BIOL 360

MOLECULAR BIOLOGY

❖**Lecturer**

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or

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General Comments

1. Lateness to class is not entertained under any circumstances. You **may be** turned away once the lectures get under way.

2. Lectures are formal sessions and students are advised to dress properly. Would not allow baseball caps, hats, track suits, sleeveless T-shirts etc.

3. It will be in your interest to use the recommended textbooks and ***ANY supplementary ones; try to visit the publishers' web sites; review regularly previous lectures and read on a topic before a new lecture.***

ALL ASSIGNMENTS SHOULD BE TAKEN SERIOUSLY.

4. ***Mobile phones MUST stay switched off during the entire period of a class. If a phone bells to cause a distraction, it will be confiscated till the end of the semester.***

5. You would be given the opportunity to ***candidly evaluate in confidence***, how the course had impacted on your scholarship getting to the end of the semester.

6. **Plagiarism and cheating** in any form by either copying from a fellow student or copying verbatim from internet sites and textbooks would ATTRACT THE SEVEREST FORM OF SANCTIONS. THE CANDIDATE SHALL LOSE ALL CONTINUOUS ASSESSMENT MARKS.

MIDSEMESTER EXAMS AND CONTINUOUS ASSESSMENT

- Both the mid-semester and the continuous assessment will make up 30% semester mark.

- The final paper (end of semester) will also make up 70%.

- Mid-semester exams will be discussed later.

COURSE OUTLINE (SYLLABUS)

Molecular Biology will focus on the following:

- DNA Structure
- Semiconservative Replication
- Replication of Eucaryotic chromosomes
- Bacterial and Viral Genetics

- Mutation
- Introduction to Gene Transfer (Transgenics)
- The Cell Cycle

INTRODUCTION & COURSE OBJECTIVES

- Through lectures, assignments and students reading on their OWN, this course will give sufficient detail that will lead to higher genetics such as **Genetic Engineering, Modification and Gene Expression (BIOL 451)** in the final year.

- It will help us know and appreciate the structure of DNA in terms of its components (i.e. nucleotides:- phosphate, pentose sugar, and a base)

- Get to know certain properties of DNA like denaturation, renaturation, bouyant density etc.

- Consider the modes of replication i.e. dispersive, conservative and semiconservative.

- Will be considering the experiments of Meselson and Stahl.
- ✓Their experiment gave a convincing evidence to support the semiconservative nature of replication).

- The course will again help us to appreciate how replication is initiated, elongated and terminated.
- ✓(Emphasis will be laid on all the enzymes involved in these processes).

- DNA repair mechanism.

- Bacterial transformation (how an exogenous DNA is taken up by a recipient cell using bacteria).

- Will consider the two types of transformation (i.e. natural and artificial)

- Viruses: Consider the definition, structure, classification, their life cycles and some properties of phage lambda (the lytic and lysogenic cycles).

- Transduction; which is also one of the major mechanisms of transferring genetic material from one bacterium to another.

- Will be considering the two types of transduction (i.e. generalized and specialized).

- Mutations; their causes and types.
- Introduction to Gene transfer (some practical applications of Molecular Biology/Genetics)
- *** The Cell Cycle***

RECOMMENDED TEXTBOOKS

1. Genetics
   Copyright © 1997, 3rd Edition,
   by Robert F. Weaver and Philip W. Hedrick

2. The Biochemistry of the Nucleic Acids
   Copyright © 1992, Eleventh Edition,
   by Roger L. P. Adams; John T. Knowler and
   David P. Leader

3. Genes V and Genes VI
Copyright © 1997
by Benjamin Lewin

4. Principles of Genetics
Copyright © 1991
by E. J. Gardner; M. J. Simmons and
D.P. Snustad

5. Molecular Biology
   Copyright © 1999
   by Robert F. Weaver

6. Dictionary of Microbiology and Molecular Biology.
   Copyright © 1987 and Reprinted in 1997
   by Paul Singleton and Diana Sainsbury

❑Questions and Answers

❑ MOLECULAR BIOLOGY

What is Molecular Biology?

- The term, has more than one definition and it depends on the person defining it.

- ***It is the study that recognizes the essential properties of genetics in terms of the structures of their macromolecules OR***

- ***It is the study of gene structure and function at the molecular level.***

It must be noted that, Molecular Biology/Genetics grew out of the disciplines of genetics and biochemistry.

BRIEF HISTORY ON THE STRUCTURE OF DEOXYRIBONUCLEIC ACID (DNA)

- All experiments described so far point to nucleic acid (DNA or RNA) as the carrier of genetic information.

- In the early 1950's, through effective investigation, scientists like Rosalind Franklin and Maurice Wilkins (X-ray crystallographers), Francis Crick, Watson, Chargaff and others were able to elucidate the structure of DNA.

- The foundations on which the structure of DNA were based, included the following:

  (i) structural and theoretical chemistry analysis of base compositions of DNA's from a variety of species

  (ii) x-ray crystallographic data

(iii) the ability of Watson and Crick to build structural models that were consistent with the chemical and physical data.

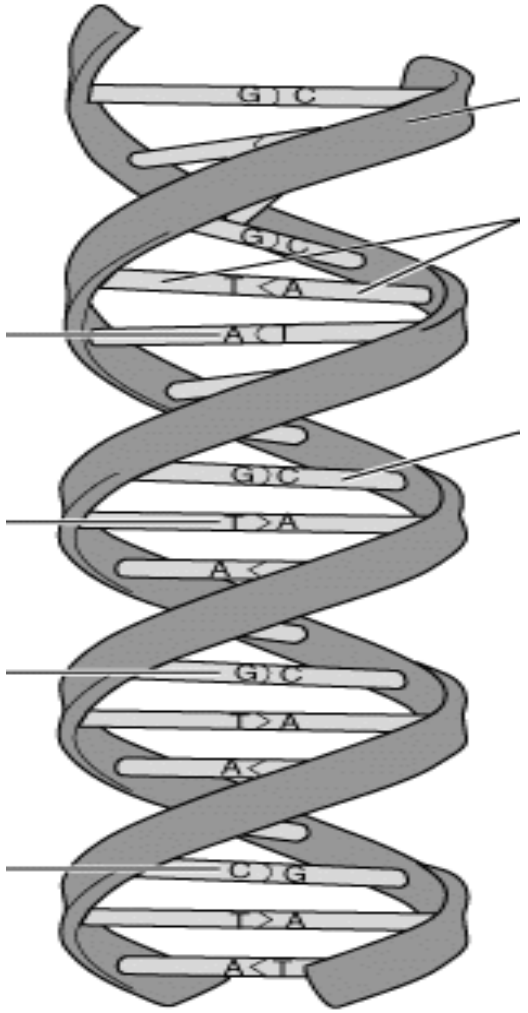
- Aaron Levene provided an important information about the chemical composition of DNA and concluded that, it is a polymer of **purine** and **pyrimidine** nucleotides.

- Using an x-ray crystallographic picture of DNA made by Franklin, Watson and Crick were able to propose a model for the structure of DNA.

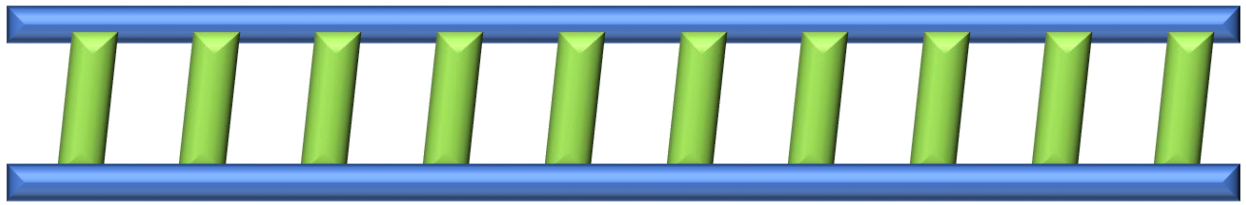
DNA is a Double Helix

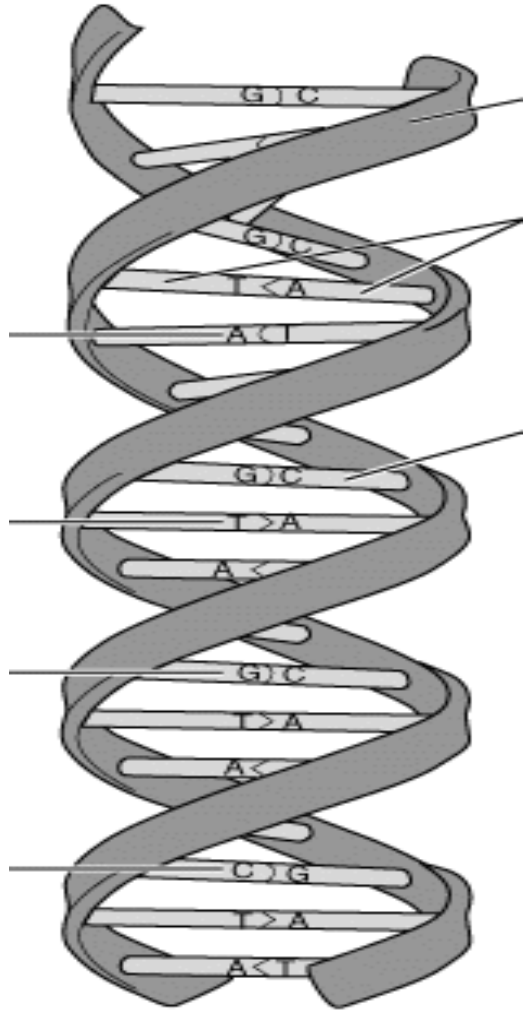
- According to the model, ***DNA is composed of two long, unbranched polymers of deoxynucleotides lying side by side.***

OR



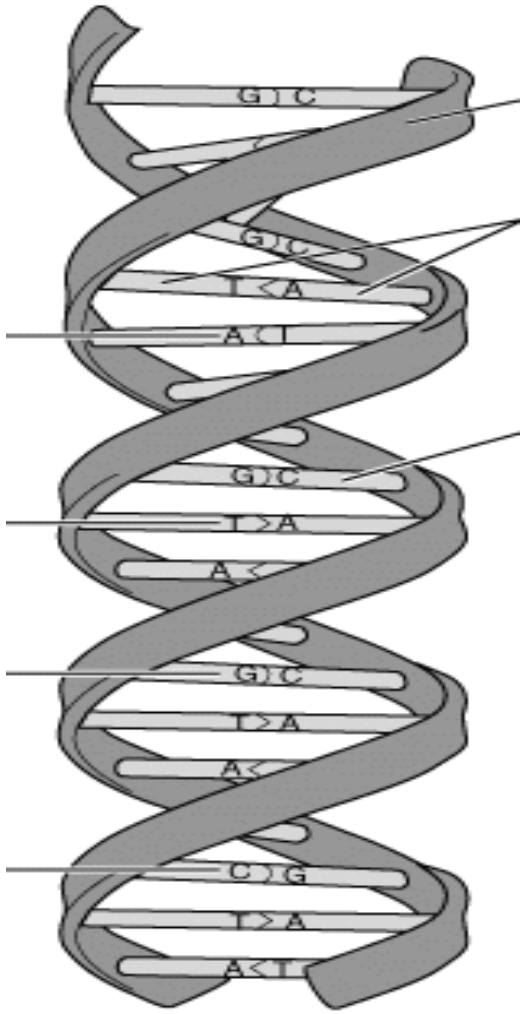
- It is a high molecular weight polymeric compound which consists of two molecules that are arranged into a ladder-like structure called a **Double Helix**.





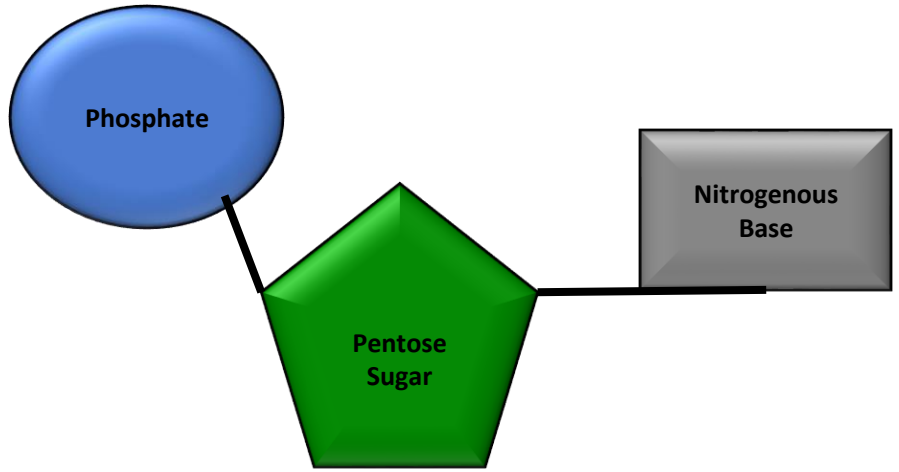
- The backbone of the helix is composed of two chains with alternating sugar-phosphate units.

- The sugar is a pentose .

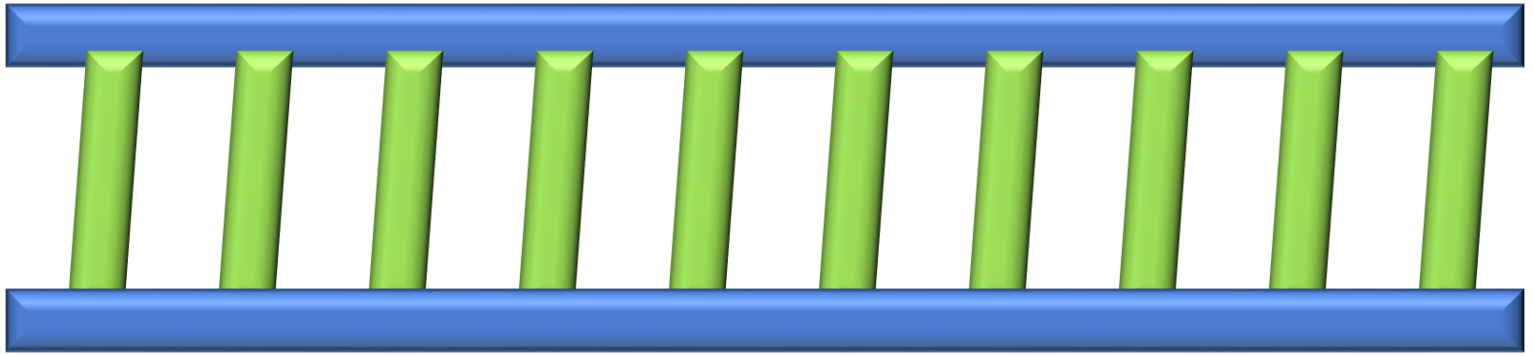


- A molecule of DNA is made up of millions of tiny subunits called **Nucleotides**.

- Each nucleotide consists of:
  1. **Phosphate group**
  2. **Pentose sugar**
  3. **Nitrogenous base**

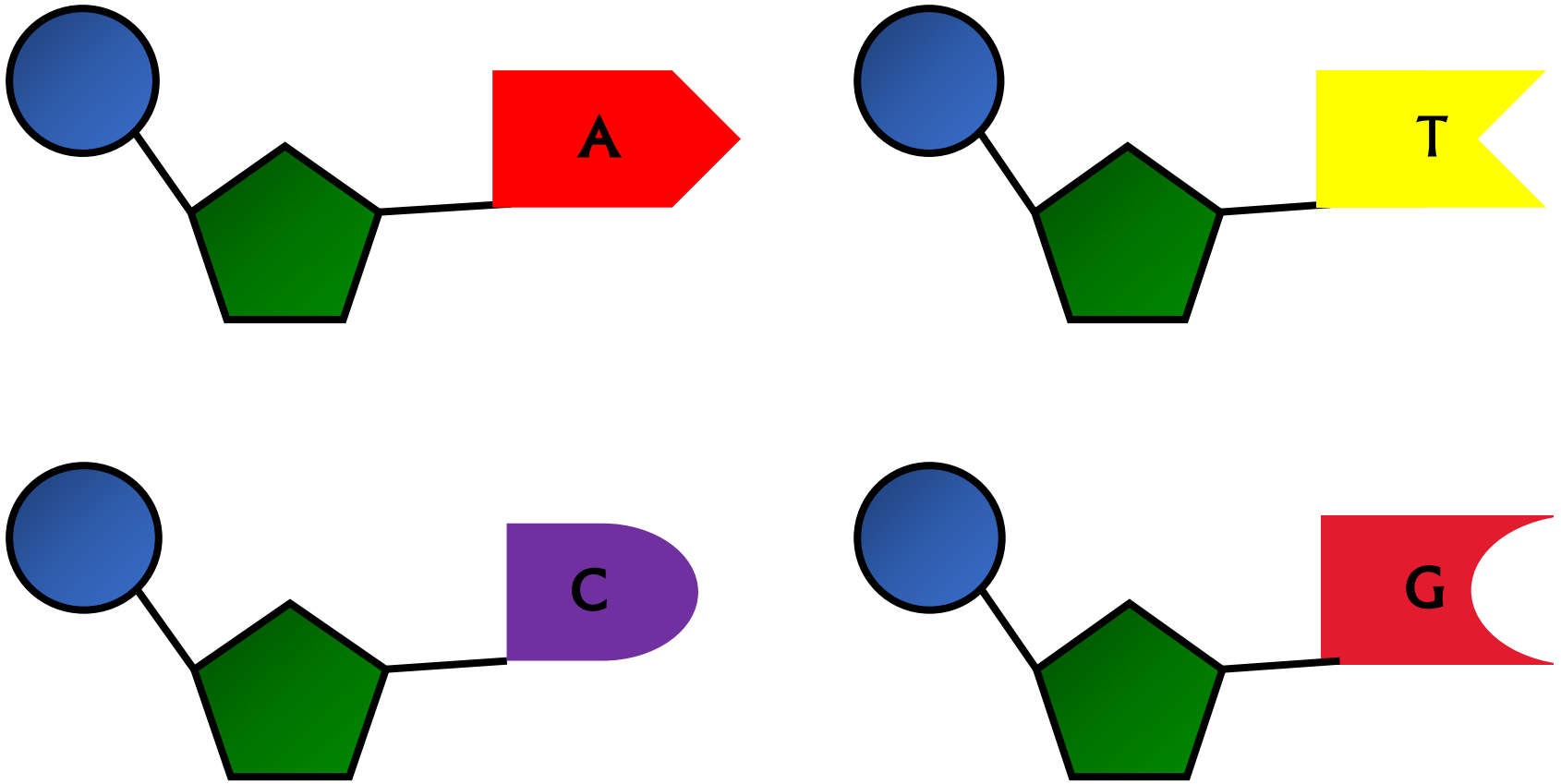


- The phosphate and sugar form the backbone of the DNA molecule, whereas the bases form the “rungs”.



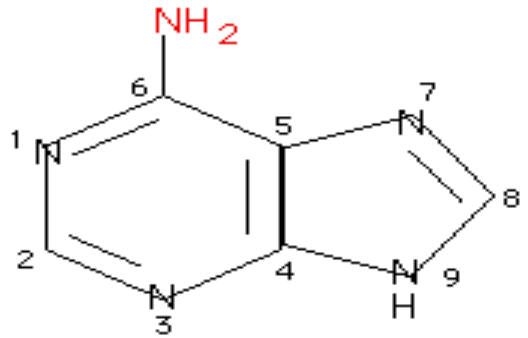
- Complete hydrolysis of DNA yield **purine and pyrimidine bases,** a **pentose sugar** , and a **phosphate group**.

- There are **four** types of nitrogenous bases.

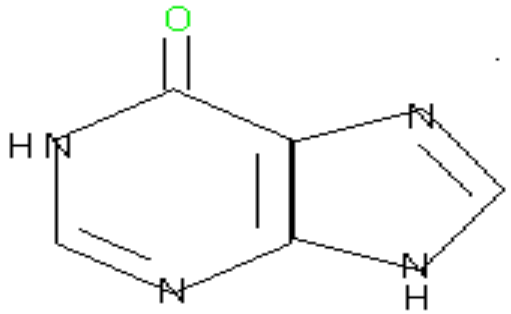


Purines (Adenine, Guanine, Hypoxanthine)

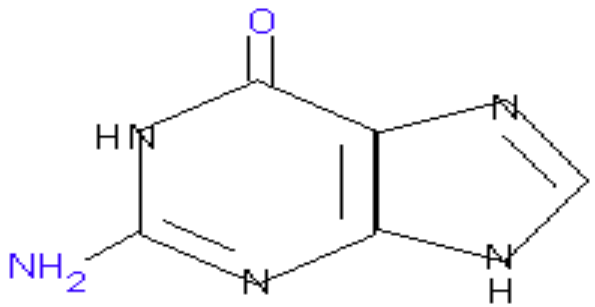
- Purines are heterocyclic ring of carbon and nitrogen atoms). Some purines are



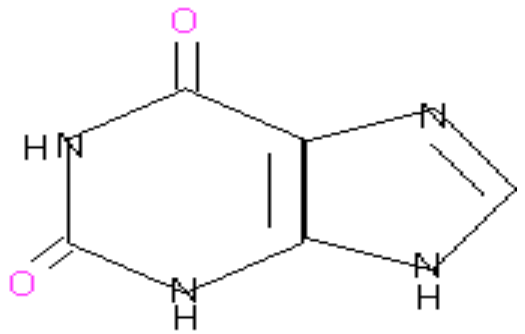
Adenine



Hypoxanthine



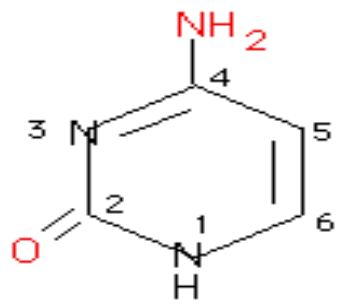
Guanine



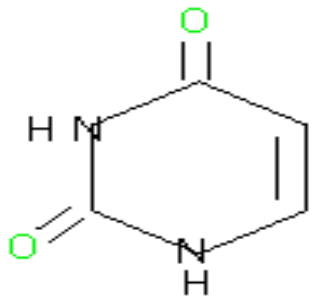
Xanthine

Pyrimidines (Cytosine, Thymine, Uracil)

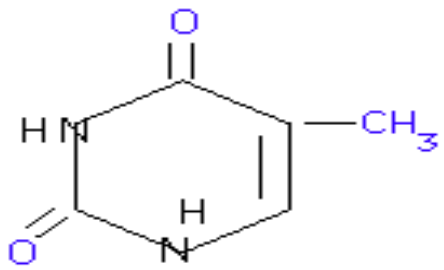
- Pyrimidines are cyclic ring of carbon and nitrogen atoms. Some examples are



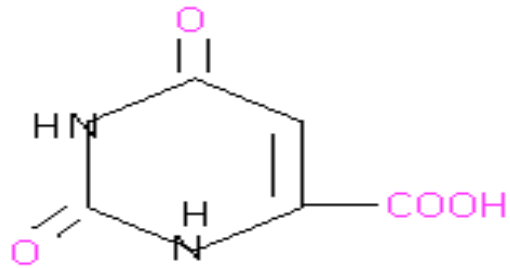
Cytosine



Uracil

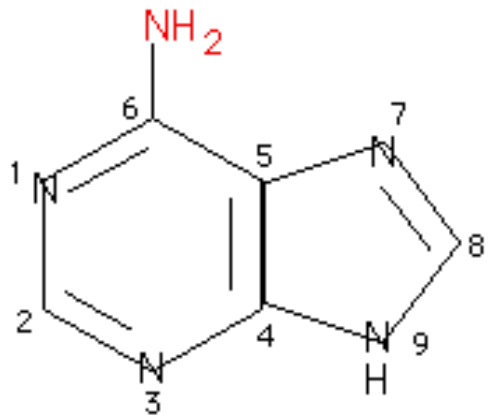


Thymine

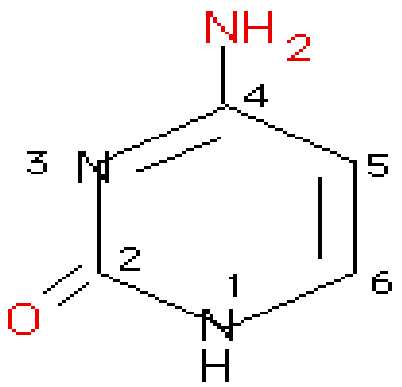


Orotic Acid

- It must be noted that, the style of numbering of the pyrimidine ring in the purines differs from that used for the pyrimidines themselves.



Adenine



Cytosine

- The major bases found in DNA are **cytosine** and **thymine** (**uracil** in RNA).

- In certain bacterial viruses, cytosine is replaced by **5-methylcytosine** or **5-hydromethylcytosine**.

- **Each base will only bond with one other specific base.**

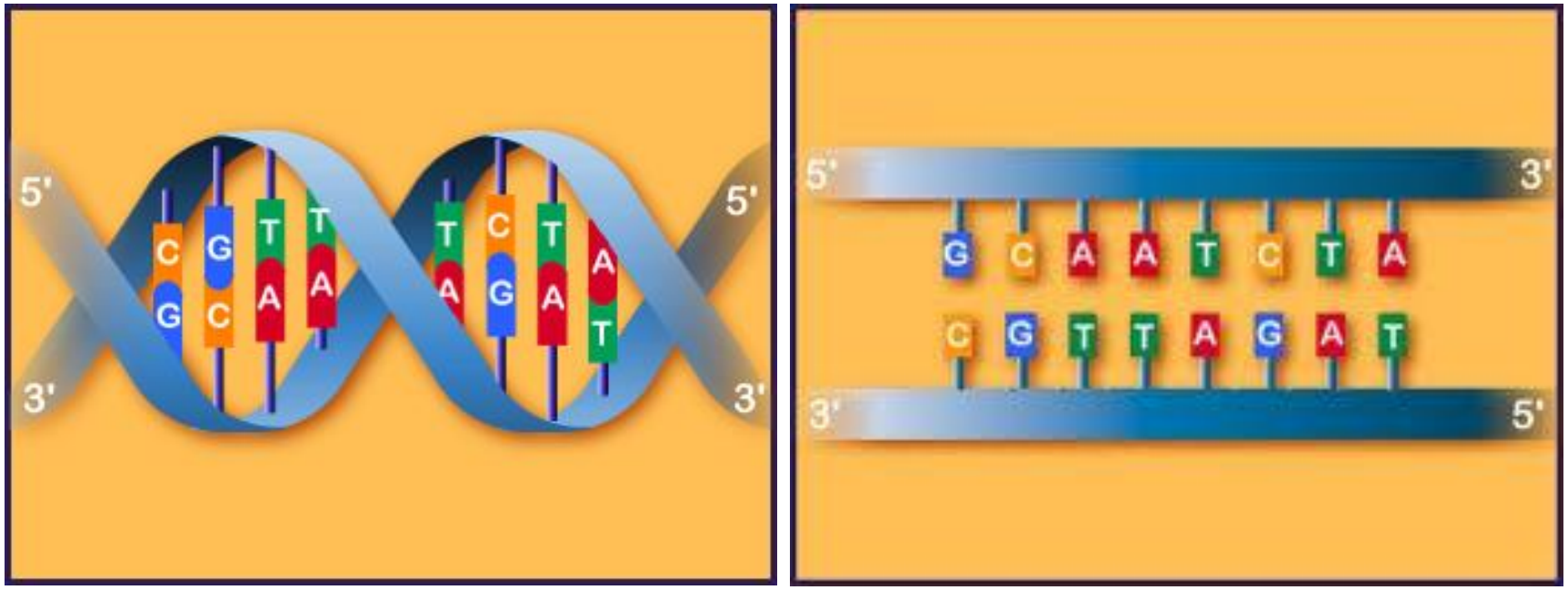
- Adenine (A)
- Thymine (T)

} Form a base pair.

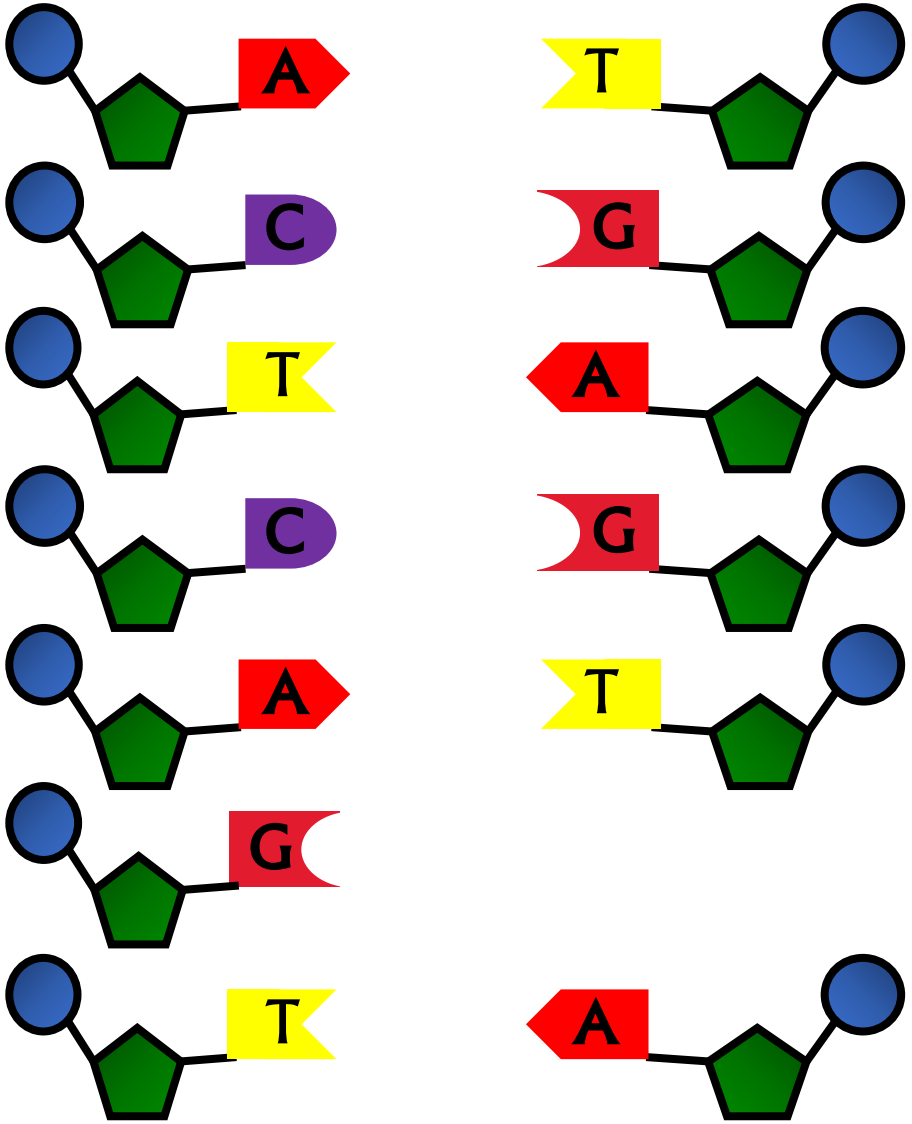
- Cytosine (C)
- Guanine (G)

} Form a base pair.

✓illustration

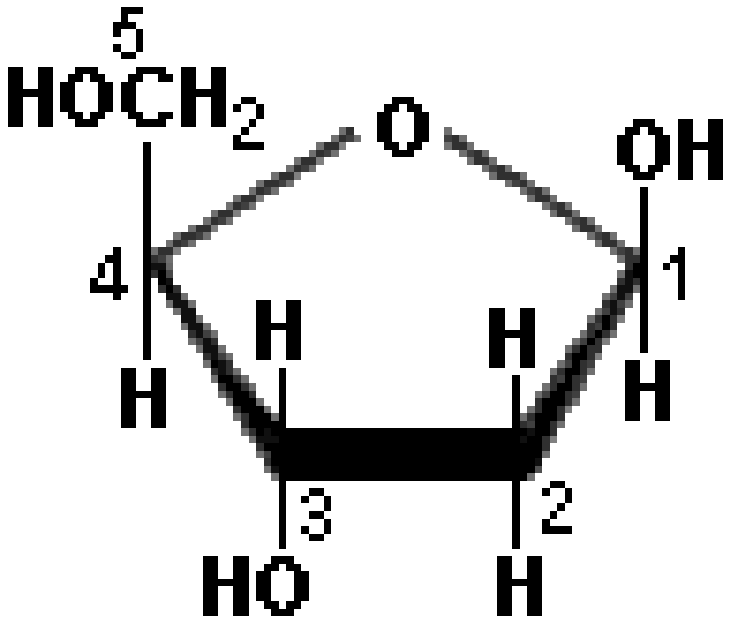


- Because of this **complementary** base pairing, the order of the bases in one strand determines the order of the bases in the other strand as shown below.

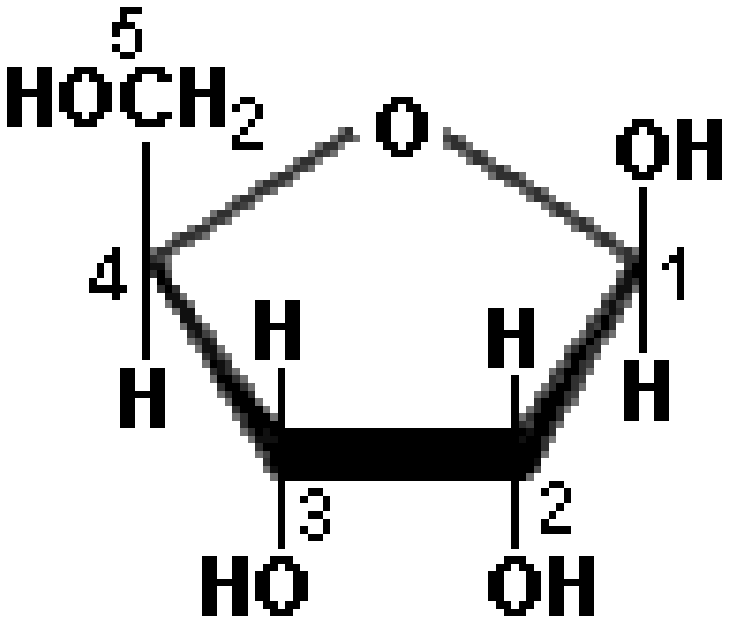

A
C
T
C
A
G
T
T
G
A
G
T
A

Pentose sugar

- A **Pentose sugar** is a five-carbon sugar in a ring form.



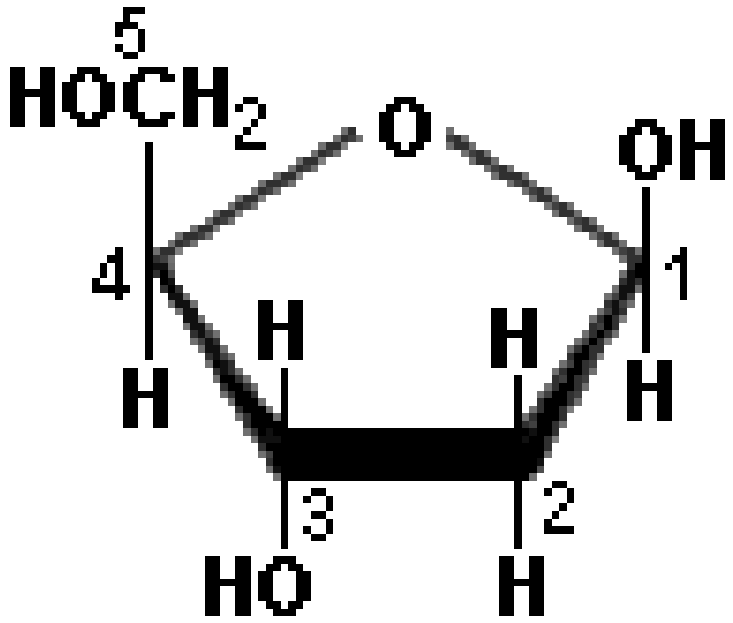
Deoxyribose



Ribose

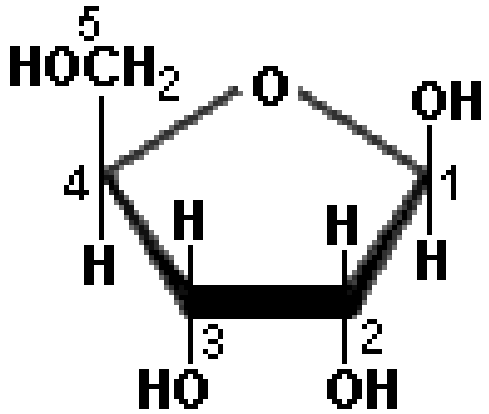
The sugar component

- The sugar component of DNA is **2-deoxyribose.**



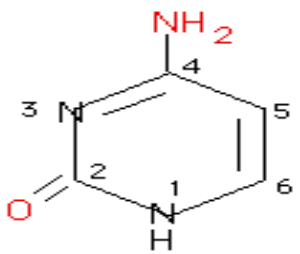
- The absence of OH- at carbon 2 has wide ranging effects on both their chemistry and structure.

- This is because the presence of the bulky hydroxyl group on the 2-position of the sugar ring (ribose) not only limits the range of possible secondary structures available to the RNA molecule,

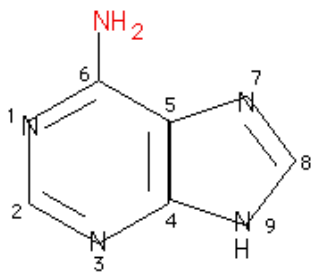


- but also makes it more susceptible to chemical and enzymatic degradation.

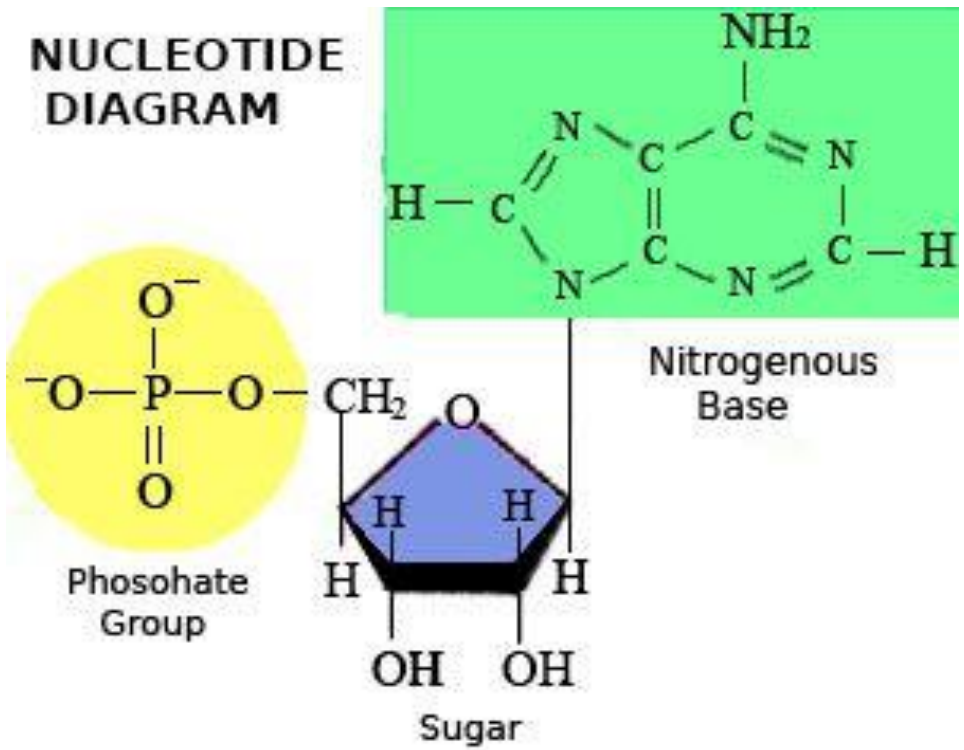
- The nitrogenous base is linked to position 1 on the pentose ring by a glycosidic bond from N_1 of pyrimidines or N_9 of purines.

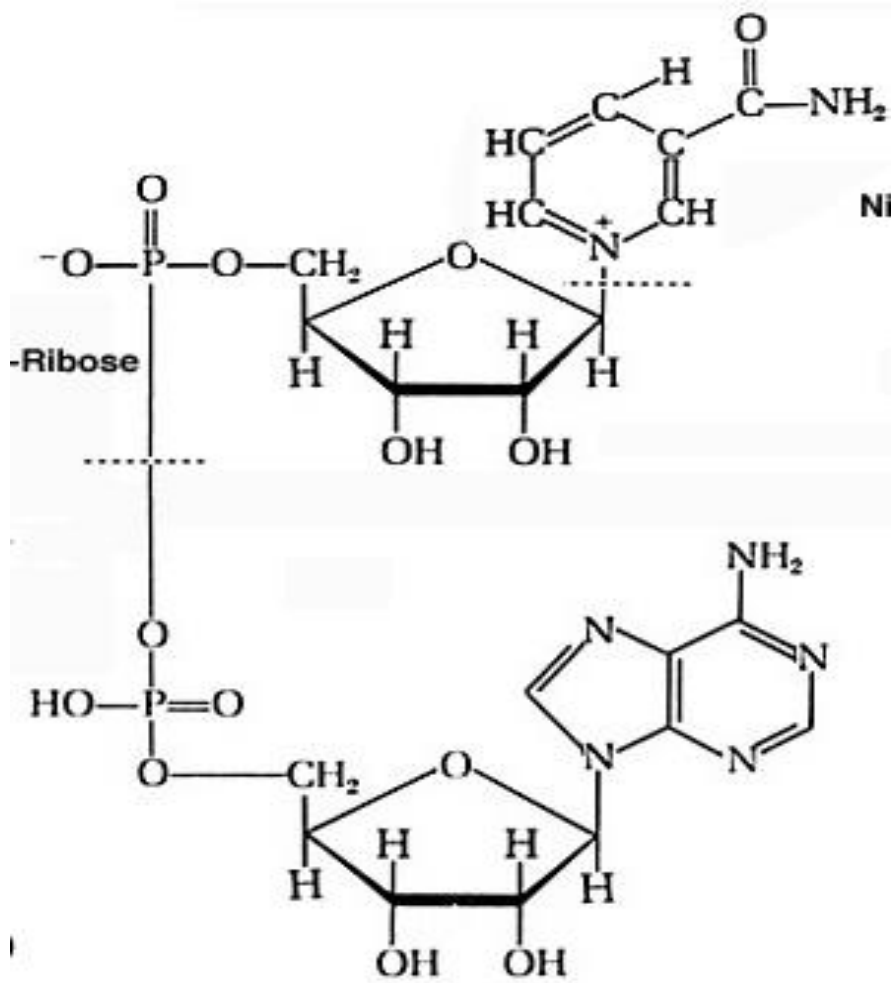


Cytosine

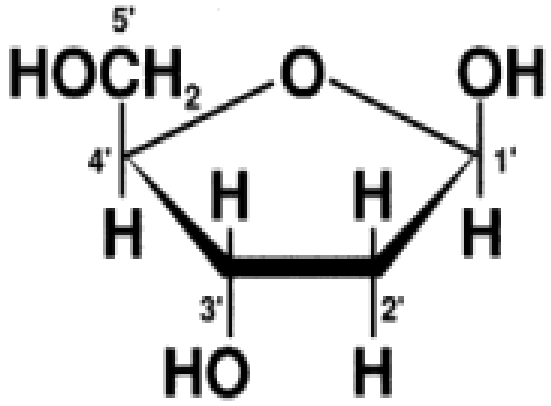


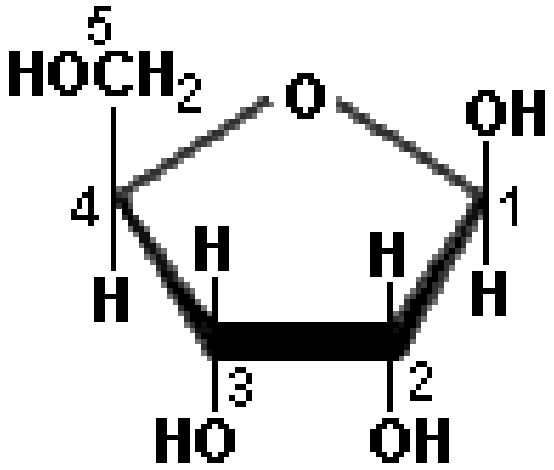
Adenine


NUCLEOTIDE DIAGRAM
NH2
N
C
C
N
H
C
C
C
N
N
C
H
Nitrogenous Base
O⁻
⁻O
P
O
O
CH2
O
H
H
H
H
OH
OH
Phosohate Group
Sugar


O
H
C—NH₂
HC
C
HC
CH
N
Ni
⁻O—P—O—CH₂
O
-Ribose
H
H
H
H
OH
OH
NH₂
N
N
N
N
HO—P=O
O
O—CH₂
O
H
H
H
H
OH
OH

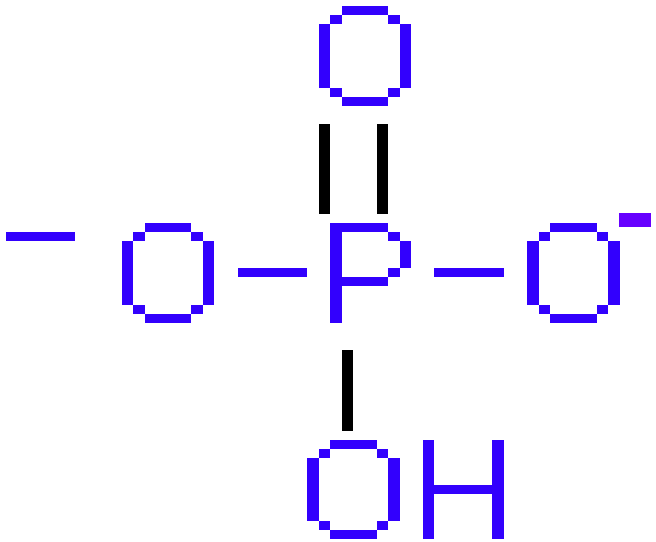
- To avoid ambiguity between the numbering systems of the heterocyclic rings and the sugar, positions on the pentose are given a prime (‘)



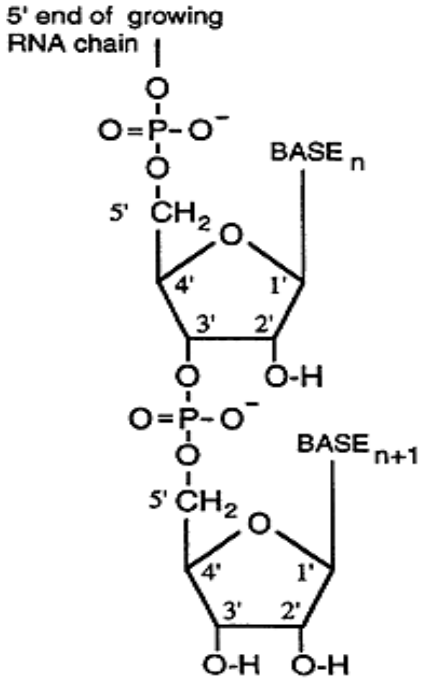


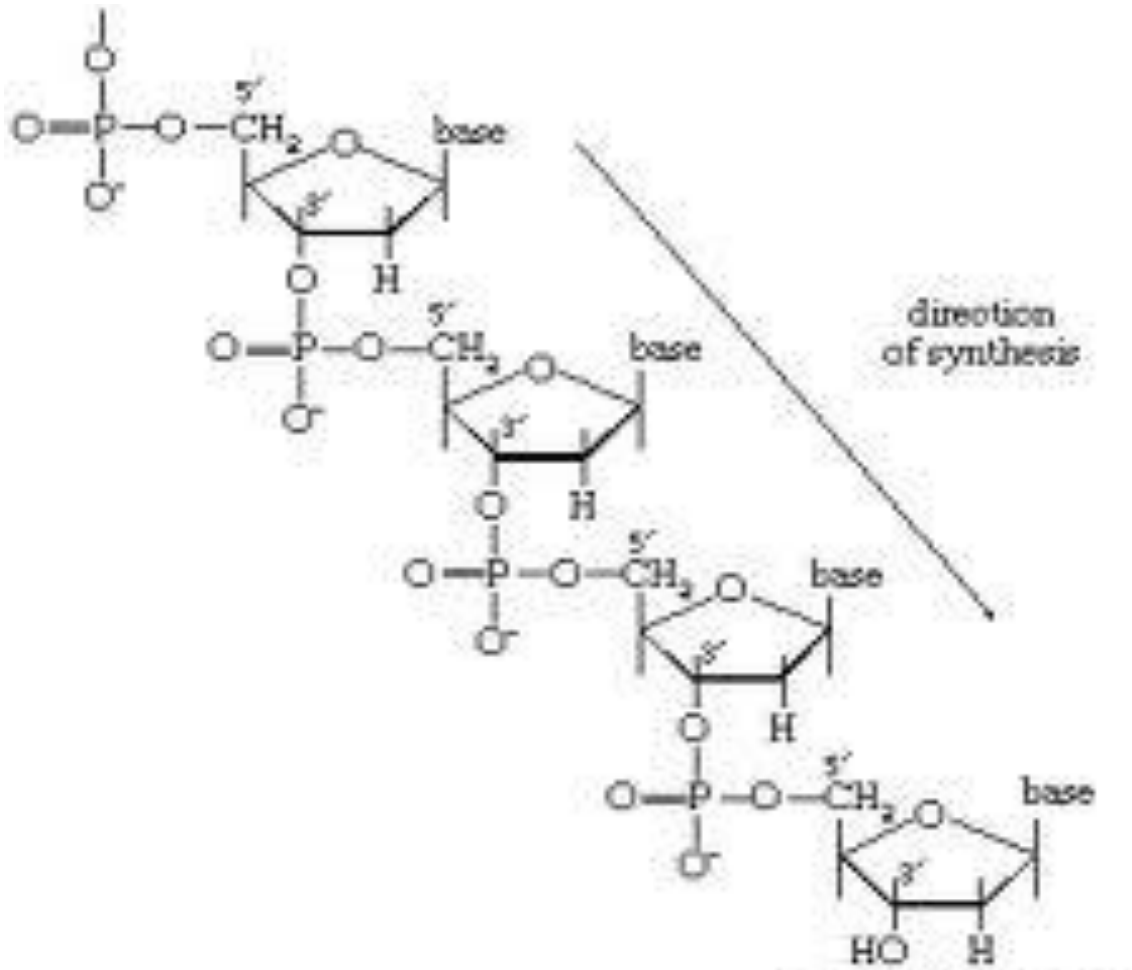
Phosphate group

- **HPO_4^{2-}**



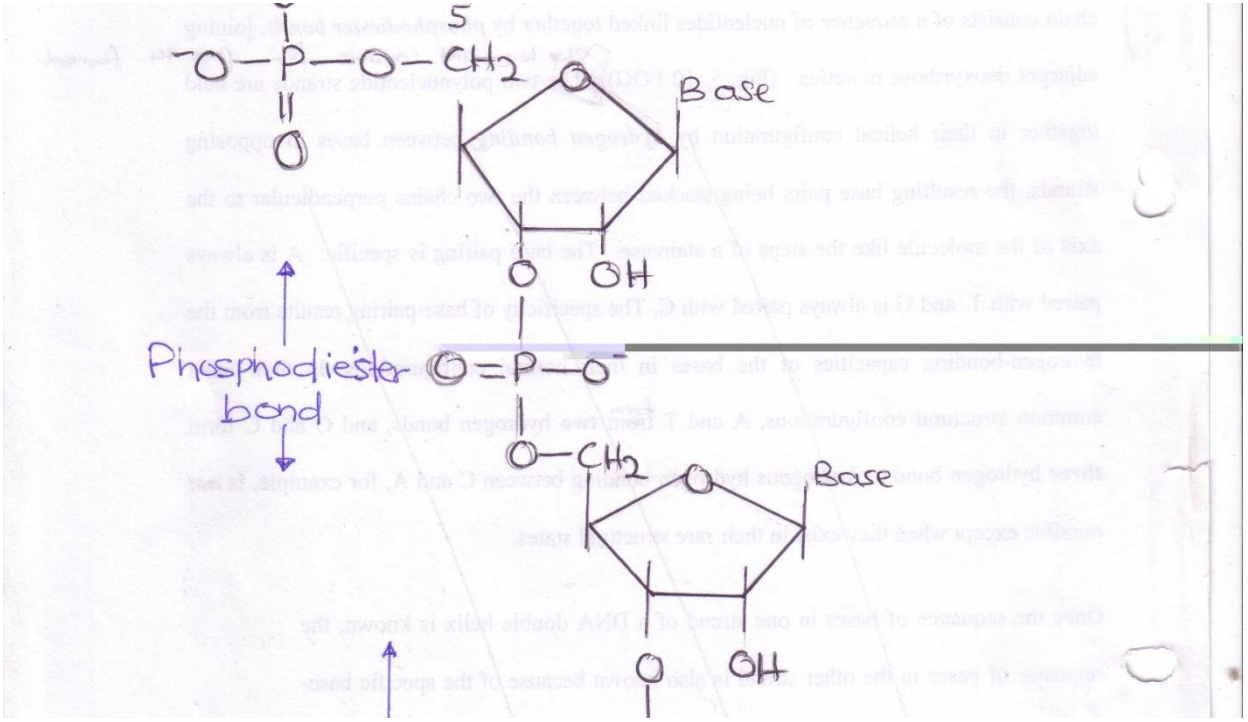
- The 5’ position of one pentose ring is connected to the 3’ position of the next pentose via a phosphate group as shown below



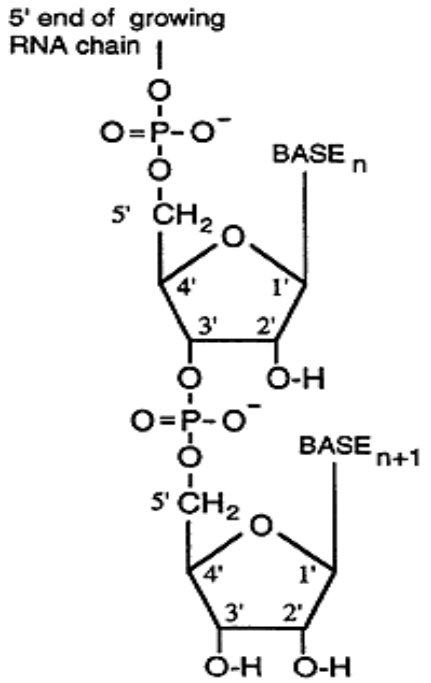


DNA strand

- Thus, the sugar-phosphate backbone is said to consist of 5′ – 3′ **phosphodiester** bond or linkages.



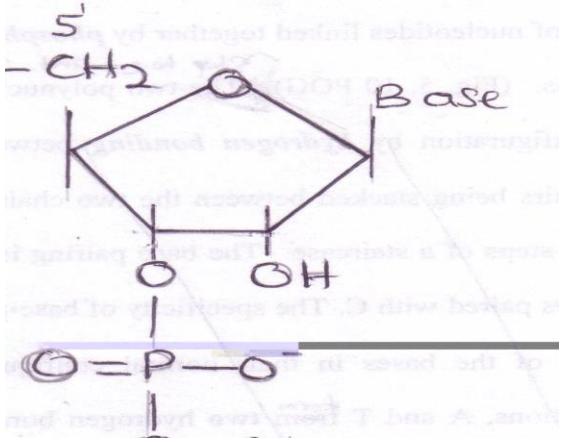
- When DNA (RNA) is broken into its constituent nucleotides, the cleavage may take place on either side of the phosphodiester bonds.

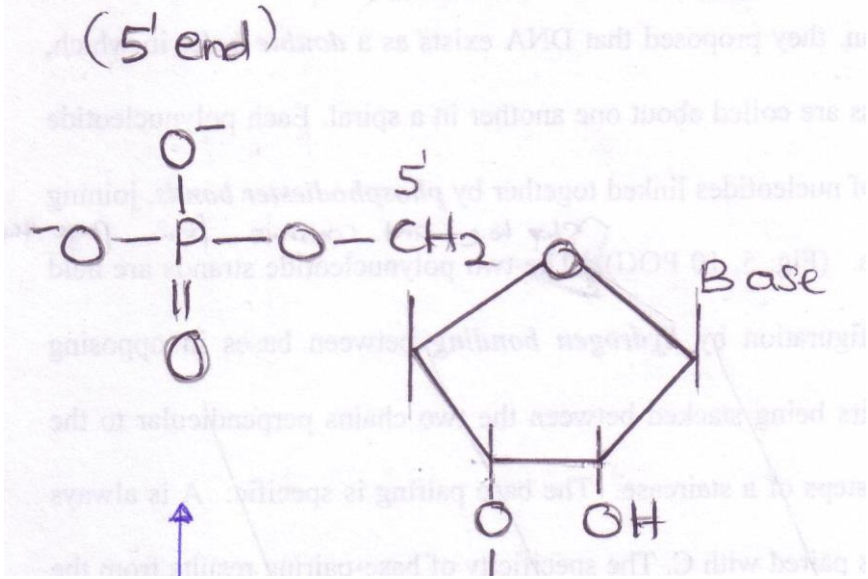


- The two types of nucleotides released from N.A. are therefore:

  (i) nucleoside-3’-monophosphate and

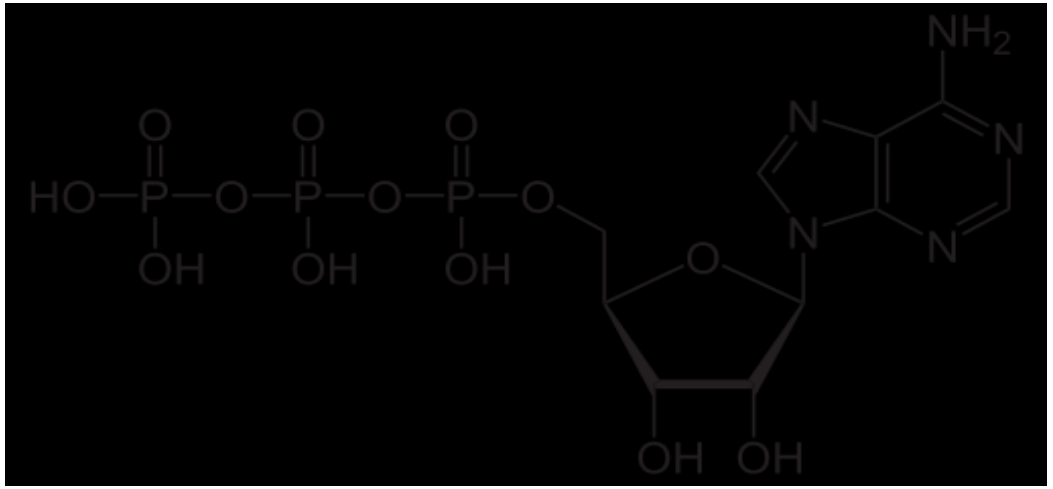
  (ii) nucleoside-5’-monophosphate.

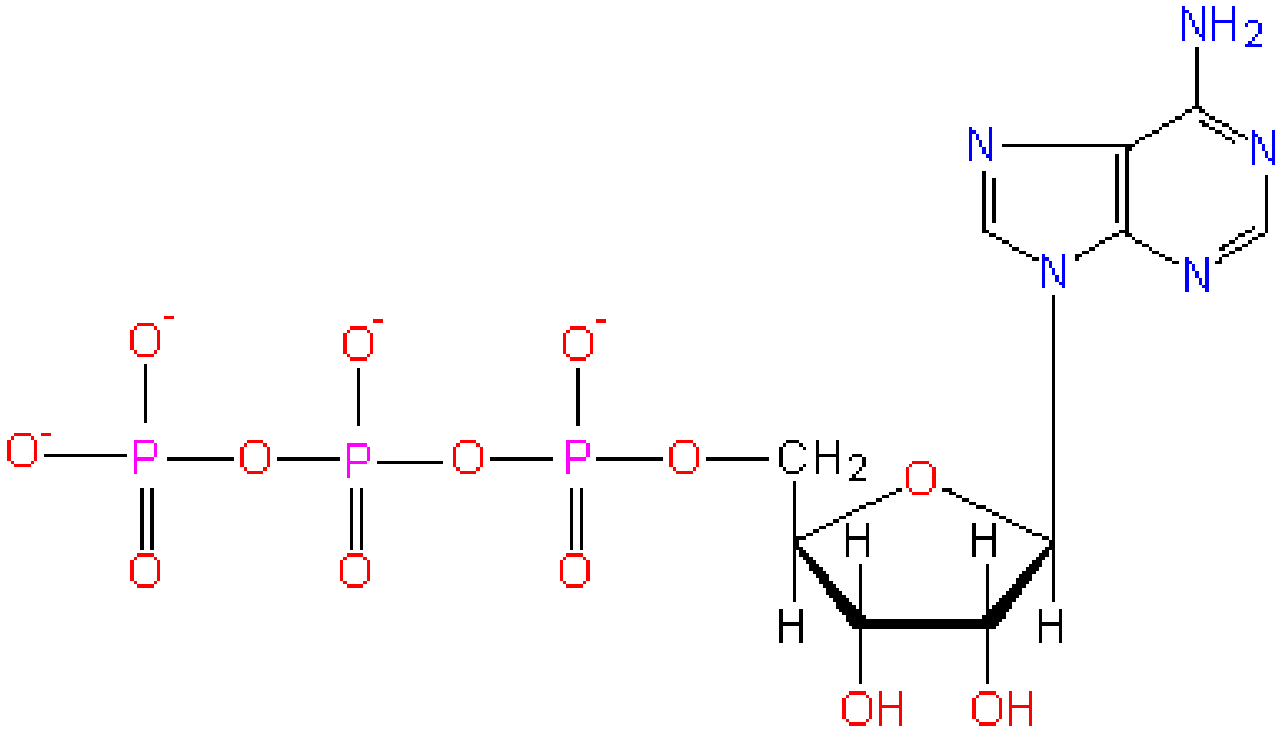




- All the nucleotides can exist in a form in which there is more than one phosphate group linked to the 5’ position.

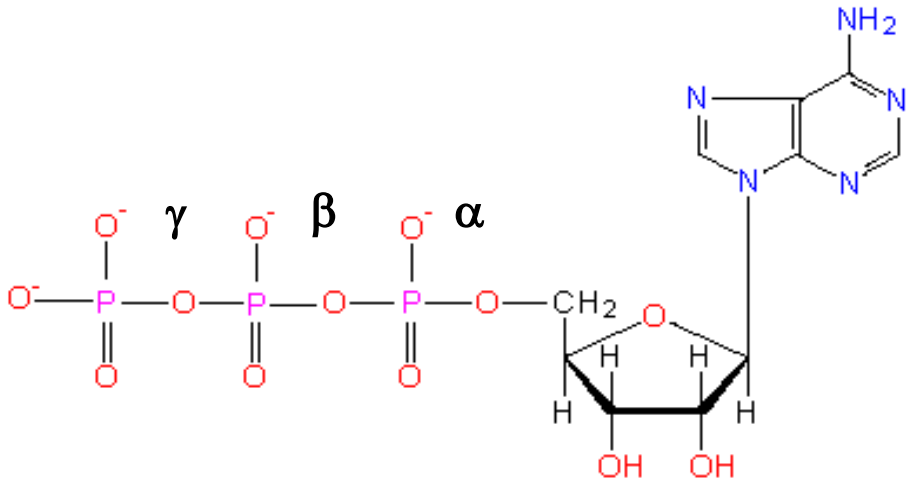
- Draw nucleoside-5’-triphosphate



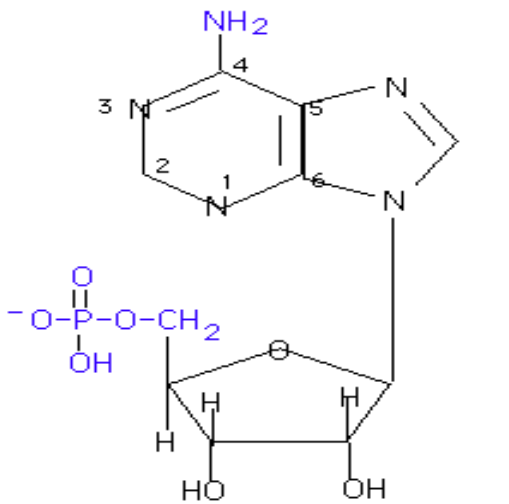


Adenosine-5-triphosphate

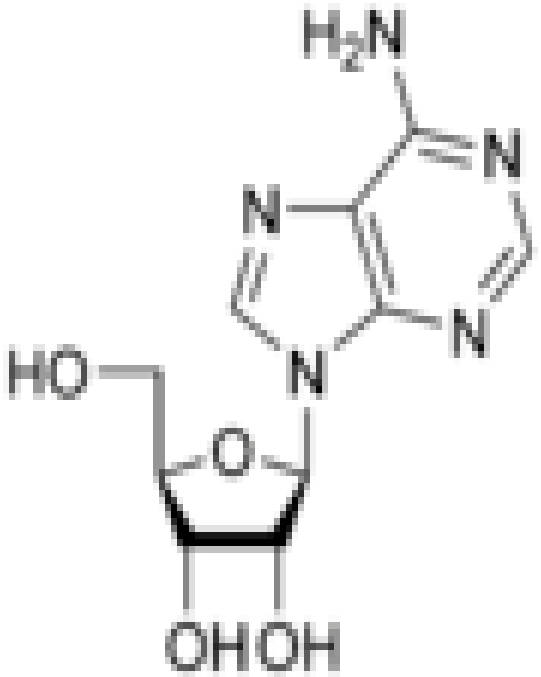
- The bonds between the first (α) and second (β), and between the second (β) and third (γ), phosphate groups are **energy-rich** and are used to provide an energy source for various cellular activities.



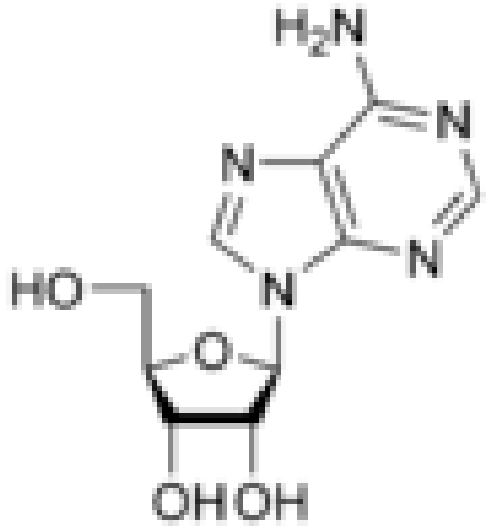
- Partial hydrolysis on the other hand yields compounds known as nucleotides and nucleosides.



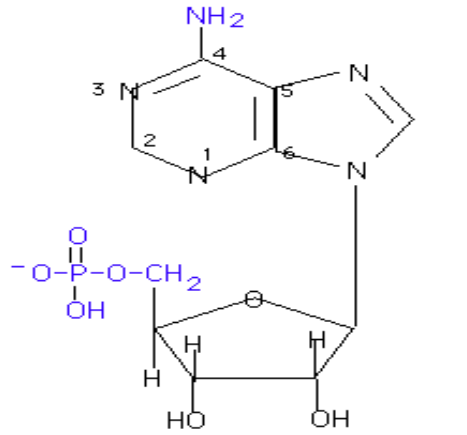
Adenosine Monophosphate
AMP



- A base linked to a sugar is called a **nucleoside**



- When a phosphate group is added, the **base-sugar-phosphate** group is called a **nucleotide.**



Adenosine Monophosphate
AMP

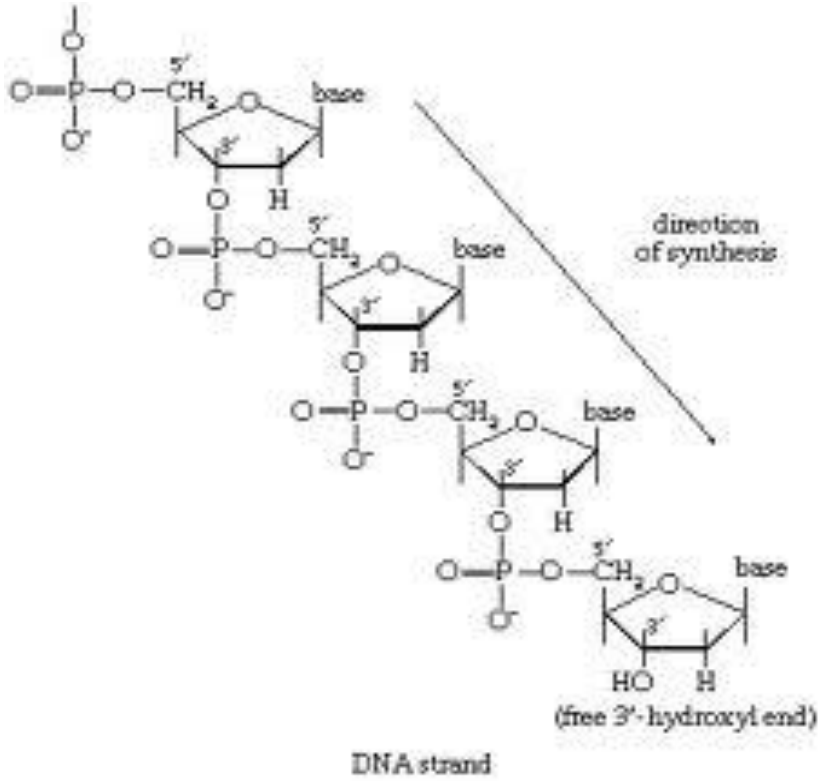
Assignment:

- Bases, nucleosides, and nucleotides have related names. Learn these nomenclature depending on the base present.

Example: adenine- adenosine- adenylic acid – dAMP

SHORTHAND NOTATION

- The representation of polynucleotide chains by complete formulae is clumsy and therefore has become necessary to use schematic systems.



- Currently, the shorthand method of describing two DNA strands has been to have the strand of 5′ → 3′ polarity on the top line of the sequence with the complementary strand of opposite polarity lying below. For example:

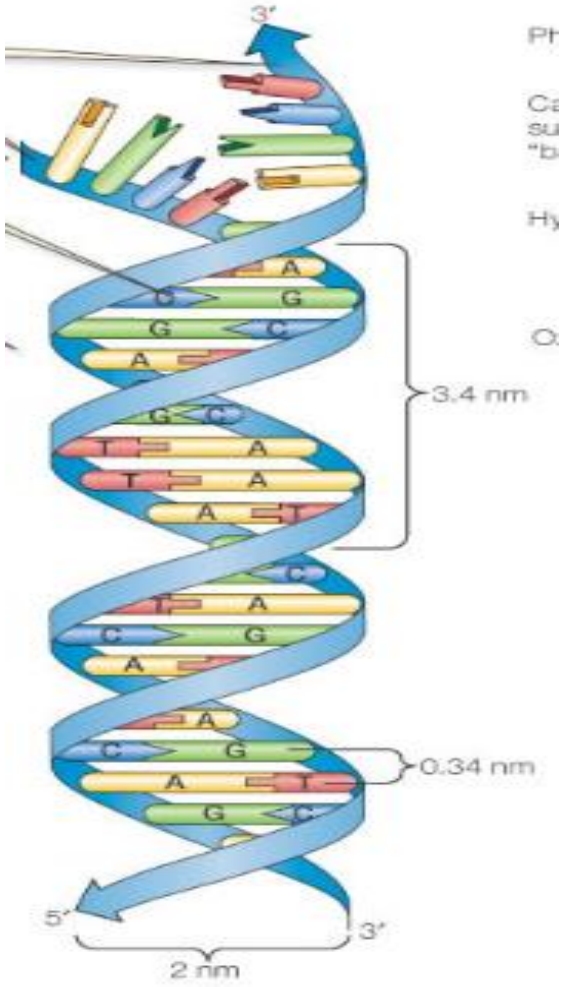
5′- AGGTC- 3′

3′- TCCAG- 5′

Some characteristics of the Double-Helix

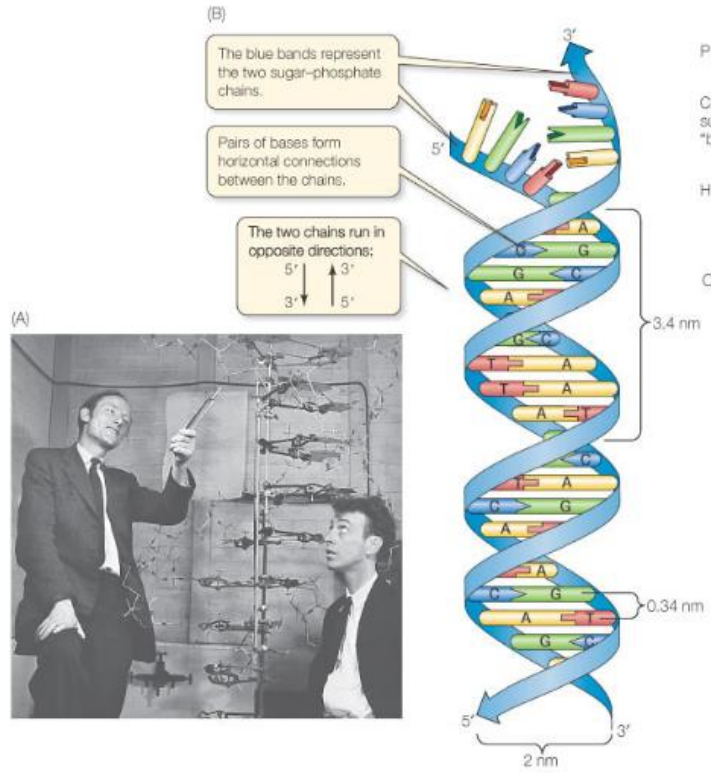
- Regular Helix has complete turn (pitch) of **34Å** or (3.4nm)

- Has a diameter of
  ≈ **20Å** or (**2nm**)

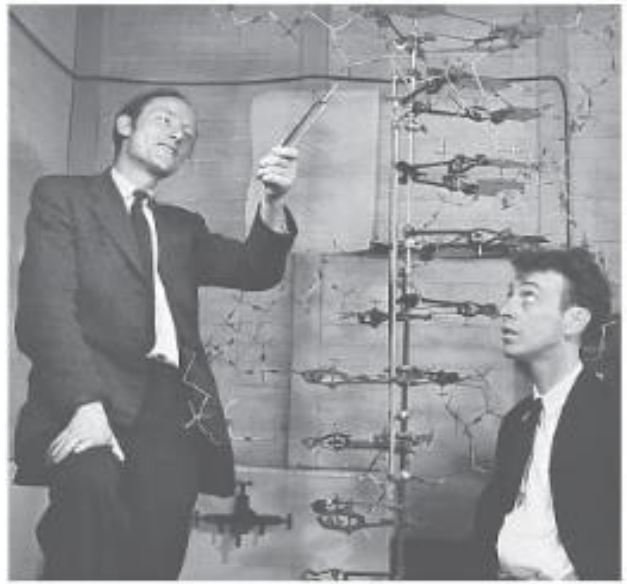


- The distance between adjacent nucleotides is **3.4Å** or (0.34nm)

- There must be **10 nucleotides** per turn



(A)



(B)

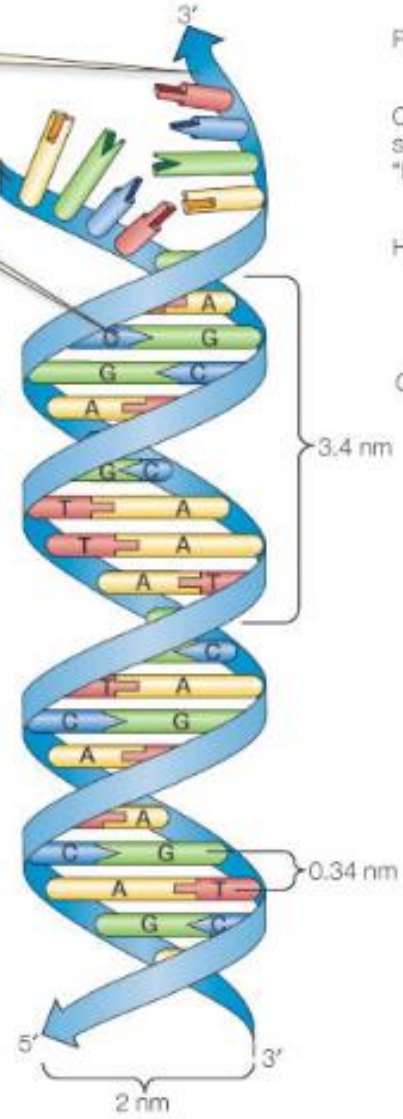
The blue bands represent the two sugar–phosphate chains.

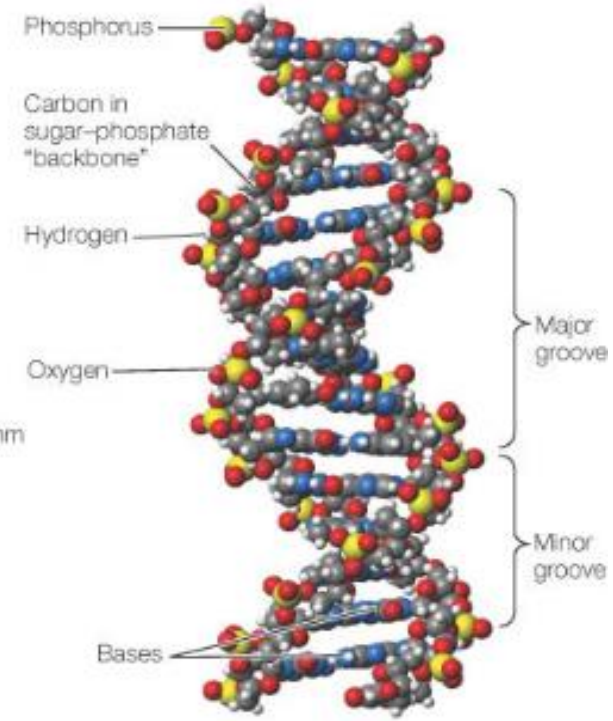
Pairs of bases form horizontal connections between the chains.

The two chains run in opposite directions:

5′ 3′

3′ 5′





- The density of DNA suggests that, the helix must contain **two** polynucleotide chains.

- The two chains are **anti-parallel** which means if one strand has 5’→3’ polarity from top to bottom, then, the other must have 3’→5’ polarity from top to bottom

- Irrespective of the actual amounts of each base, the proportion of G is always the same as the proportion of C in DNA, and that of A is always the same as T.

Assignment: Read on Chargaff's Rule.

Contributions of the Base pairs to the Double Helix

- The base pairs affect the thermodynamic stability of the double helix in two ways:

(i) Hydrogen bonding between the bases in each pair releases energy corresponding to 3 H-bonds per G-C and 2H-bonds per A-T pair.

- ***Due to the increased number of hydrogen bonds holding together a G:C nucleotide pair, regions of DNA rich in G + C are more stable than regions rich in A + T.***

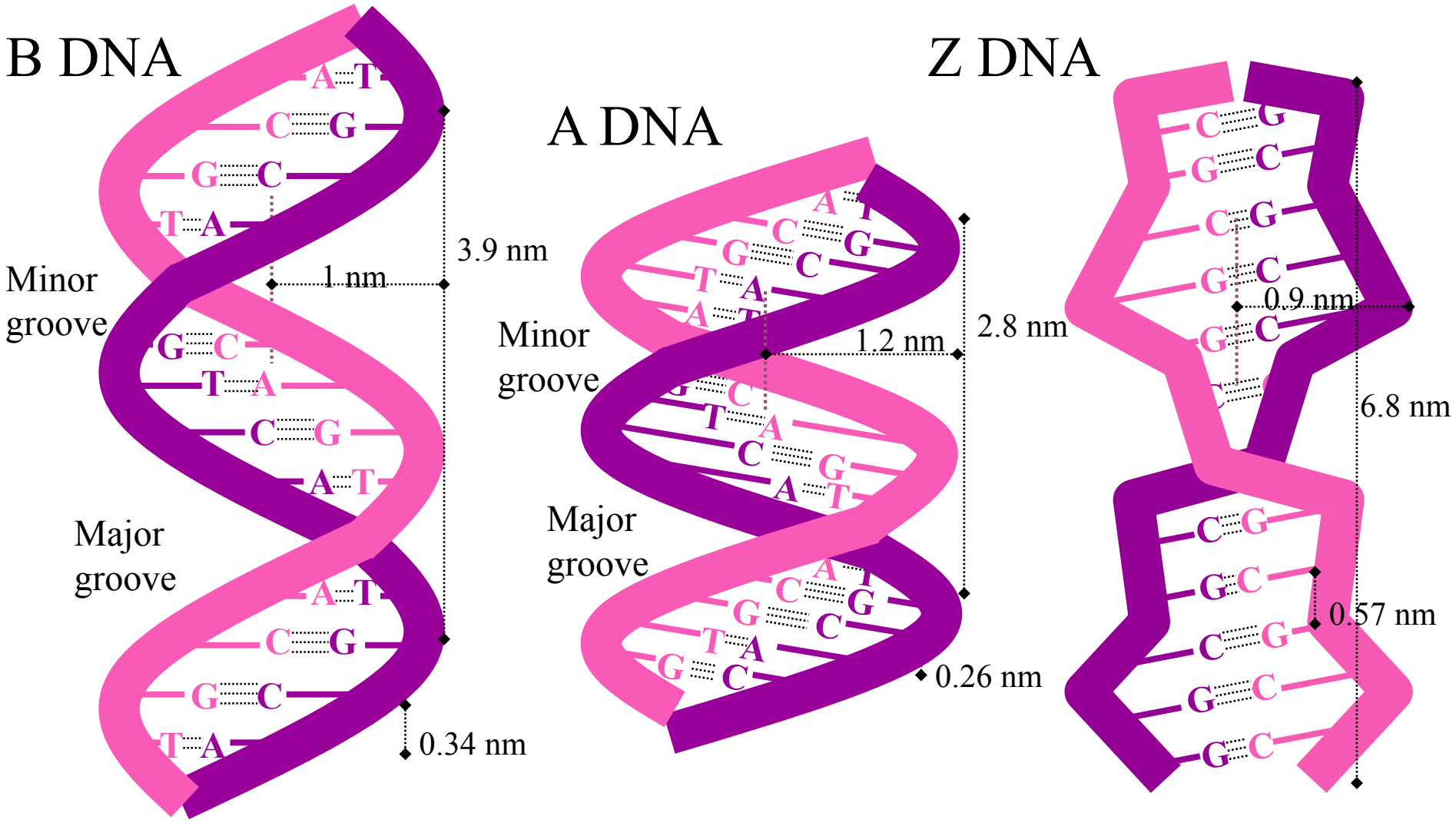
- ***On denaturation, the A + T-rich regions will melt first.***

(ii) The interaction between the electron systems of the base pairs also results in hydrophobic base-stacking.

Different Forms of DNA

- Although the basic model put forward by Watson and Crick remains close to the accepted structure of the DNA molecule in solution,
- Refined X-ray studies have shown that, depending on the **conditions** chosen to produce the DNA molecule, we can have a variety of possible structures like **A-**, **B-**, **C-**, and **Z-** forms.

Forms of the Double Helix



10.4 Bp/turn	11 Bp/turn	12 Bp/turn
+34.6° Rotation/Bp	+34.7° Rotation/Bp	-30.0° Rotation/Bp

Assignment:

- Read and summarize the different forms of DNA under the following headings:

(i) Relative Humidity (ii) Pitch in nm

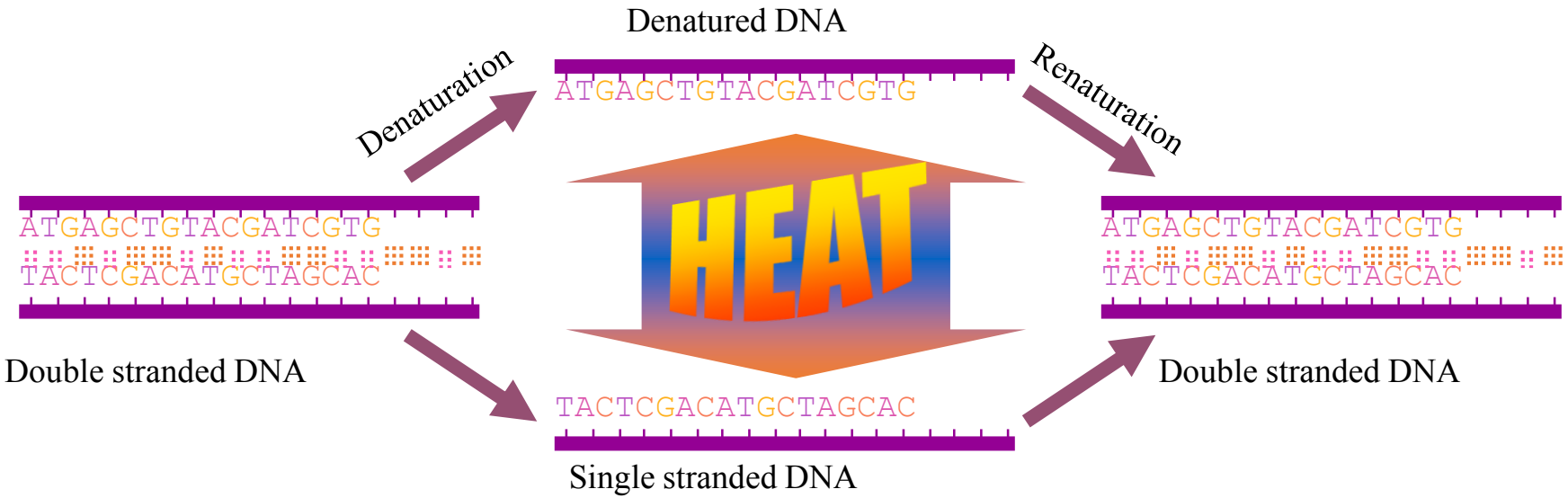
(iii) Residues per turn and

(iv) Inclination of b.p. from horizontal.

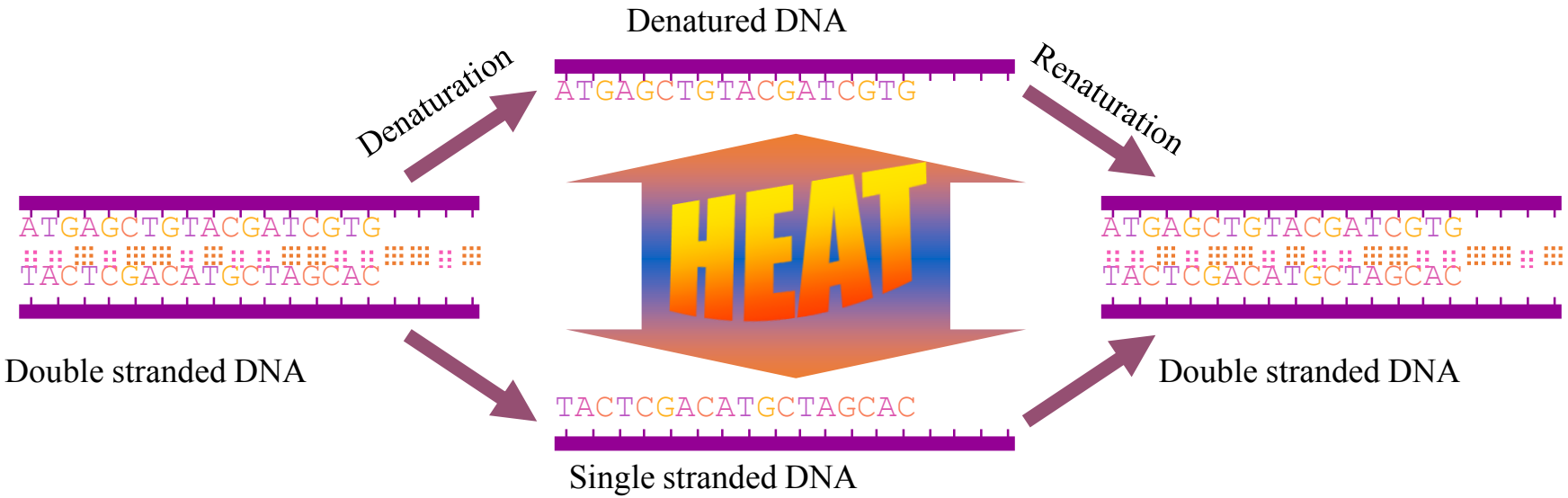
Example: The B-form under R.H of 92% has a pitch of 3.4nm, 10 bases per turn and has 0^{o} angle of inclination to the horizontal.

DNA Denaturation and Renaturation

- When double-stranded DNA molecules are subjected to extremes of temperature or pH, the hydrogen bonds of the double helix are ruptured and the two strands are no longer held together.



- **The DNA is said to denature and changes from a double helix to a single stranded DNA random coil.**



strand separation
denaturation
melting

dsDNA  ssDNA

HELIX

annealing
renaturation
hybridization

COIL

HELIX - COIL TRANSITION

- When heat is used as the denaturant, the DNA is said to **melt** and the temperature at which the strands separate is the **melting temperature** or **transition temperature** (**Tm**).

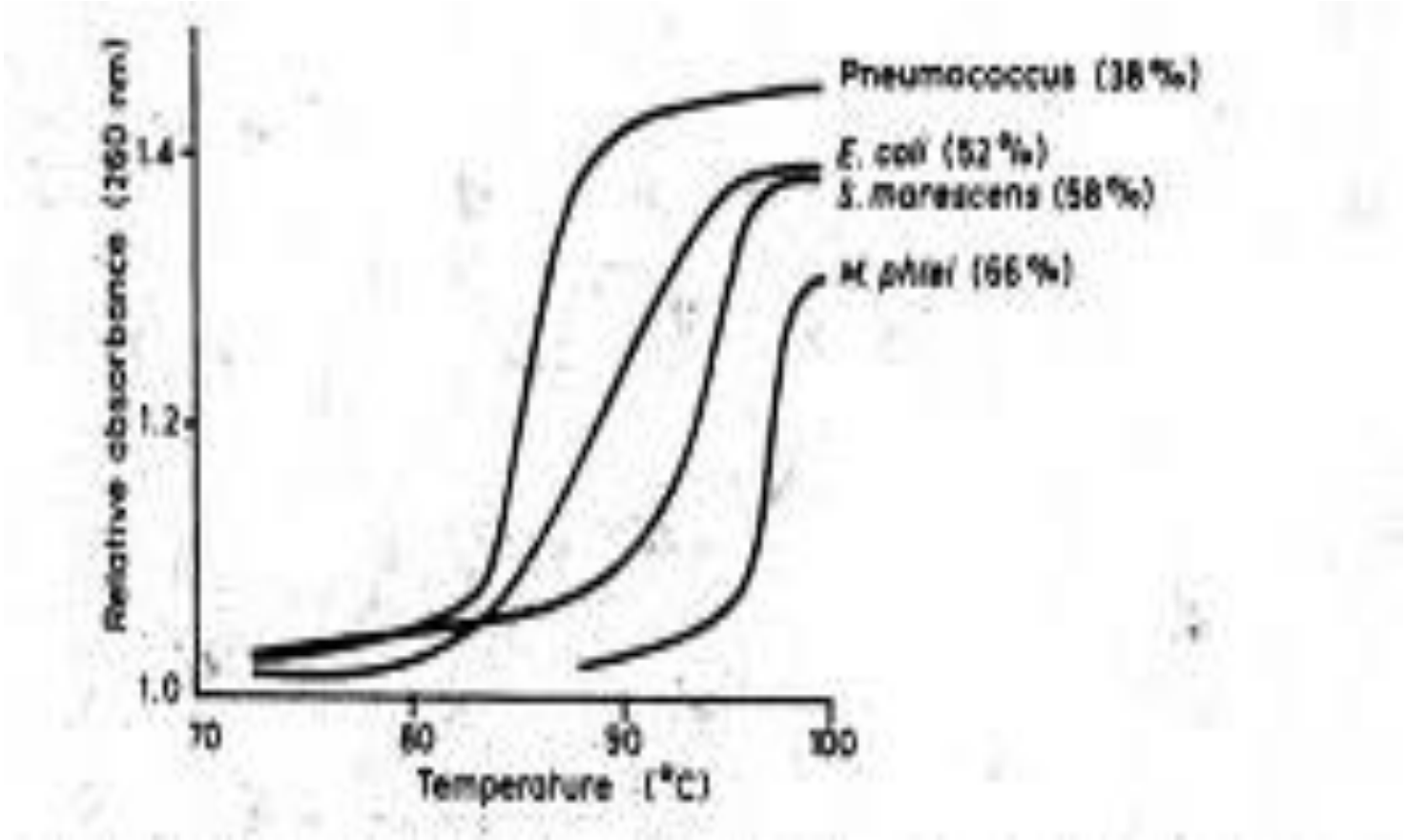
- When duplex DNA melts, the hydrogen bonds break and the bases unstack with the consequence that the absorption at 260 nm rises by 30-40% (20-30%).

- This rise in absorption is the **hyperchromic effect** or **shift** and is used to monitor the melting of DNA.

- The nature of the melting transition is affected by several factors:

(i) G + C content:- the higher the G+C content of DNA, the more stable the molecule will be and hence, the higher the melting temperature.

Denaturation by heat of DNAs from different organisms.



Read and make notes on the other factors

- (ii) The nature of the solvent
- (iii) The nature of the DNA

Functions of DNA

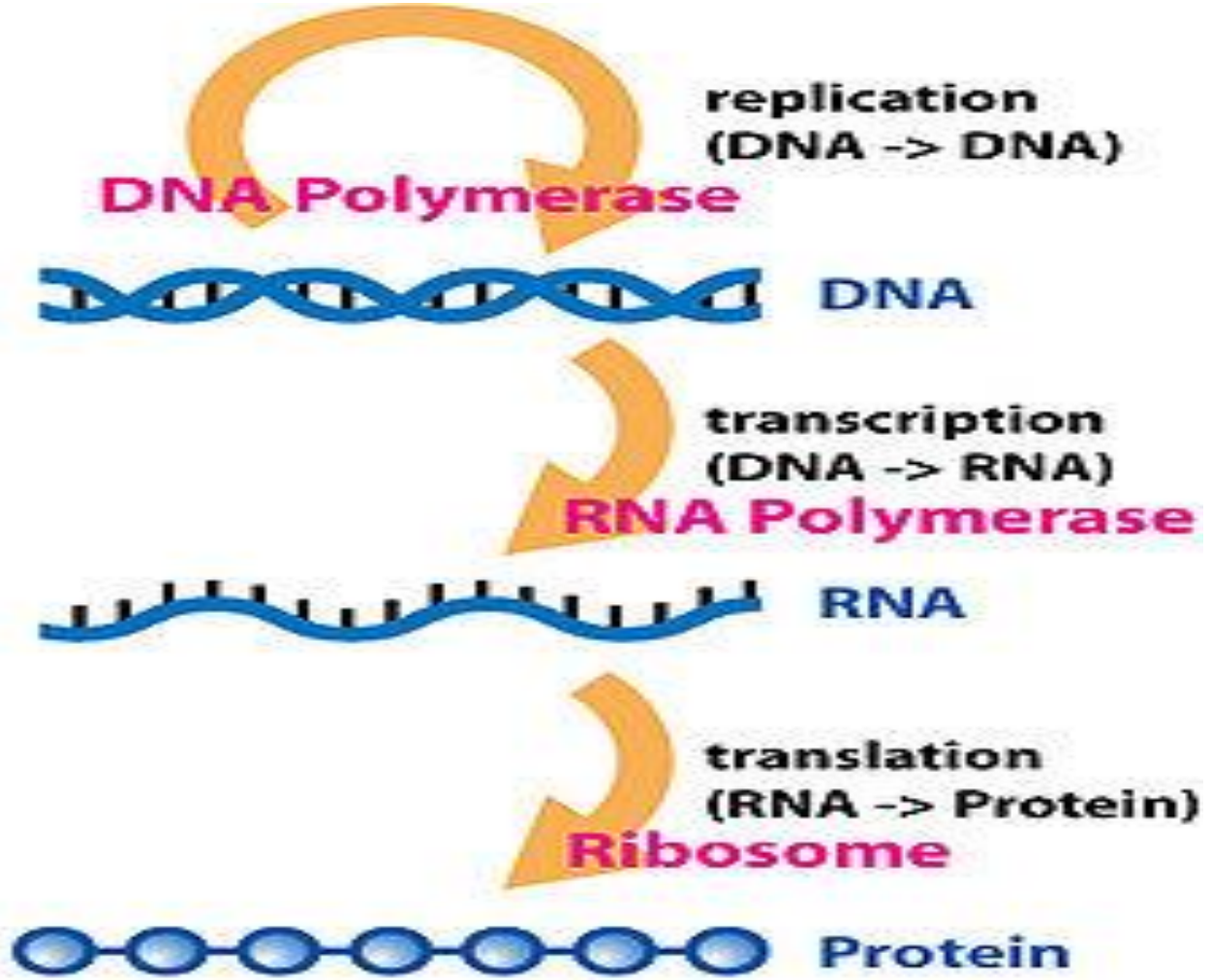
1. Storage of genetic information

2. **Self-duplication (replication)** & inheritance.

3. Expression of the genetic message.

✓**DNA's major function is to code for proteins.**

The Central Dogma of Gene Expression or Genetics

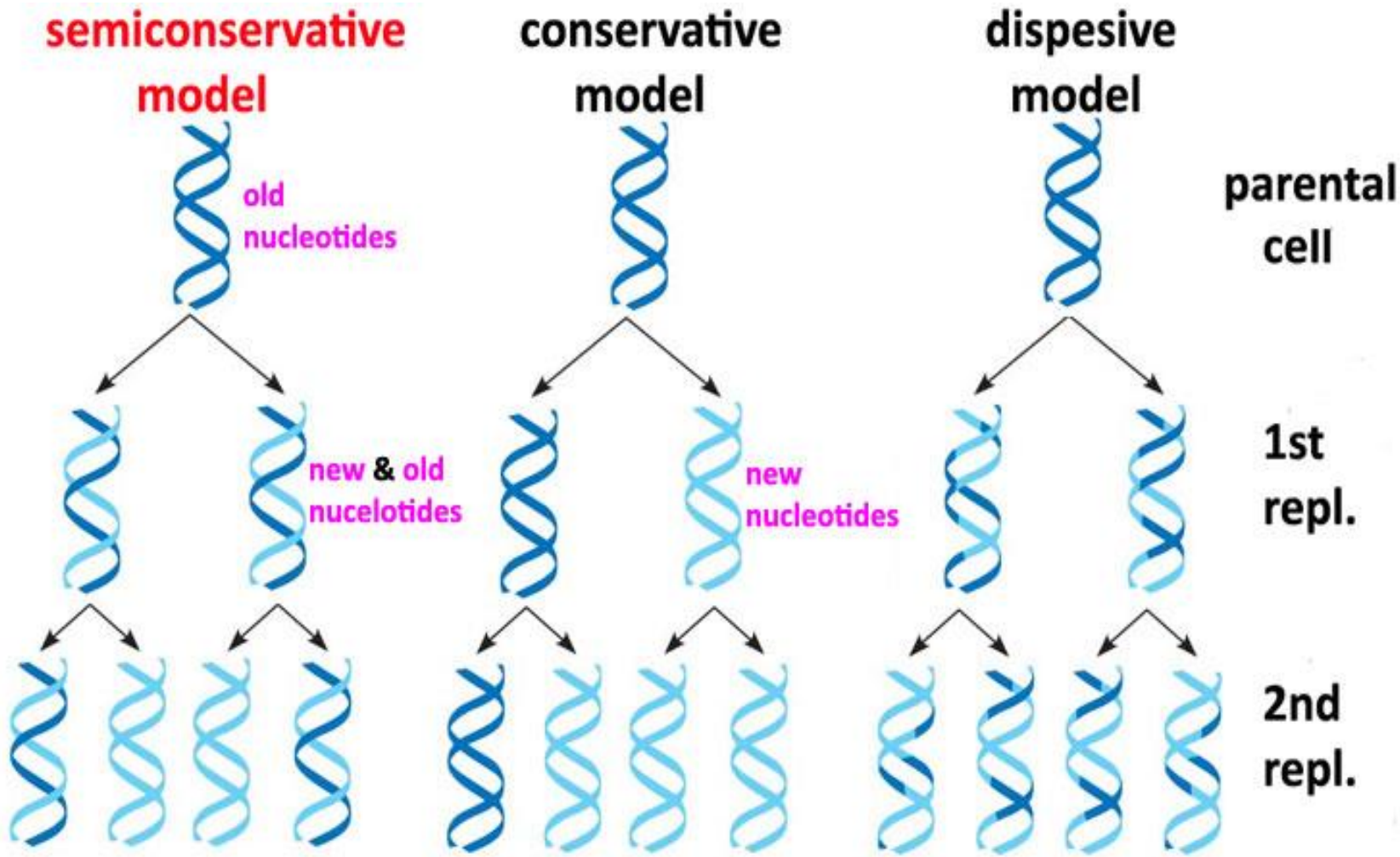


Replication

- To explain the phenomenon of heredity, biological information must be accurately copied (**replicated**) and transmitted from each cell to all of its progeny.

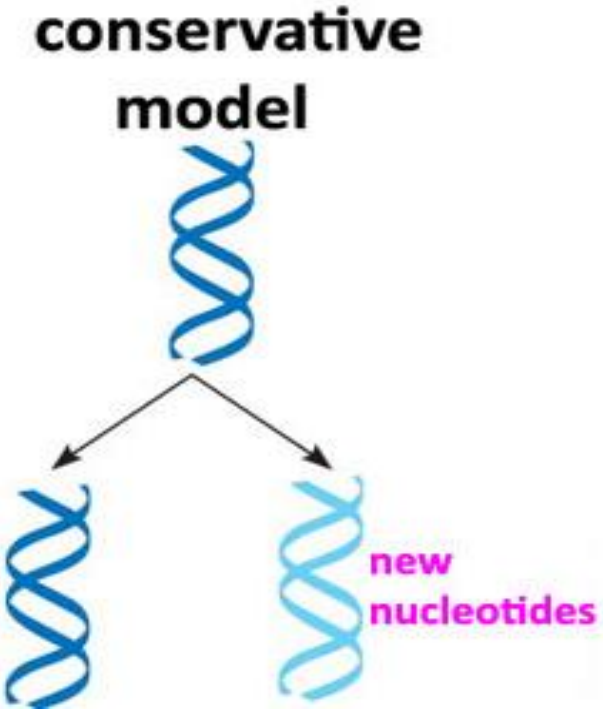
- Three hypotheses had been previously proposed for the method of replication of DNA.

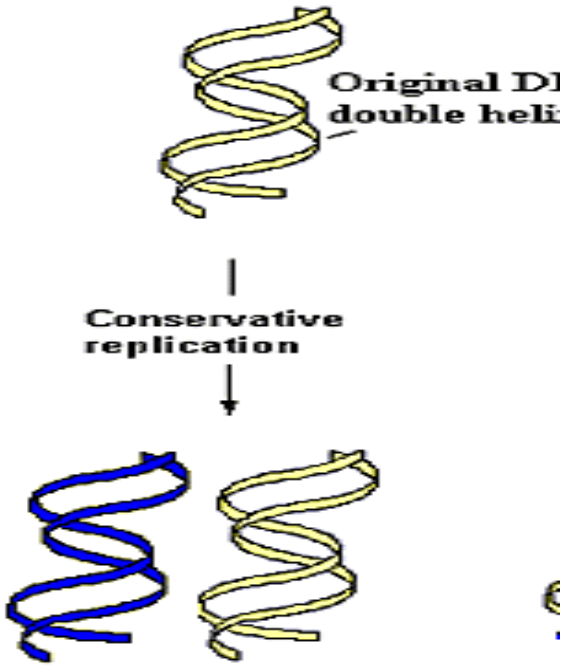
- These are **Semiconservative**, **Conservative** and **Dispersive** replication.


semiconservative model
conservative model
dispesive model
parental cell
old nucleotides
new & old nucelotides
new nucleotides
1st repl.
2nd repl.

Conservative Replication

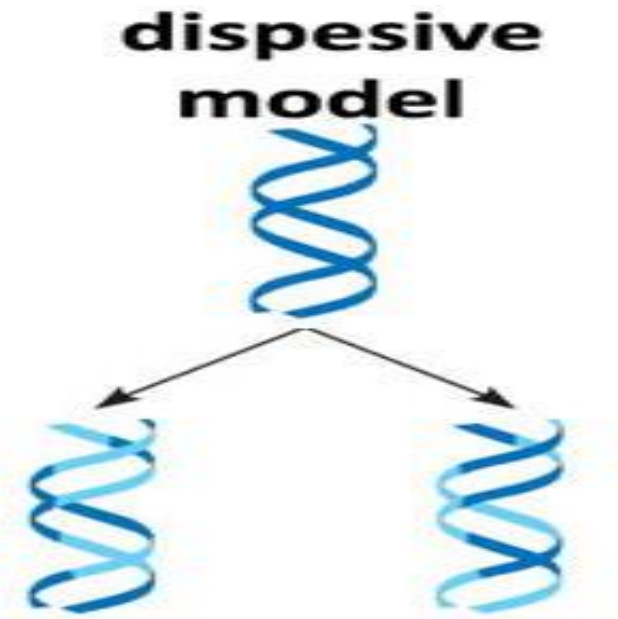
- Conservative replication would leave intact the original DNA molecule and generate a completely new molecule.

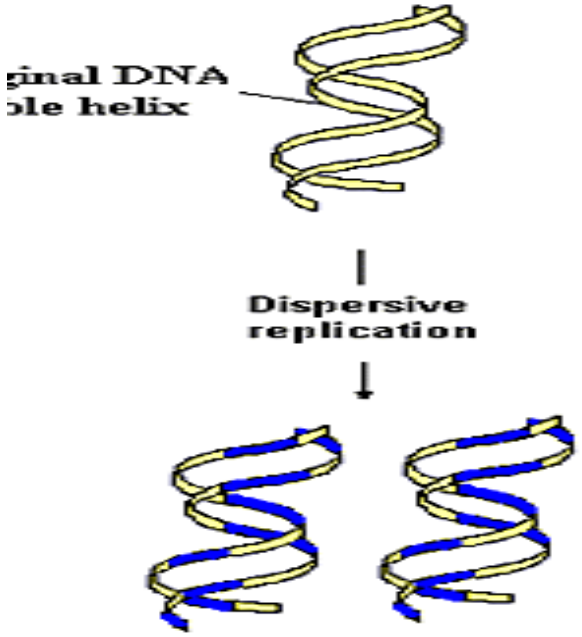




Dispersive Replication

- Dispersive replication would produce two DNA molecules with sections of both old and new DNA interspersed along each strand.



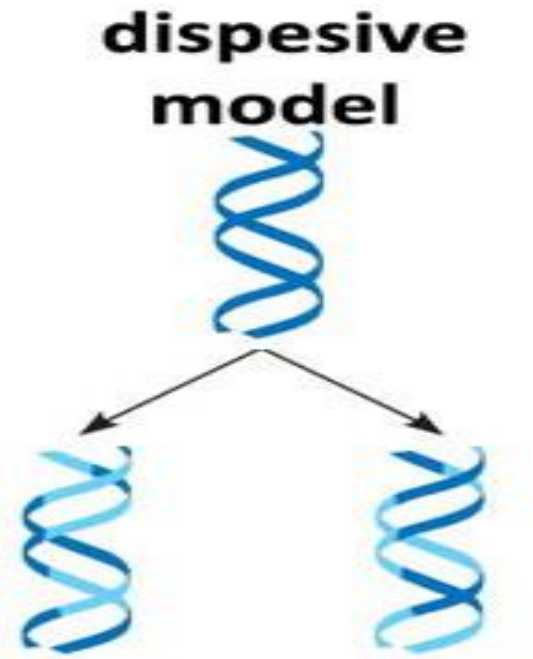


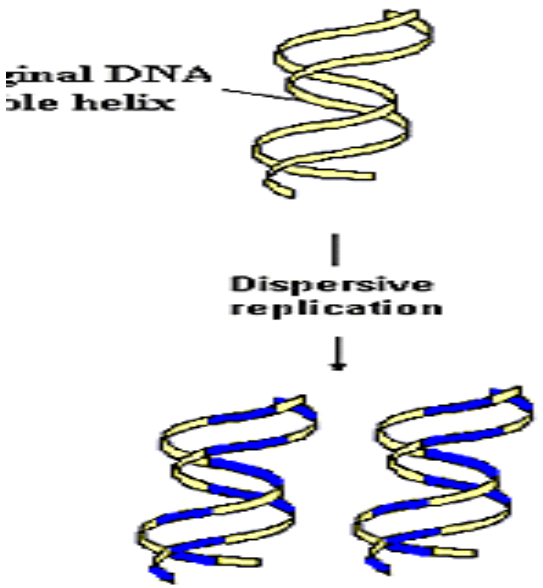
- The dispersive hypothesis is exemplified by a model proposed by Max Delbrück.

- He attempted to solve the problem of unwinding the two strands of the double helix by a mechanism that breaks the DNA backbone every 10 nucleotides.

- Untwists the molecule, and attaches the old strand to the end of the newly synthesized one.

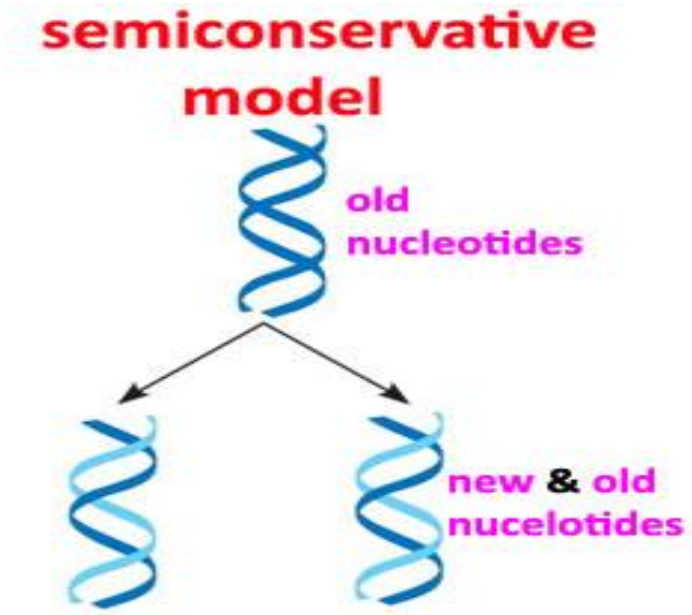
- This would synthesize the DNA in short pieces alternating from one strand to the other

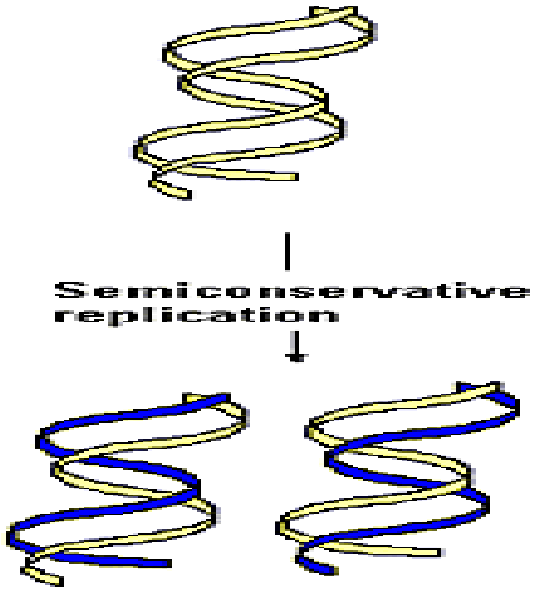




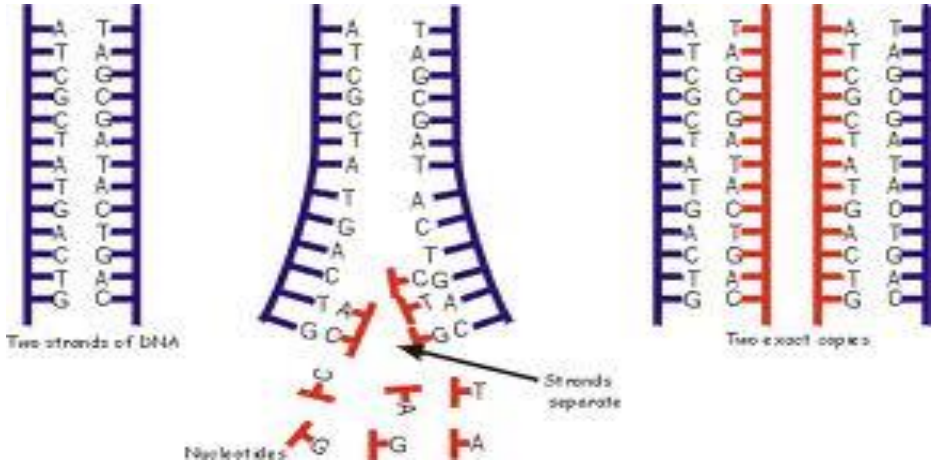
Semiconservative Replication

- Semiconservative replication would produce molecules with both old and new DNA, but each molecule would be composed of one old strand and one new one.



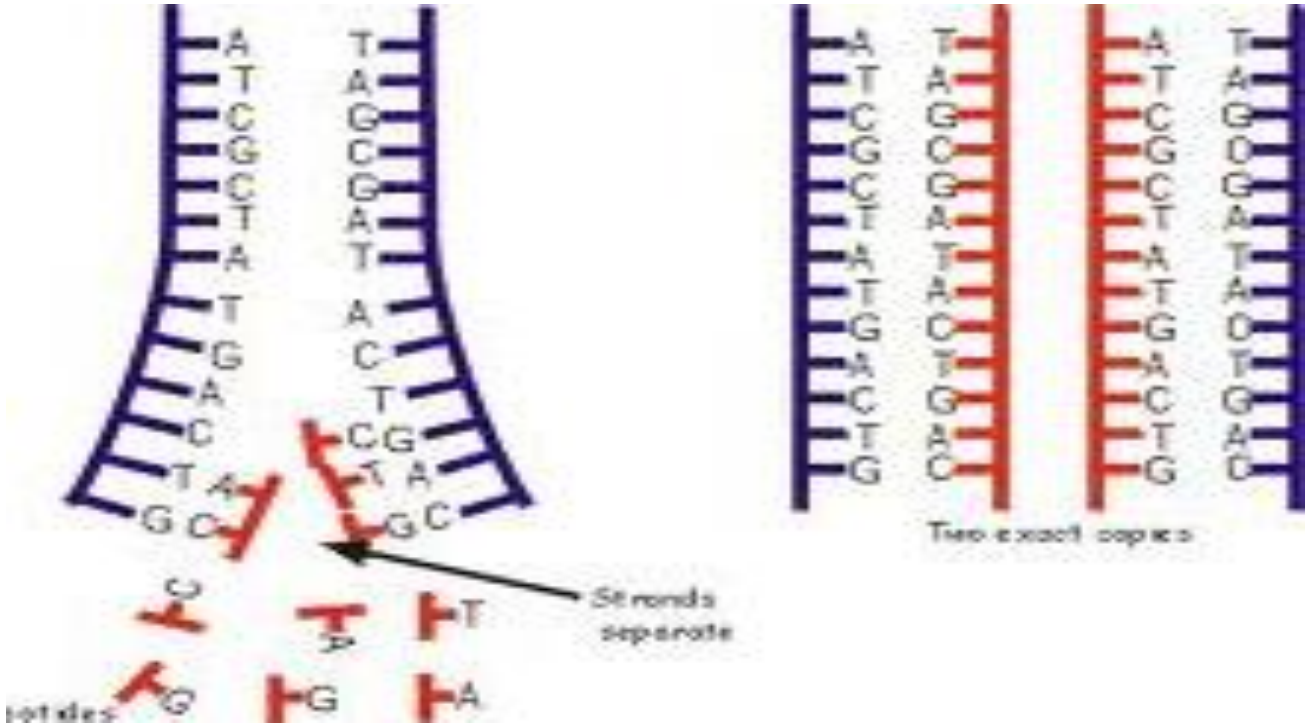


- Each strand acts as a **template** or **guide** for the synthesis of a new DNA molecule by the sequential addition of complementary base pairs.



- Thereby generating a new DNA strand that is the complementary sequence to the parental DNA.

- Each daughter DNA molecule ends up with one of the original strands and one newly synthesized strand.



Evidence for Semi Conservative Replication

- In 1958, **Matthew Meselson** and **Franklin Stahl** worked out a clever procedure to distinguish semi conservative DNA replication from conservative or dispersive replication, using a nonradioactive heavy isotope of nitrogen.

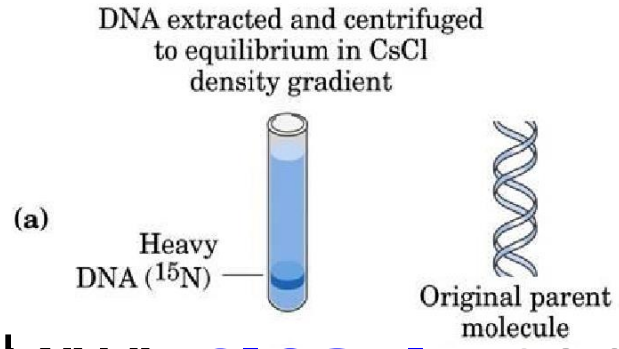
- Meselson and Stahl opted for nitrogen because it is an essential chemical component of DNA;

- Therefore, every time a cell divides and its DNA replicates, it incorporates new N atoms into the DNA of either one or both of its two daughter cells, depending on which model was correct.

- Ordinary nitrogen, the most abundant isotope, has an atomic weight of 14, so it is called ^{14}N.

- A relatively rare isotope ^{15}N has an atomic weight of 15.

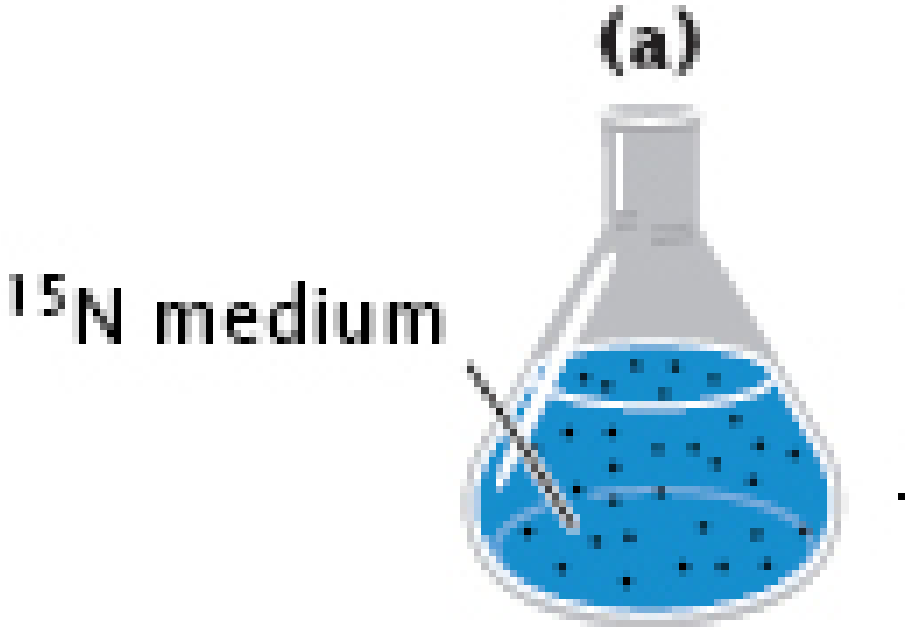
- Meselson and Stahl found that if bacteria are grown in a medium enriched in ^{15}N, they incorporate the heavy isotope into their DNA, which becomes denser than normal.



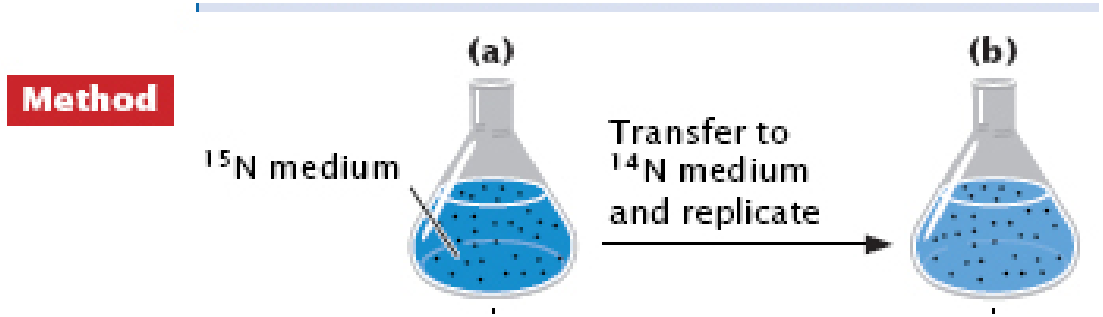
- This labeled DNA **clearly** separates from ordinary DNA in gradient of Cesium Chloride (CsCl) spun in an ultracentrifuge.

- CsCl is used because it is a very dense salt and therefore makes dense enough solution that DNA will float somewhere in the middle rather than sinking to the bottom.

- The aim of the experiment was to grow ^{15}N labeled bacteria in ^{14}N-medium and then to look at the density of the DNA products.



- That is, *E. coli* cells with only ^{15}N in their DNA were transferred to a ^{14}N medium and were allowed to divide or replicate;

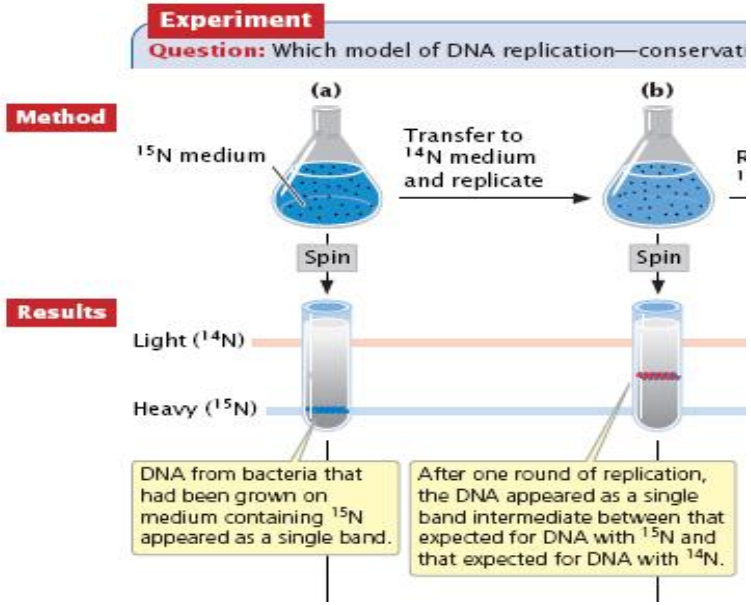


- The progress of cell division was monitored by measuring the optical density of the cell suspension.

Experimental Results

- DNA was extracted periodically and was compared to pure ^{14}N DNA and ^{15}N DNA.

- After one replication, the DNA was found to be close to the **intermediate density**.



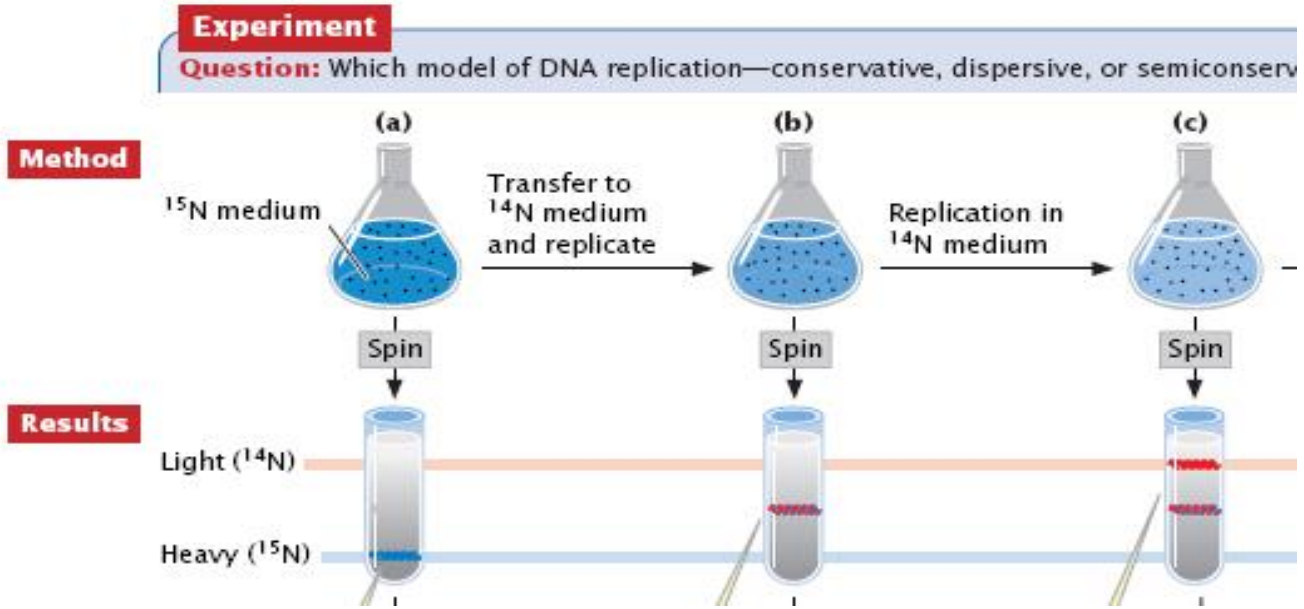
- Based on these findings, the scientists were immediately able to **exclude the conservative model of replication as a possibility.**

- After all, if DNA replicated conservatively, there should have been two distinct bands after a single round of replication

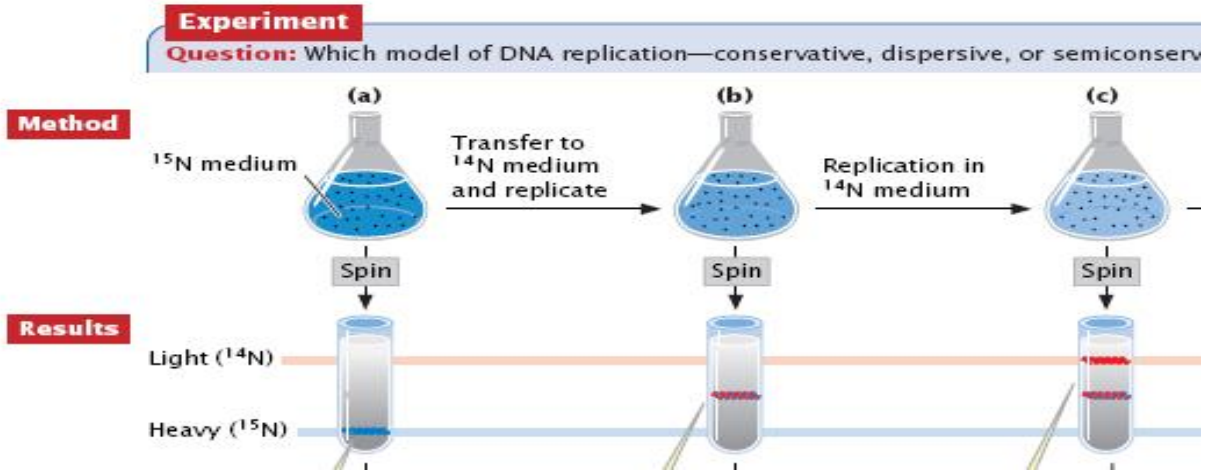
- However, this result was consistent with both semiconservative and dispersive replication.

- To differentiate between the two, Meselson and Stahl had to let the cells divide again and then sample the DNA after a second round of replication.

- DNA from cells after two replications had been completed was found to consist of equal amounts of DNA with two different densities.



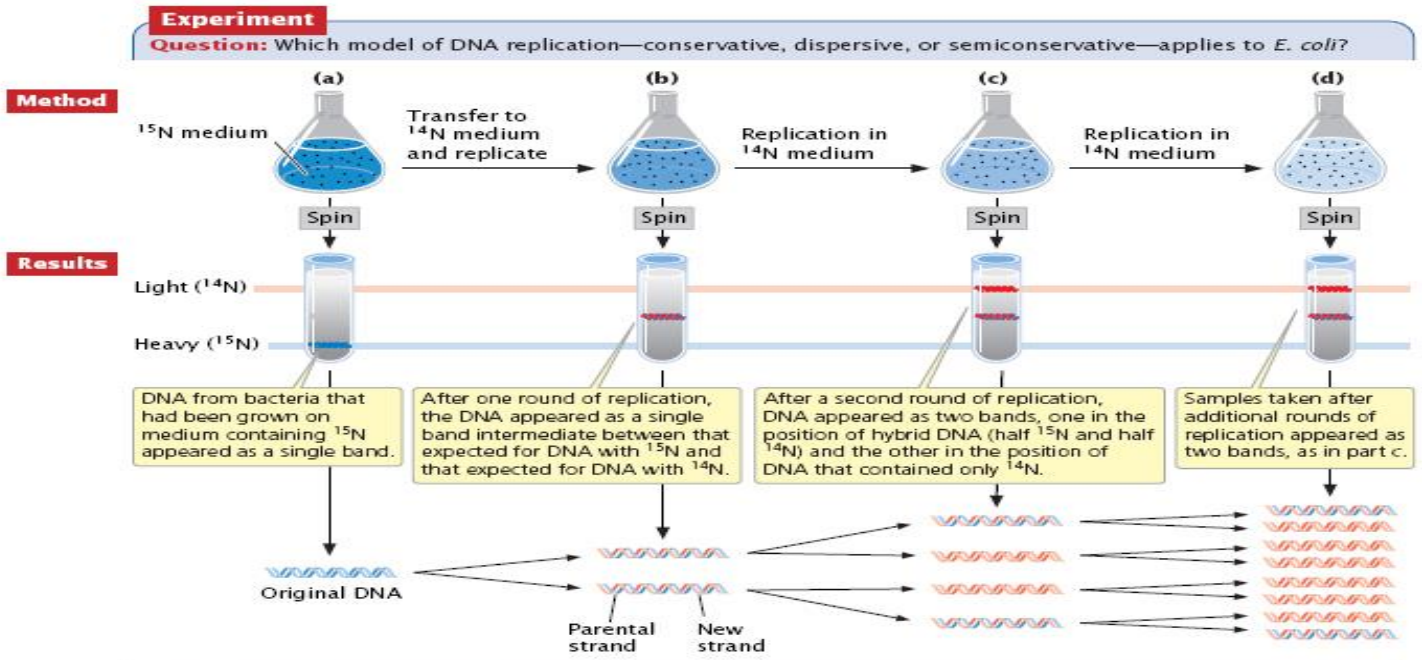
- One corresponding to the intermediate density of DNA of cells grown for only one division in ^{14}N medium, the other corresponding to DNA from cells grown exclusively in ^{14}N medium.



- This was inconsistent with dispersive replication.

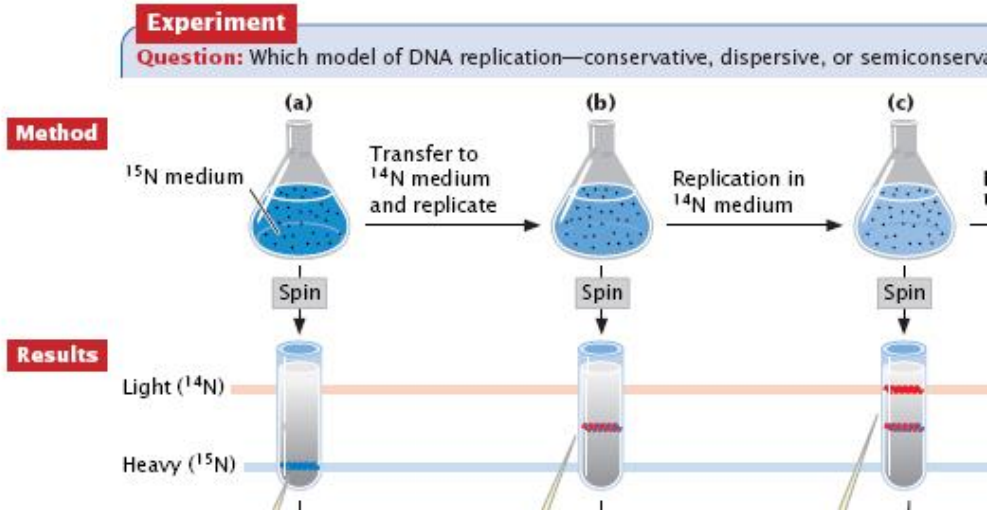
- After all, if the dispersive model were the correct model, the scientists would have continued to observe only a single band after every round of replication.

- Dispersive replication would have resulted in double-stranded DNA with both strands having mixtures of ^{15}N and ^{14}N DNA, either of which would have appeared as DNA of an intermediate density.



- Semiconservative replication would result in double-stranded DNA with one strand of ^{15}N DNA, and one of ^{14}N DNA.

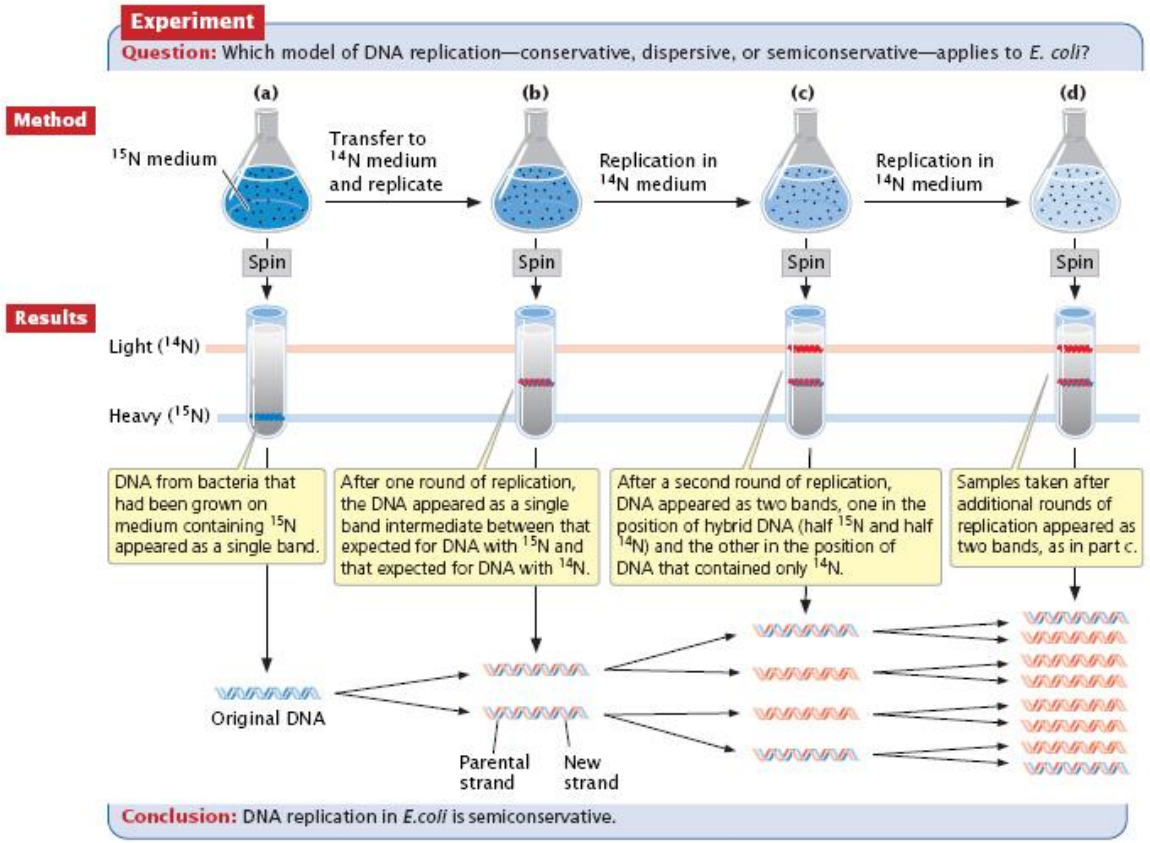
- This will result in equal amounts of DNA with two different densities.



- The scientists continued to observe the same two bands after several subsequent rounds of replication.

- These results were consistent with the semiconservative model of replication and the reality that, when DNA replicated, each new double helix was built with one old strand and one new strand.

➢The result was consistent with the semiconservative replication hypothesis



Replication of Eucaryotic chromosomes

- Before a cell can divide, it must duplicate or replicate all its DNA.

- In eukaryotes, this occurs during S phase of the cell cycle

- Eukaryotic DNA replication is very slow compared to *E. coli* DNA replication: only about **75 nucleotides/second**.

Mechanism of DNA replication

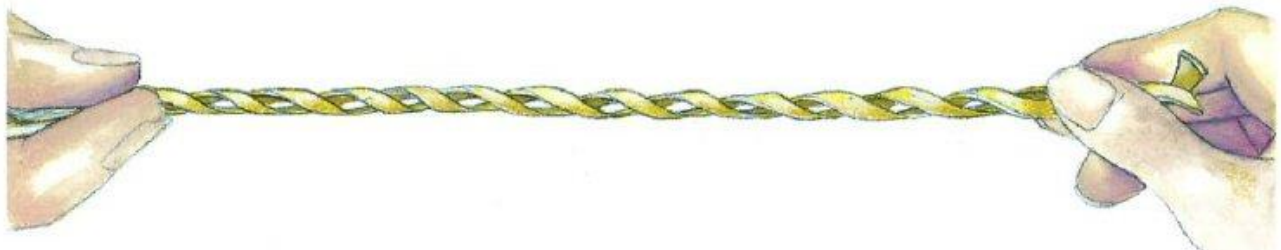
- Replication is a huge task, whether in bacteria or in eukaryotes and requires many proteins or enzymes to act together.

- There are several physical and biochemical challenges the cell must overcome.

- First, the site or sites at which to begin replication must be located and the proper enzymes collected or deposited there.

- Second, the double helix must be unwound to expose the two strands.

- This imposes twisting strain on the portions of the helix farther away from the unwinding site, much like untangling a twisted phone cord does, and those forces must be relieved to prevent breakage of the DNA strands.



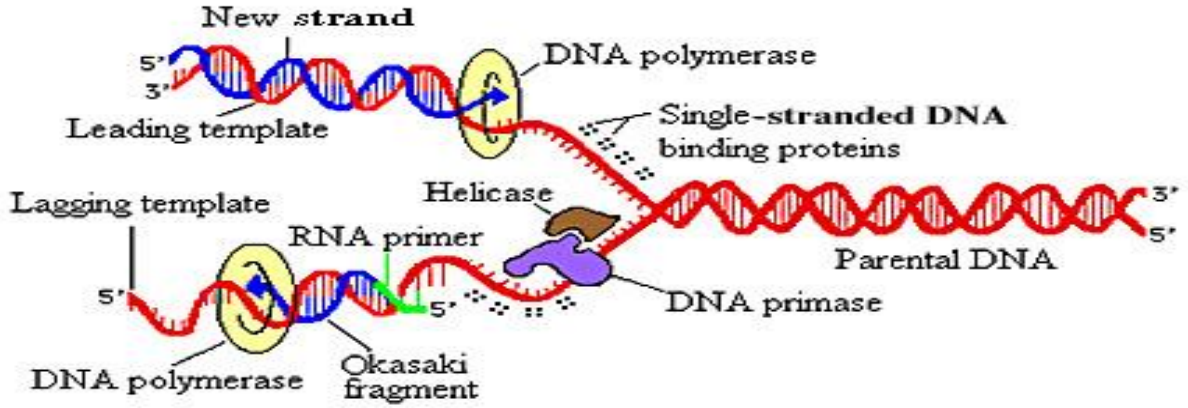
- DNA replication begins at specific points known as the ***Origins***.

- These sites are recognized by certain proteins in the cell.

- The Origins are rich in Adenine and Thymine bases, as breaking two hydrogen bonds between the Adenine and Thymine is easier than breaking the triple hydrogen bonds between Cytosine and Guanine.

- Six proteins arranged in a ring shape known as **Helicase**, unwind the double stranded DNA helix into single strands by breaking the hydrogen bonds between them.

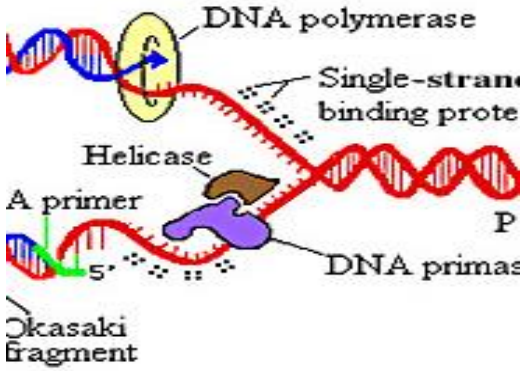
- This results in the formation of a **replication fork.**

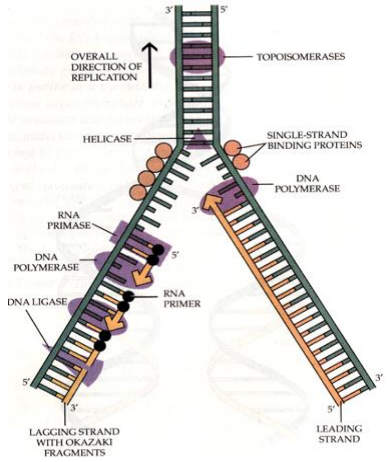


- The replication fork is a structure that is formed during the DNA replication process.

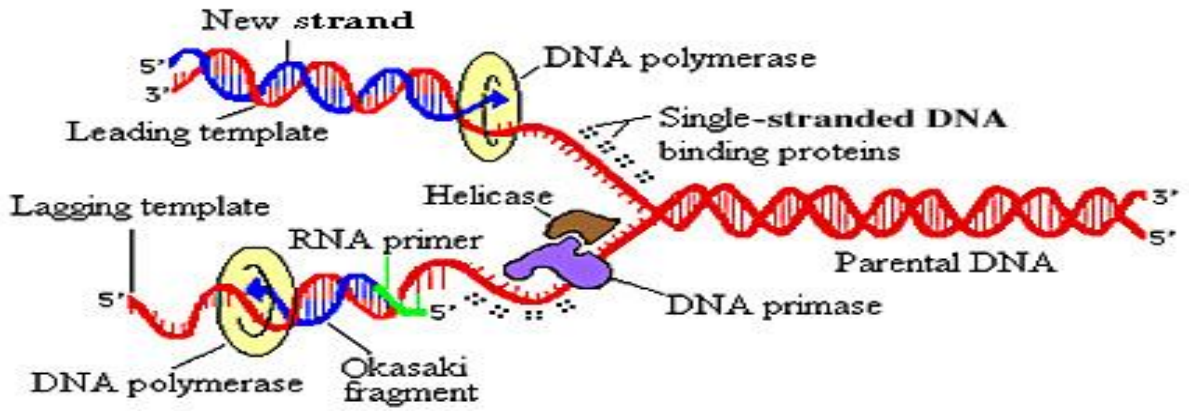
- The fork is made with the action of helicase, that breaks the hydrogen bonds, that hold the two DNA strands together.

- This results in a structure that has two branching 'prongs' of a single strand DNA each.

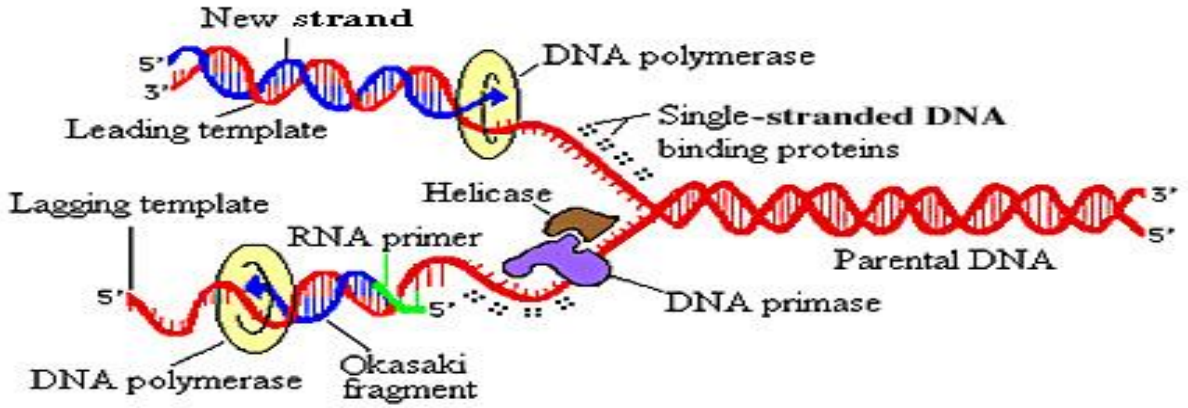




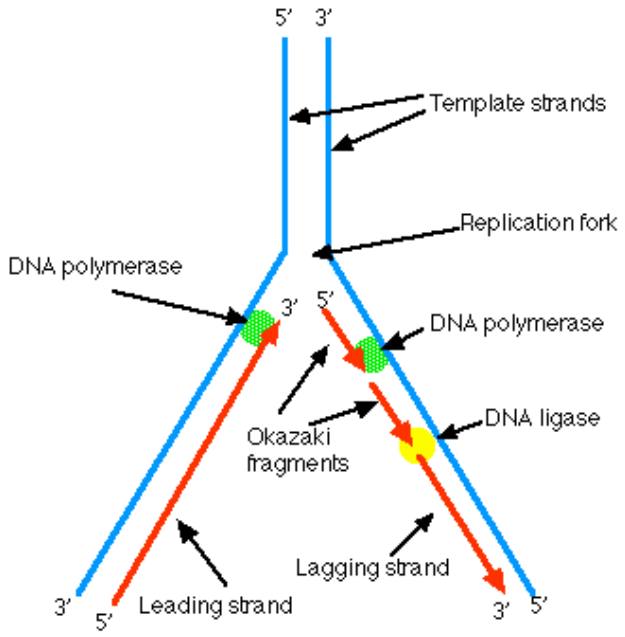
- Tetramers, known as the **single stranded binding proteins**, cover the single-stranded DNA.
- This prevents the DNA strands from re-annealing and forming the double stranded molecule.



- The two single DNA strands act as templates individually, that are used for producing two complementary DNA strands.
- The double helix consists of two anti-parallel DNA strands with complementary 5' to 3' strands.



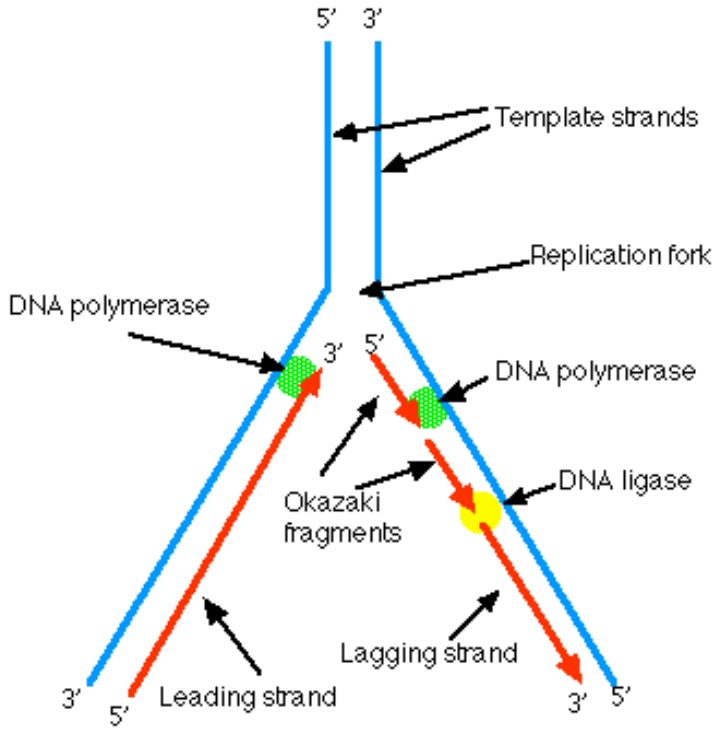
- An enzyme called **DNA polymerase** binds to one strand of the DNA and begins moving along it in the 3' to 5' direction, using it as a template for assembling a **leading strand** of nucleotides and reforming a double helix.



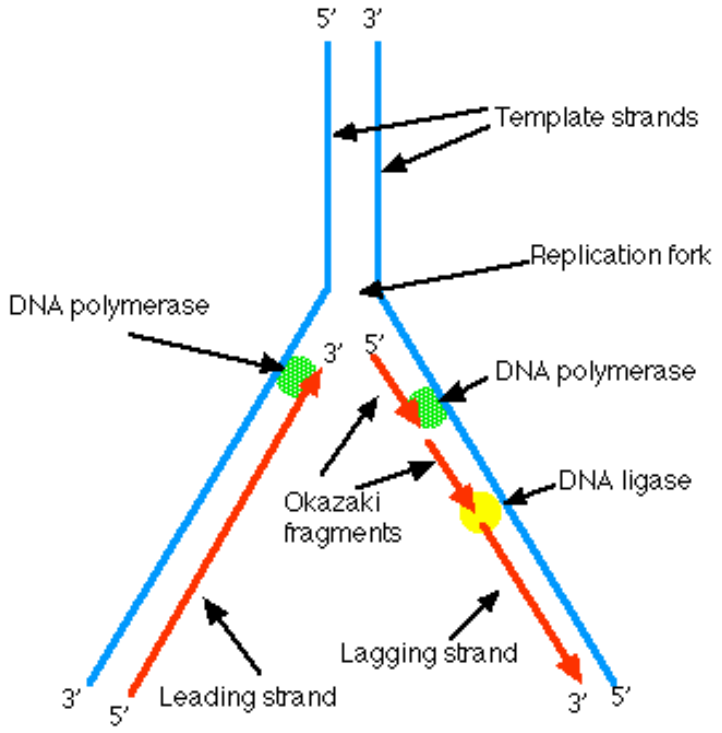
- In eukaryotes, this molecule is called **DNA polymerase delta (δ)**

- Because DNA synthesis can only occur in 5' to 3‘ direction, a molecule of a second type of DNA polymerase (**epsilon, ε**, in eukaryotes) binds to the other template strand as the double helix opens as shown below.

- This molecule synthesize discontinuous segments of polynucleotides (**called Okazaki fragments**).



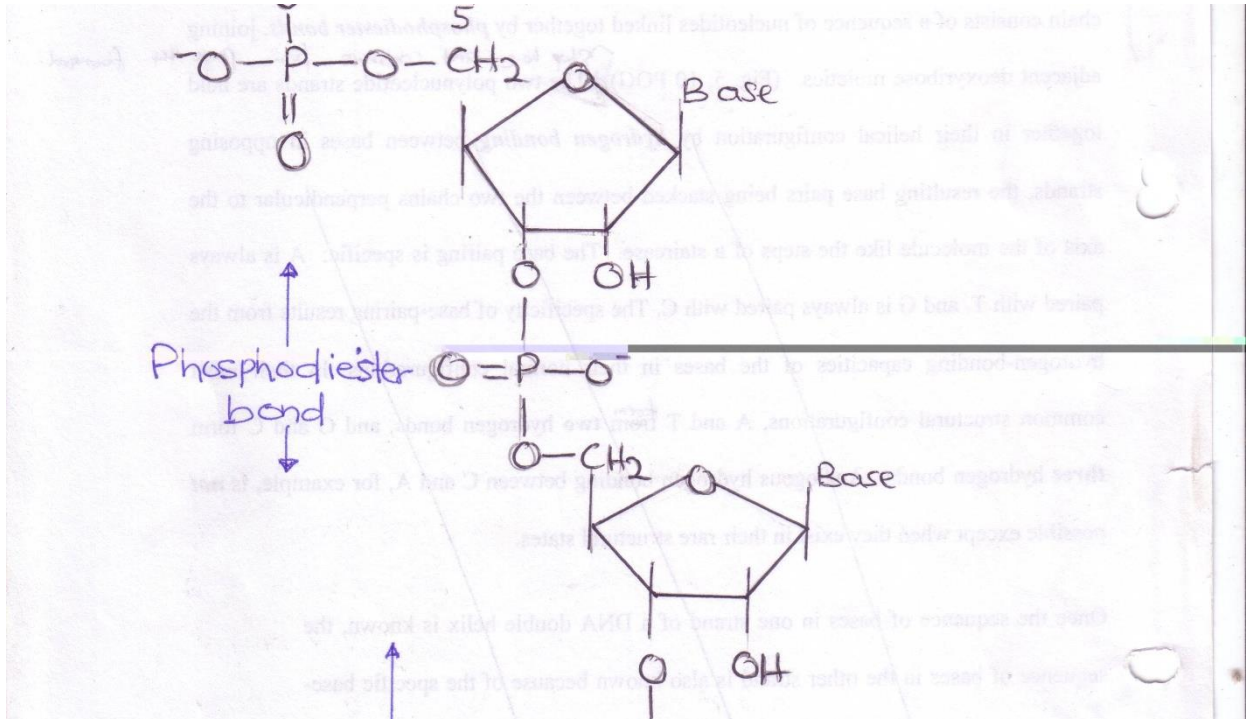
- Another enzyme, **DNA ligase** then stitches or seals these together into the **lagging strand**.



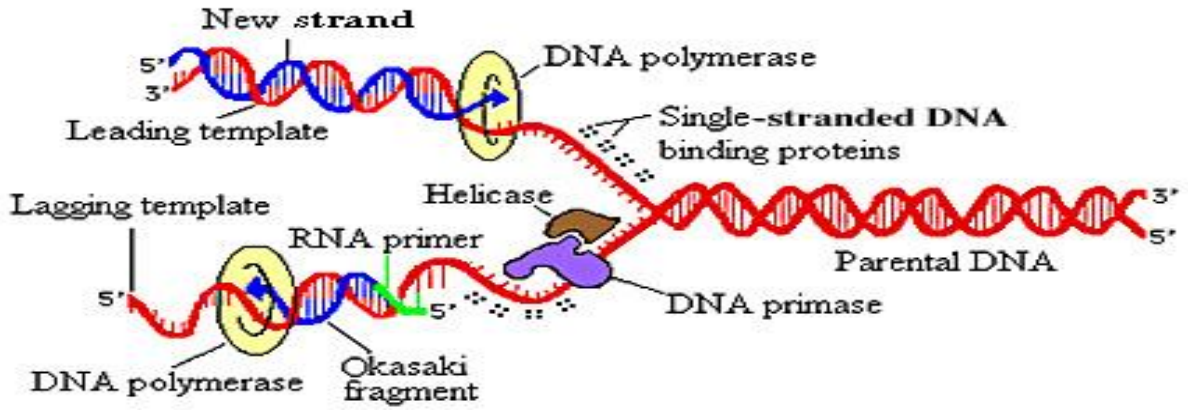
- The original DNA strand is used as a template to synthesize the DNA strand in the 5' → 3' direction with the help of an extension formed by **RNA primer**.

- DNA polymerase can synthesize the strand in **5' → 3' direction only**.

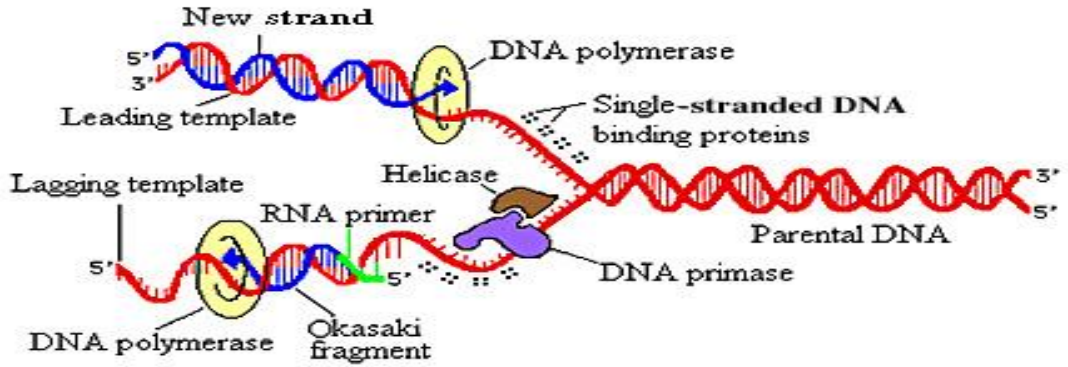
- It hooks the 5' phosphate group of an incoming nucleotide onto the 3' hydroxyl group at the end of the growing nucleic acid chain.



- The DNA polymerase starts synthesizing a new strand called the **Leading Strand** by adding new deoxyribonucleotides at a continuous stretch against one of the parent strands.



- The new strand that is formed using the other parent strand as a template is known as the **Lagging Strand** as it is formed in segments or fragments.



- It is also referred to as the **Okazaki Fragments**.

- DNA polymerase cannot begin synthesizing the DNA strand initially.

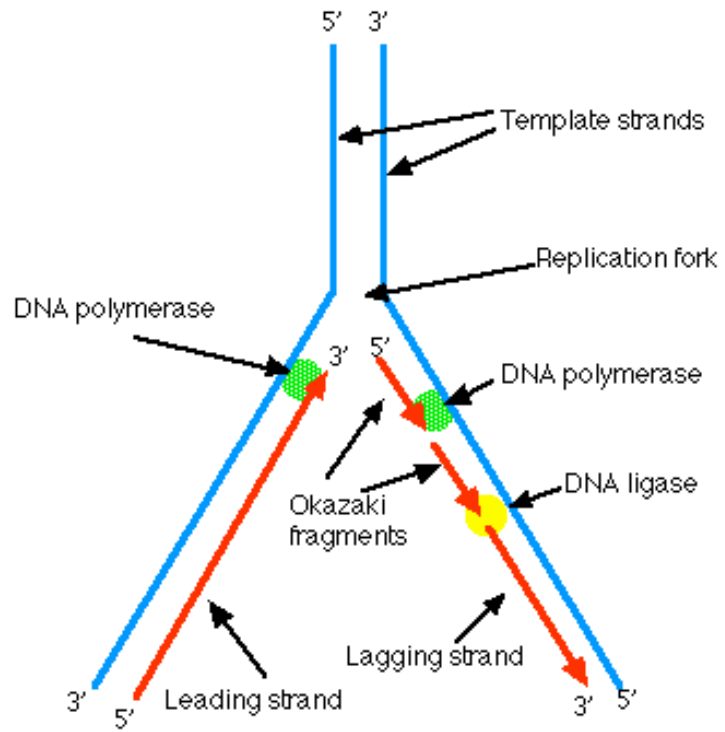
- It needs a nucleic acid chain in the beginning to begin copying the strand.

- An RNA polymerase known as **primase**, synthesizes short **RNA primers** (about 60nt long) that initiate the DNA replication process.

- This gives the DNA polymerase the required platform to begin copying the DNA strand.

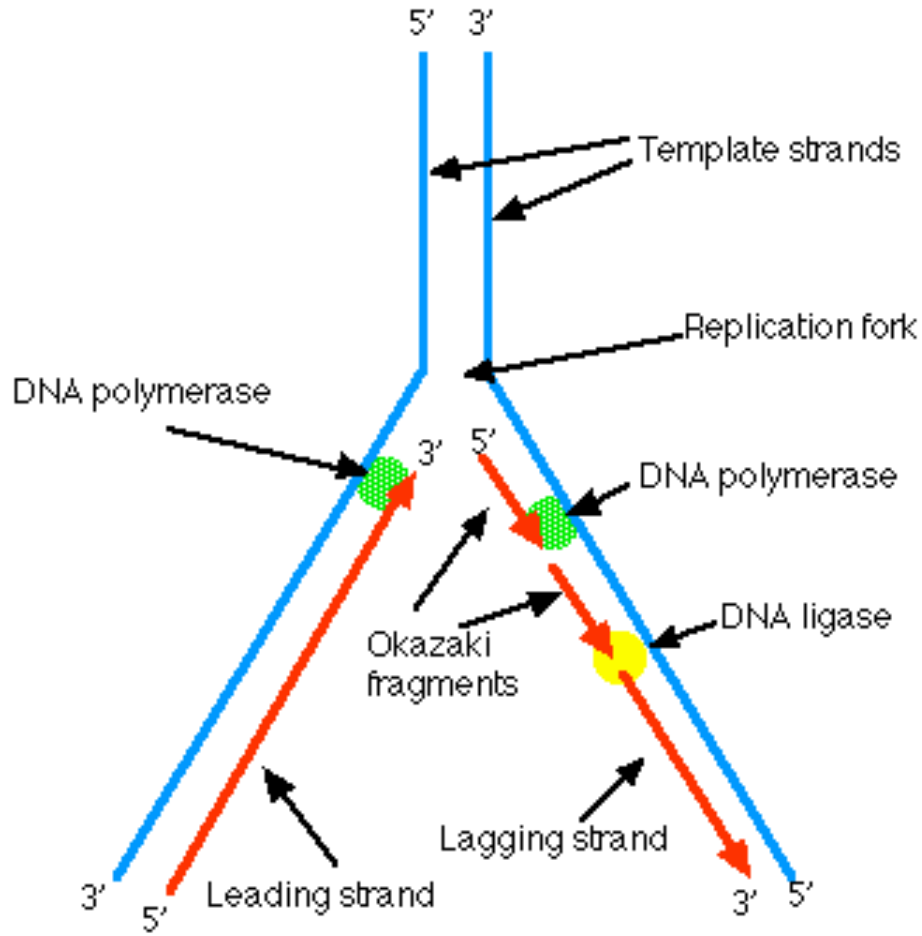
- It begins at the 3' end of the RNA primer.

- Two simple DNA replication enzymes are required for each parental DNA strand. The two polymerase enzymes move in opposite direction of the two strands.



- During the synthesis, only one polymerase remains on the DNA template and copies the DNA in a continuous strand.

- The other polymerase copies only a short stretch of DNA, before running into the primer of the initially sequenced fragment.

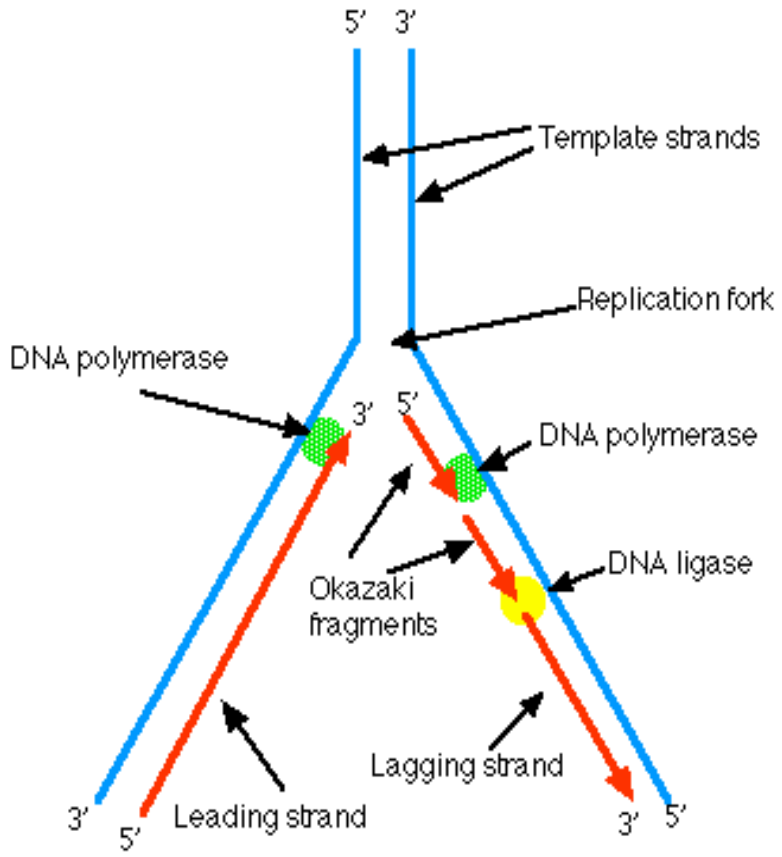

5′
3′
Template strands
Replication fork
DNA polymerase
3′
5′
DNA polymerase
Okazaki
fragments
DNA ligase
Lagging strand
3′
5′
Leading strand
3′
5′

- The strand that is synthesized continuously is called the leading strand and the strand that is synthesized in short pieces is called the lagging strand.

- The short pieces of synthesized DNA, that make up the lagging strand, are called the Okazaki fragments.

Synthesis of the Leading Strand

- The DNA strand that is read in the 3' → 5' and synthesized in the 5' → 3' direction continuously, is known as the leading strand.



- **DNA polymerase III** synthesises the DNA using the 3'- OH group, donated by the single RNA primer.

- The DNA replication continues in the direction of the replication fork, in a continuous manner.

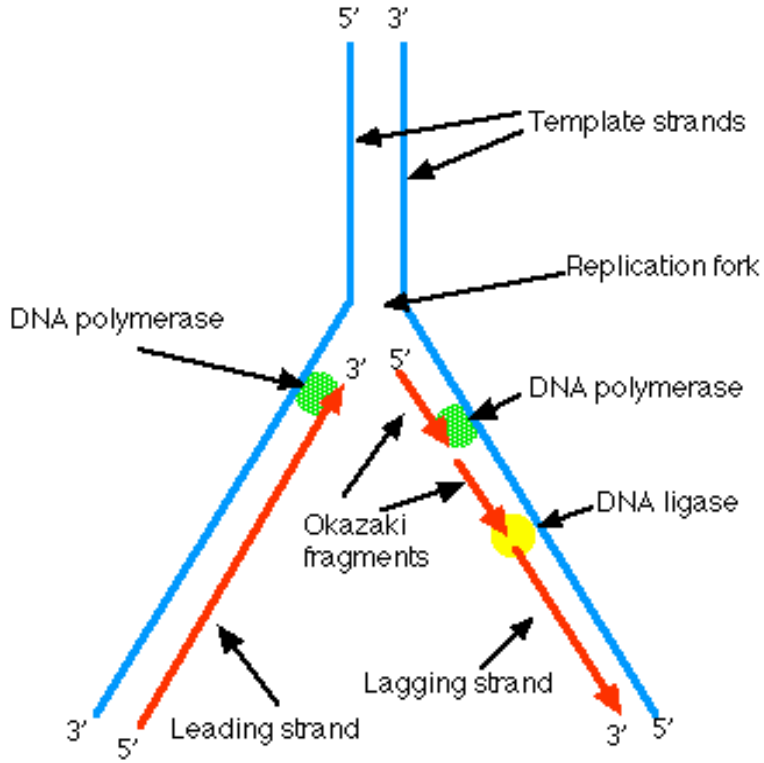
- RNAse H and DNA polymerase I (exonuclease) recognizes the RNA polymers that are bound to the DNA template and removes the primers by RNA hydrolysis.

❖READ ON DNA POL I, POL II AND POL III

Synthesis of the Lagging Strand

- The lagging strand is the DNA strand of the replication fork, that is opposite to the leading strand.

- It is synthesized in the opposite direction, that is, 5' to 3' instead of the 3' end as in the leading strand.



- The DNA polymerase cannot synthesize the strand 5' → 3' as explained above.

- Thus, the strand is synthesized in short fragments forming a lagging strand known as the Okazaki fragment.

- Primase builds RNA primers in short bursts over the lagging strand, which is synthesized in the 5' → 3' by DNA polymerase.

- The RNA primers are then removed and new deoxyribonucleotides are added to the gaps, where the RNA was present.

- DNA Polymerase continues with the synthesis of the new DNA strand.

- Finally, DNA ligase (an enzyme) joins the deoxyribonucleotides together, thus completing the lagging strand.

- In eukaryotes, termination of replication is poorly understood.

- Eukaryotes have linear DNA, and therefore use telomeres, which are GT rich repeating units that 'protect' the end of the DNA.

Rate of Replication

- The single molecule of DNA, that is the *E. coli* genome contains 4.7 x 10^6 nucleotide pairs.

- DNA replication begins at a single, fixed location in this molecule, called the **replication origin**, proceeds at about 1000 nucleotides per second, and thus is done in no more than 40 minutes.

- Due to the precision of the process (which includes a "proof-reading" function), the job is done with only about one incorrect nucleotide for every 10^9 nucleotides inserted.

- In other words, more often than not, the E. coli genome (4.7×10^6) is copied without error!

- The Genome of complex eukaryotes is huge as compared to procaryotes.

- The speed of DNA replication for human is about 50-75 nucleotides per second per replication fork.

- However, the genome can be copied only in a few hours because many replication forks take place at the some time (multiple initiation sites).