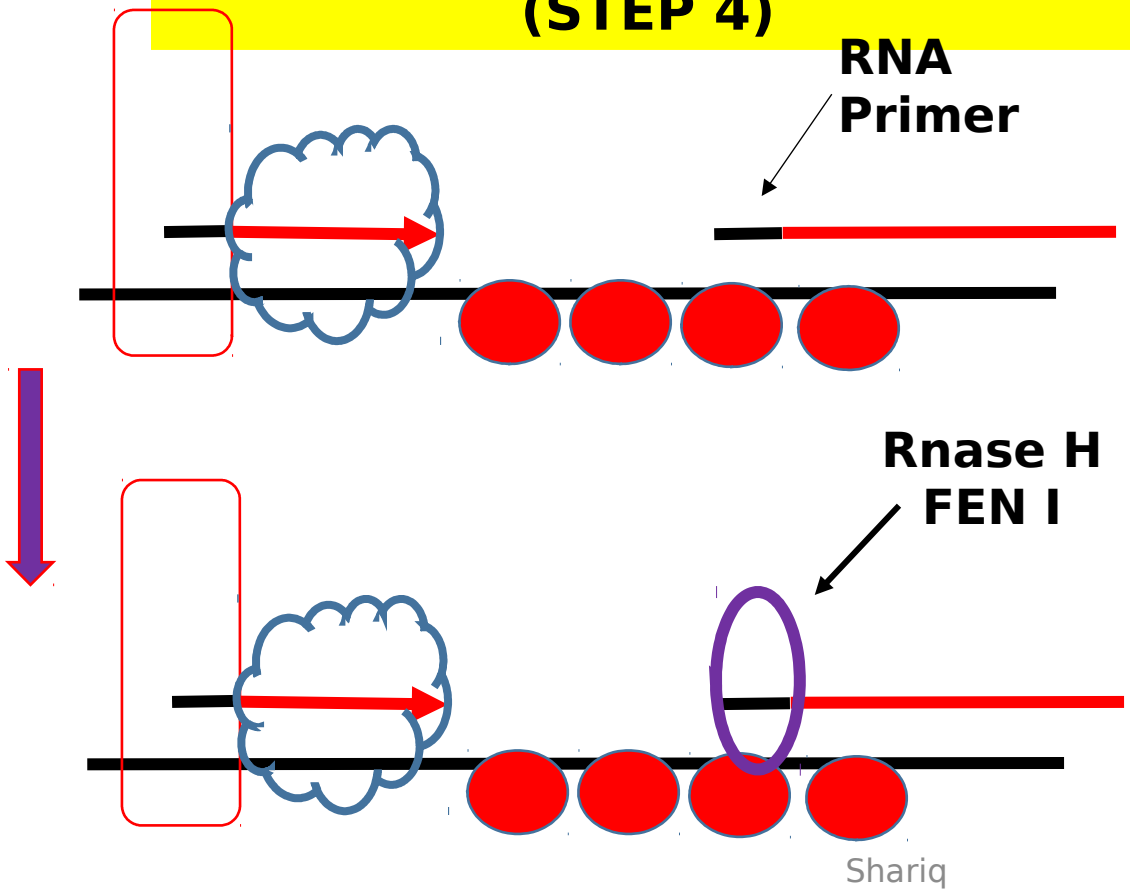
Replication at Lagging Strand (STEP 3)



- RFC (Replication Factor) binds to elongated primer
- PCNA also gets added to RFC
- DNA Polymerase –δ binds to RFC (acts as sliding clamp)
- DNA Polymerase –δ elongates DNA fragment (150-200 bp)

Eukaryote DNA Replication is *Different*

Replication at Lagging Strand (STEP 4)

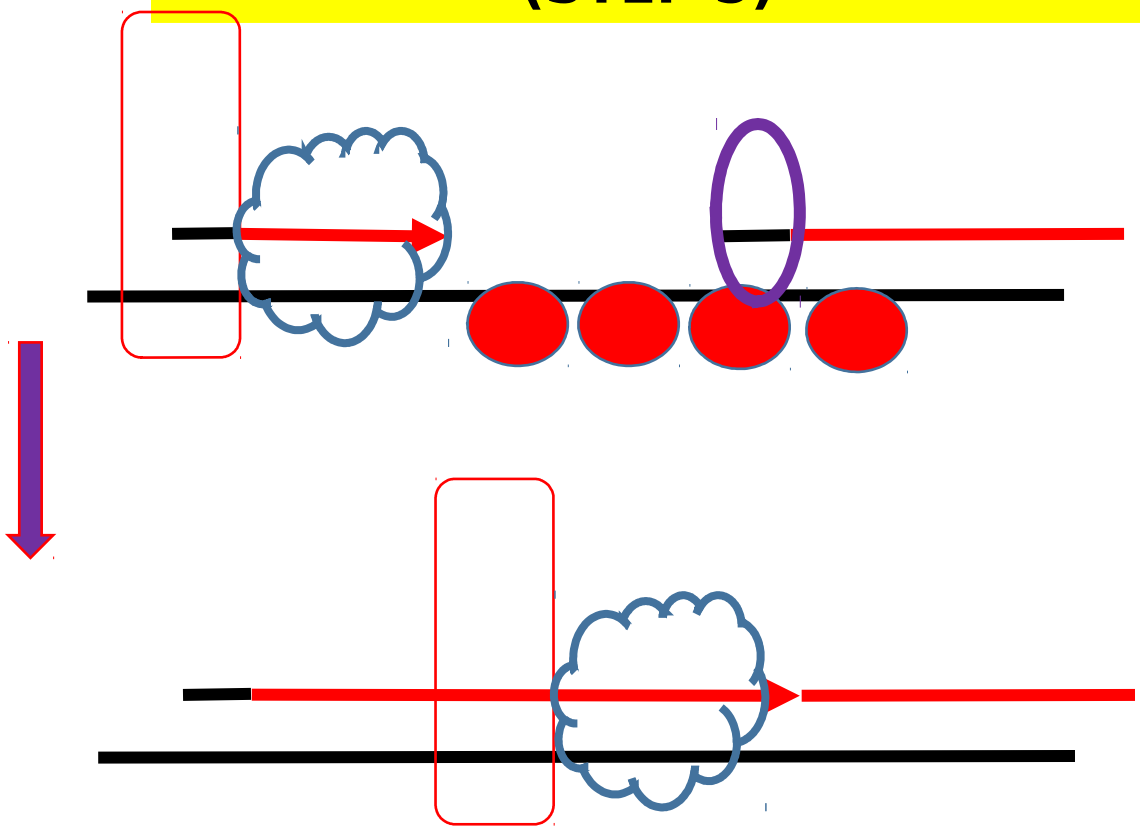


- Replicating DNA approaches primer of previous DNA fragment
- RNA primer is removed by enzymes *Rnase + FEN I*
- Gap filled by new DNA synthesized by DNA polymerase –δ
- Small nicks finally sealed by *DNA Ligase*

Shariq

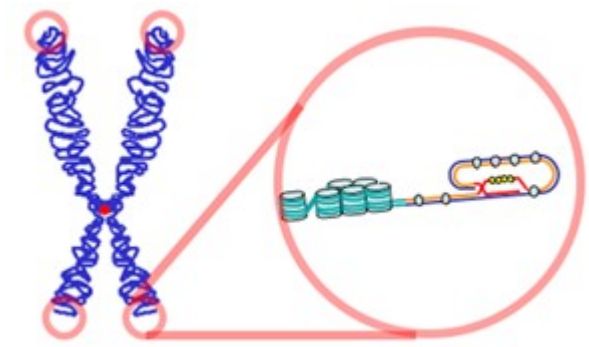
Eukaryote DNA Replication is *Different*

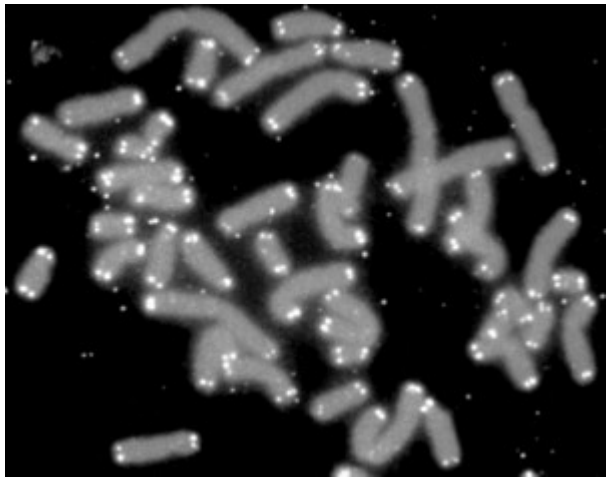
Replication at Lagging Strand (STEP 5)



- Replication DNA approaches primer of previous DNA fragment
- RNA primer is removed by enzymes *Rnase + FEN I*
- Gap filled by new DNA synthesized by DNA polymerase –δ
- Small nicks finally sealed by *DNA Ligase*

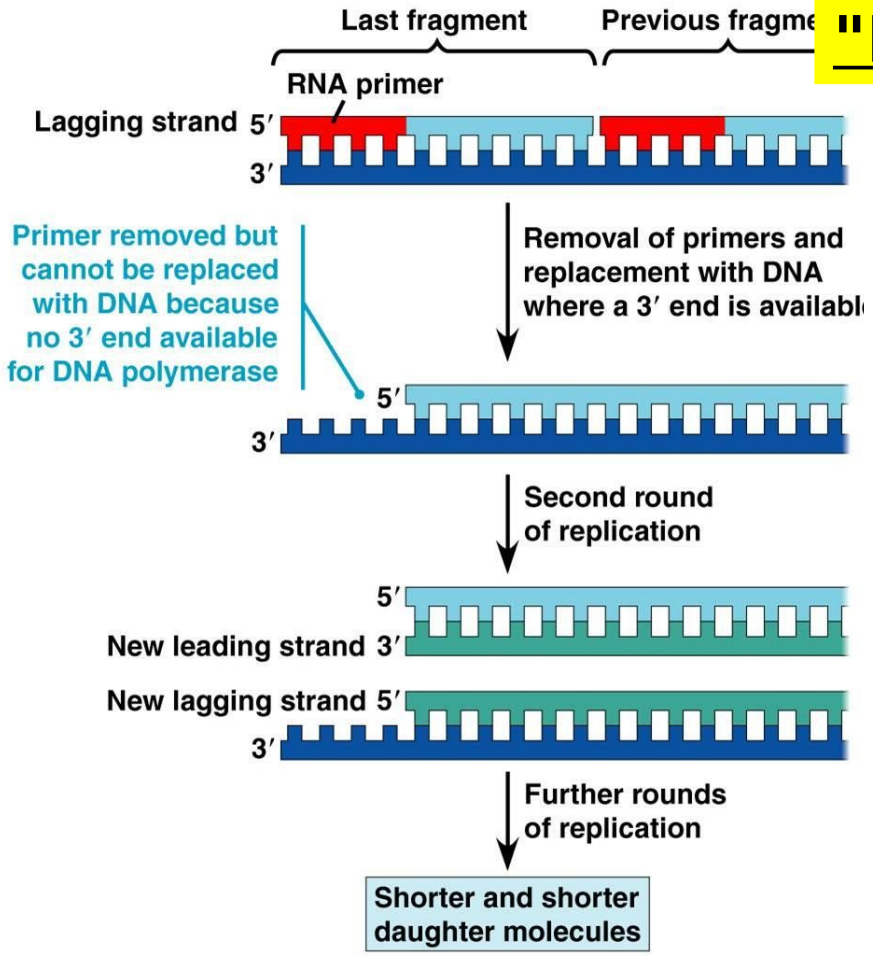
Telomeres



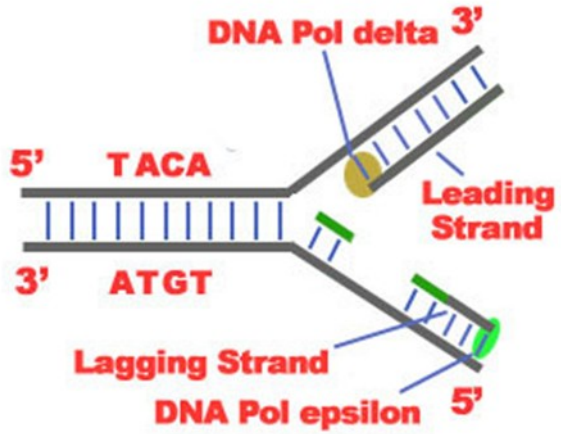


- Telomeres are end of chromosomes (*Telo* = end)
- Special structures to prevent continuous loss of DNA
- Protect ends of DNA
- Prevent chromosome fusing with others
- Sequence of TTAGGG is repeated X1000
- Are disposable buffers at the ends of chromosomes which are truncated during cell division
- They protects the genes before them on the chromosome from being truncated instead

Telomerase

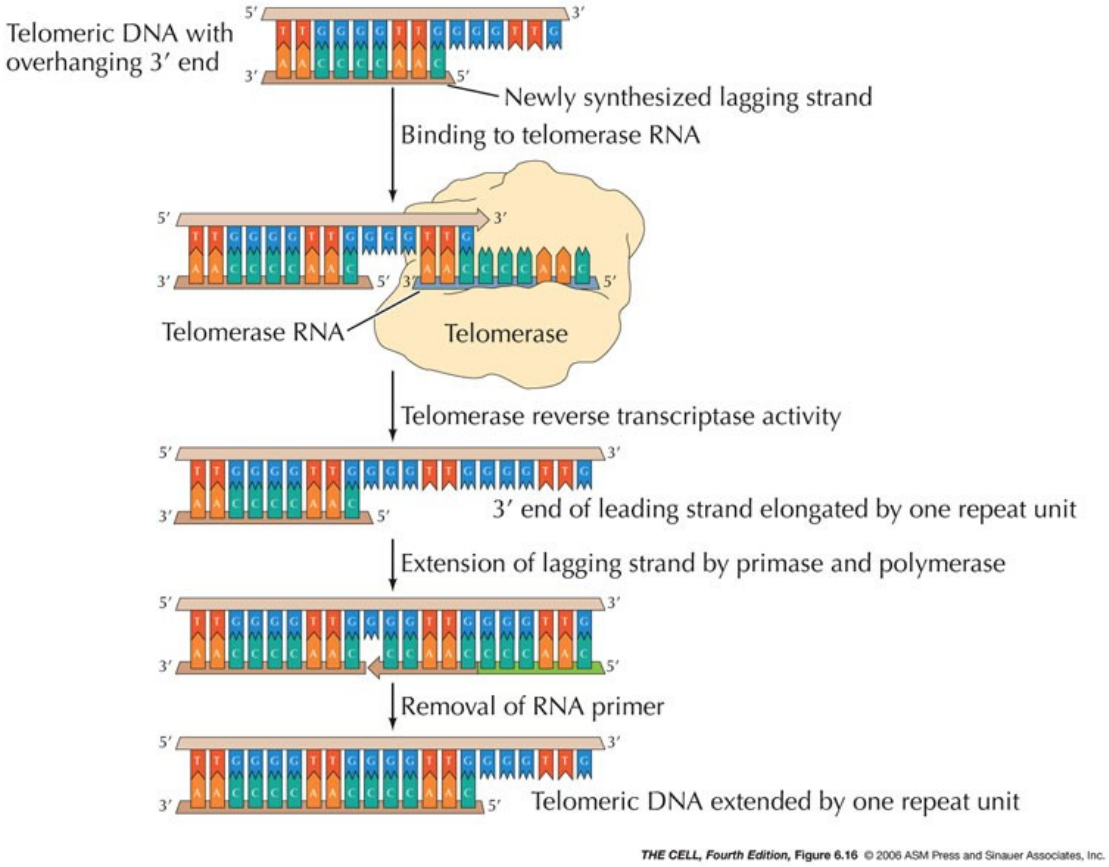


"END-REPLICATION" PROBLEM



- DNA polymerases operate only in the **5' to 3' direction**
- Synthesis of the lagging strand occurs through a **"backstitching" mechanism** that produces short fragments of DNA
- Without its complement, the **hanging piece** of unpaired DNA from the parent strand might be recognized by the cell as a broken piece of DNA and then **damaged or even chopped off** in the cell's

Telomerase



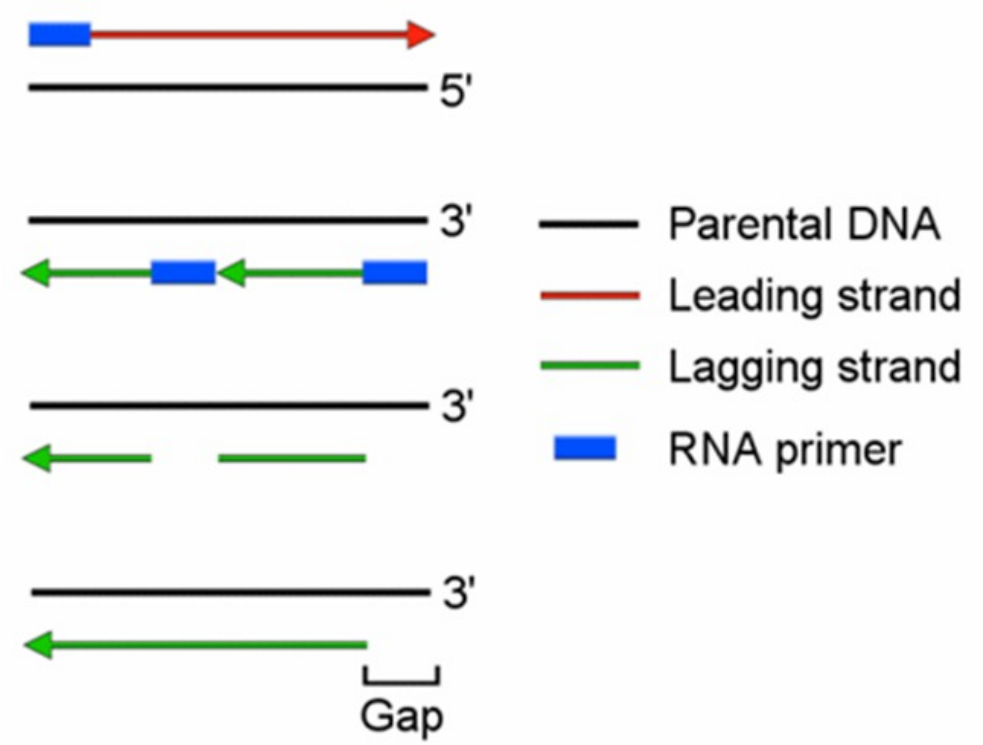
- Telomerase: <u>Unique protein +RNA template</u>
- Reverse transcriptase activity (Create DNA from RNA)
- DNA created is based on telomere template (TTAGGG) at leading strand
- Synthesis of DNA on lagging strand by DNA Polymerase
- At the end RNA primer is removed
- Some shortening will NOT create a problem
- Since Telomere do not encode proteins they Do NOT have to be of same length

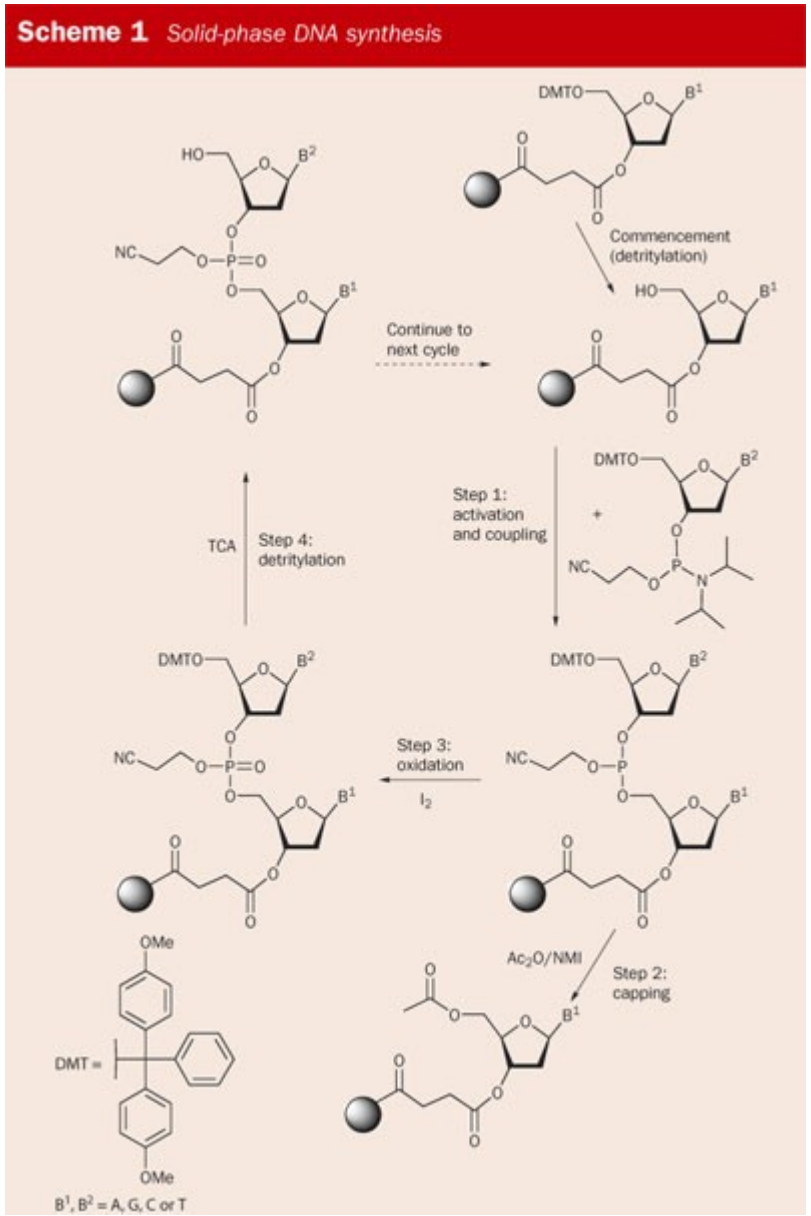
Telomerase

"END-REPLICATION" PROBLEM

During DNA Replication

After DNA Replication





http://www.atdbio.com/content/17/Solid-phase-oligonucleotide-synthesis