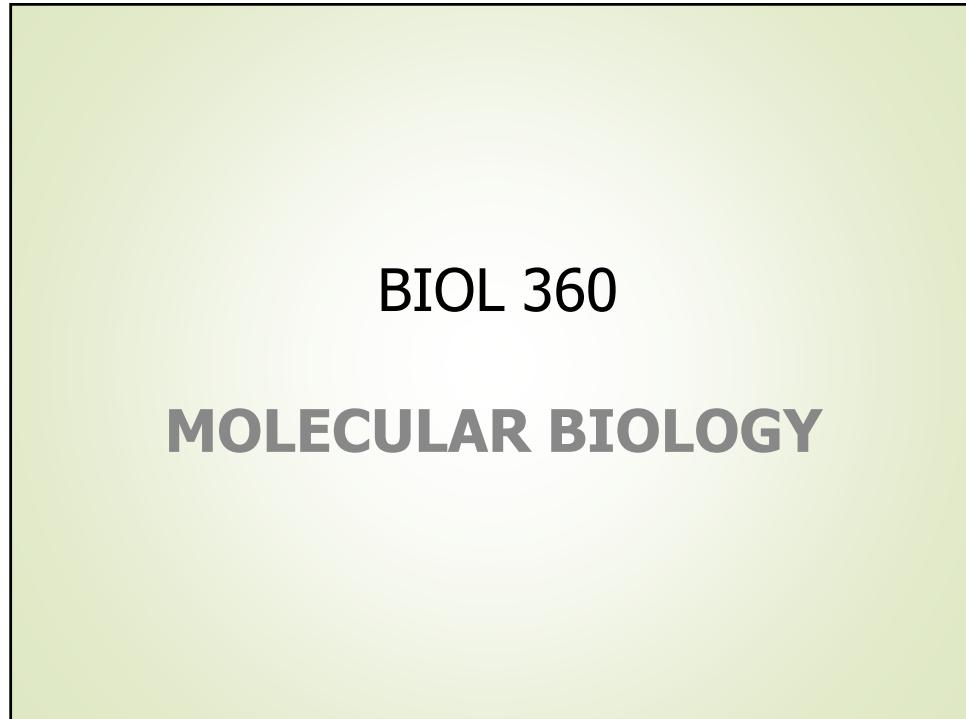


**BIOL 360**

**MOLECULAR BIOLOGY**



### 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

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

## COURSE OBJECTIVES

- 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, buoyant 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
- \*\*\* The Cell Cycle\*\*\*

RECOMMENDED  
TEXTBOOKS

1. Genetics

Copyright © 1997, 3<sup>rd</sup> 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?

#### WHAT IS MOLECULAR BIOLOGY?

- Understanding life at the molecular level

What about life

- Functions and processes for evolutionary success

What are the components of life?

- biomolecules

How do the components achieve the functions?

❖ 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***

molecular biology.....

- the study of the chemical and physical structure of biological macromolecules (Astbury, W. 1945)

-the branch of biology that studies the structure and activity of macromolecules essential to life (deals with the molecular basis of biological activity)

IT IS THE STUDY OF GENE STRUCTURE AND  
***function at the molecular level.***

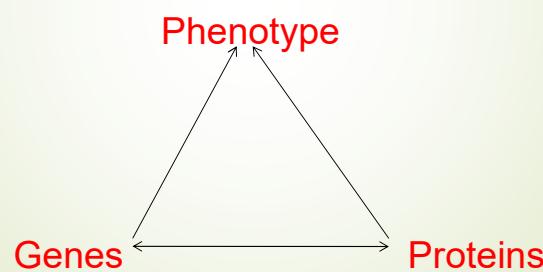
It must be noted that, Molecular Biology/Genetics grew out of the disciplines of genetics and biochemistry.

**Modern  
Molecular Biology combines:**

Biochemistry

Genetics

Cell Biology



## 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.

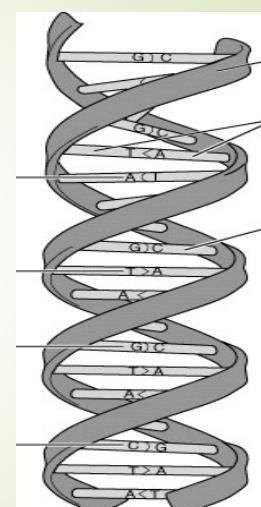
➤ Aaron Levene provided an important information about the chemical composition of DNA and concluded that, it is a polymer of **purine** and **pyrimidine** nucleotides.

- 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.

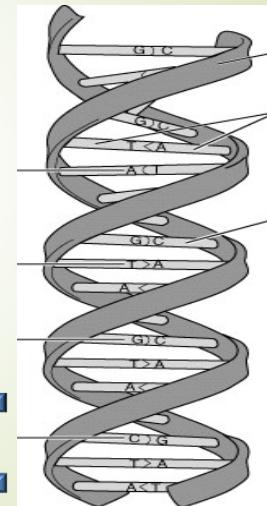
### DNA is a Double Helix

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

OR

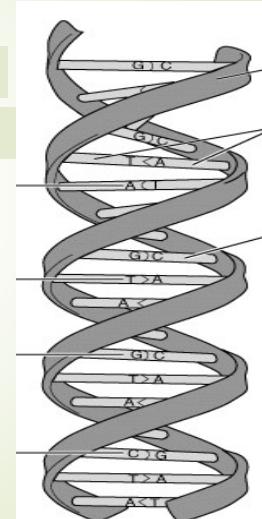


- It is a high molecular weight polymeric compound which consists of two molecules that are arranged into a ladder-like structure called a **Double Helix.**



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

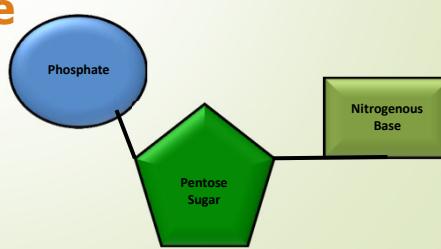
- The sugar is a pentose .



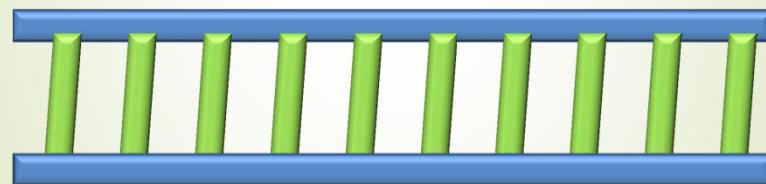
➤ A molecule of DNA is made up of millions of tiny subunits called **Nucleotides**.

➤ Each nucleotide consists of:

1. **Phosphate group**
2. **Pentose sugar**
3. **Nitrogenous base**



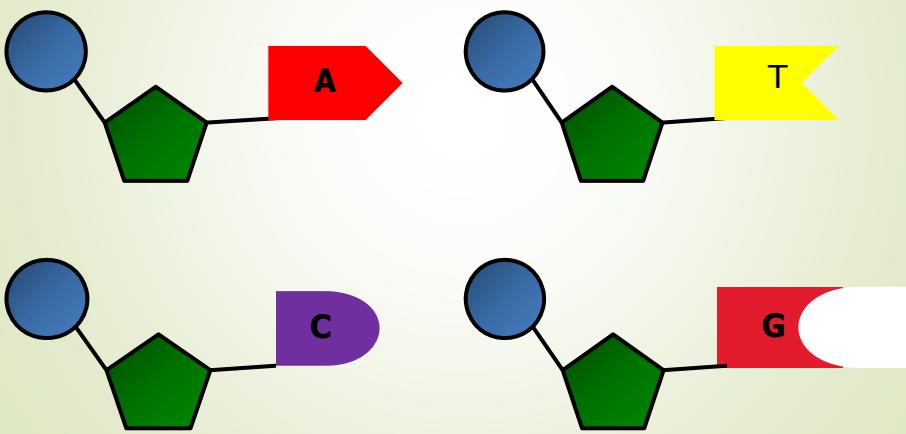
➤ The phosphate and sugar form the backbone of the DNA molecule, whereas the bases form the “rungs”.



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

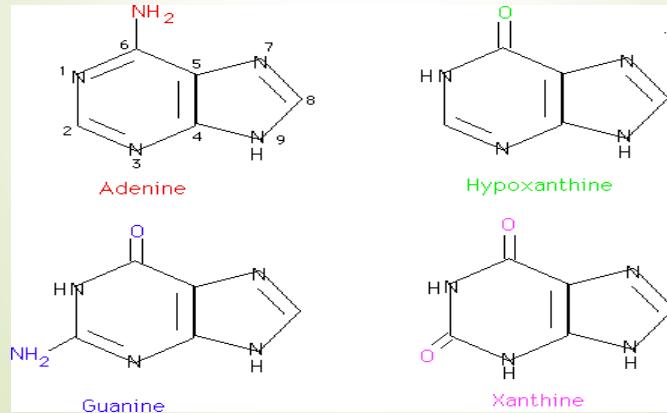


- There are **four** types of nitrogenous bases.



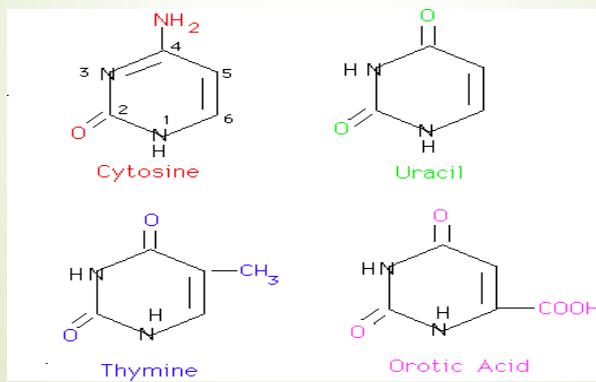
## Purines (Adenine, Guanine, Hypoxanthine)

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

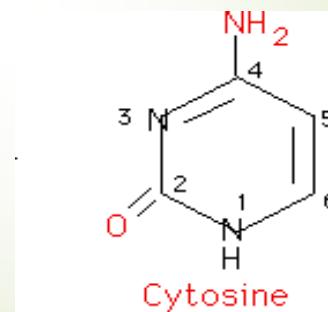
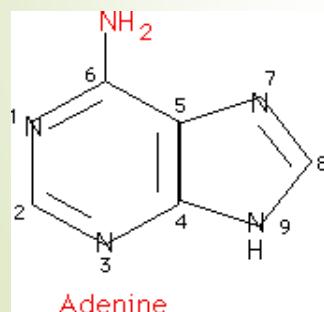


## Pyrimidines (Cytosine, Thymine, Uracil)

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



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

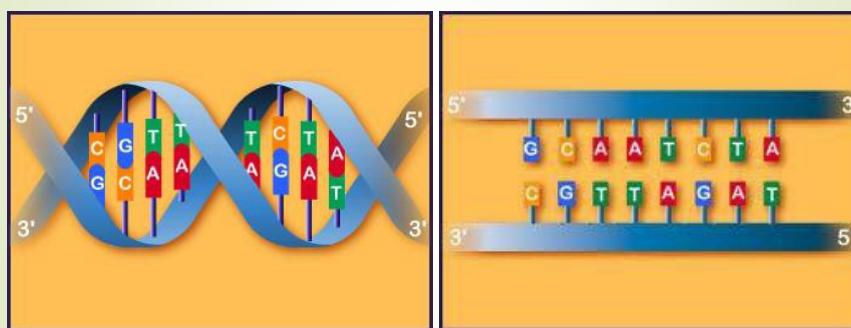


- The major bases found in DNA are **Guanine, adenine, cytosine** and **thymine** (**uracil** in RNA).
- In certain bacterial viruses, cytosine is replaced by **5-methylcytosine** or **5-hydromethylcytosine**.

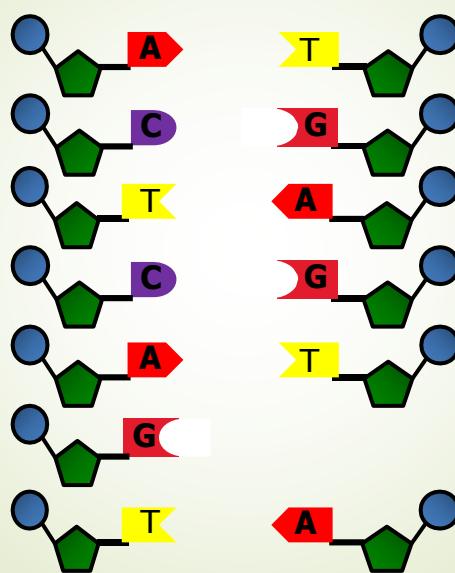
➤ Each base will only bond with one other specific base.

- Adenine (A)
  - Thymine (T)
- } Form a base pair.
- Cytosine (C)
  - Guanine (G)
- } Form a base pair.

✓ illustration

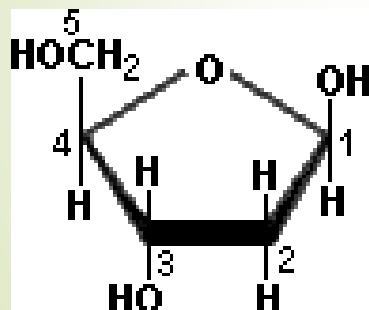


➤ Because of this **complementary** base pairing, the order of the bases in one strand determines the order of the bases in the other strand as shown below.

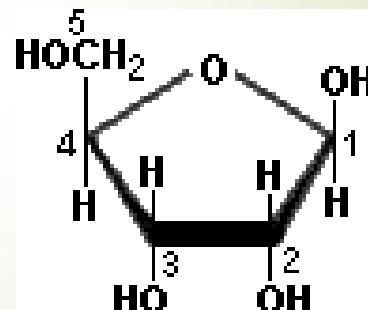


## Pentose sugar

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



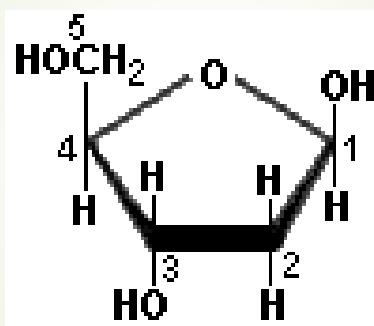
**Deoxyribose**



**Ribose**

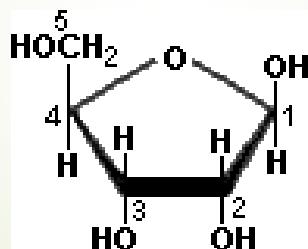
## The sugar component

➤ 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.

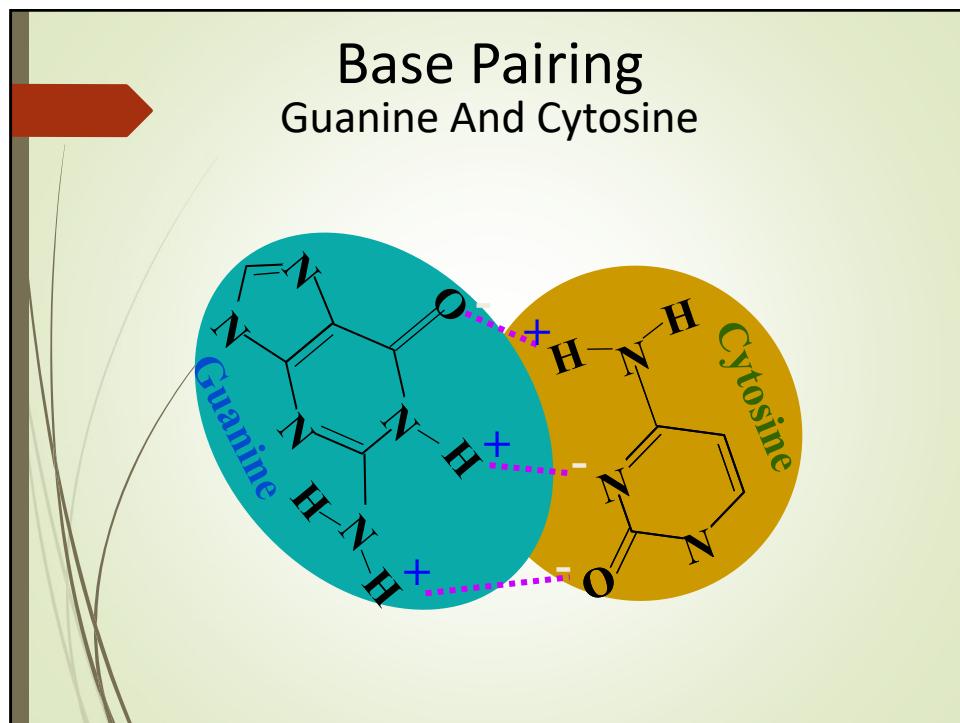
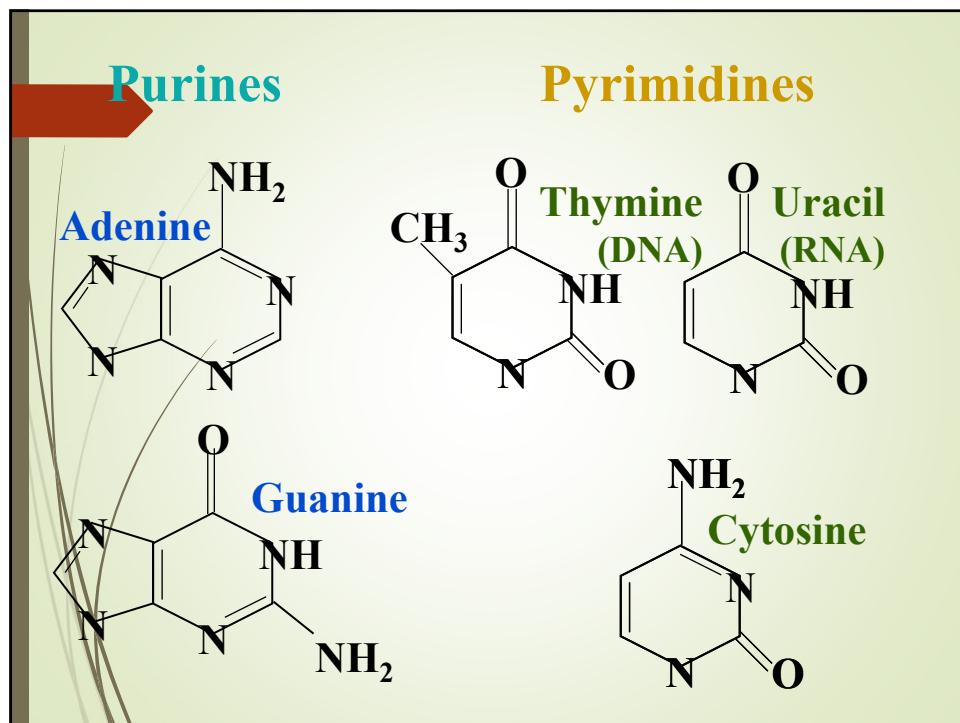
- This is because the presence of the bulky hydroxyl group on the 2-position of the sugar ring (ribose) not only limits the range of possible secondary structures available to the RNA molecule,



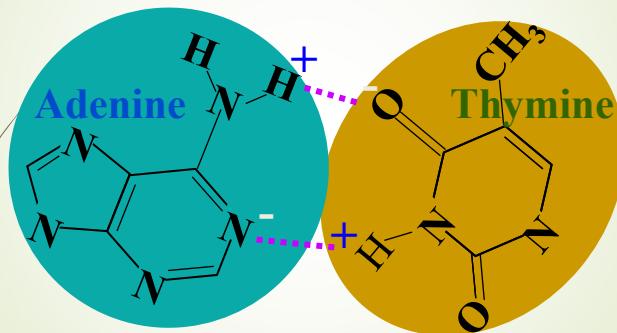
- but also makes it more susceptible to chemical and enzymatic degradation.

## Organic Bases

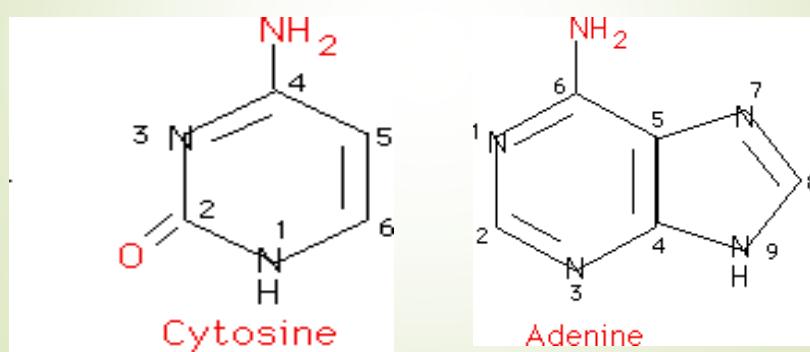
- Purines - adenine and guanine
- Pyrimidines - uracil, thymine and cytosine
- All heterocyclic
- All contain nitrogen, which is not ionized at physiological pH

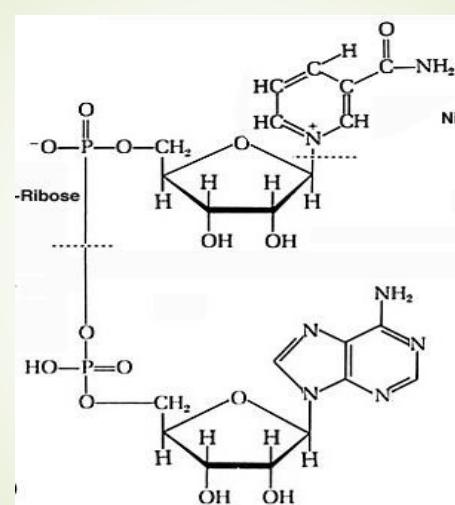
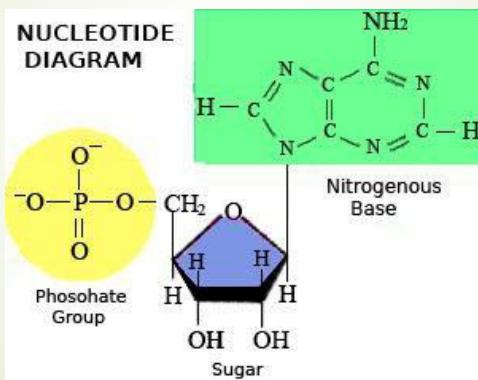


## Base Pairing Adenine And Thymine

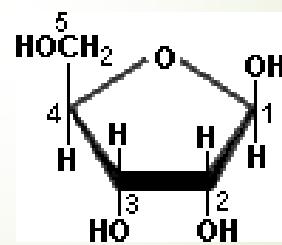
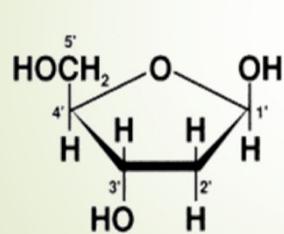


➤ The nitrogenous base is linked to position 1 on the pentose ring by a glycosidic bond from  $\text{N}_1$  of pyrimidines or  $\text{N}_9$  of purines.



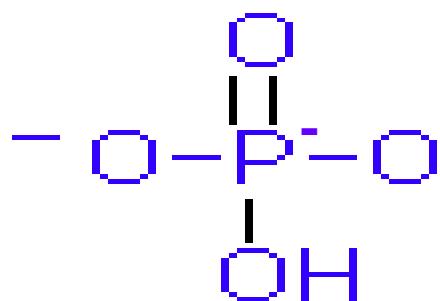


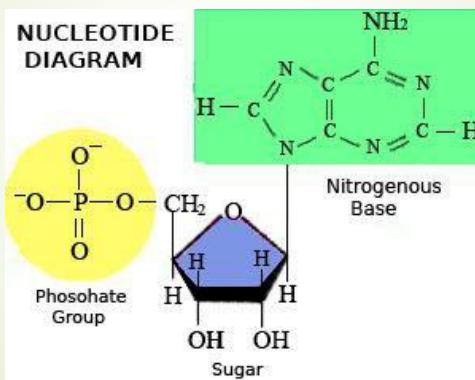
➤ To avoid ambiguity between the numbering systems of the heterocyclic rings and the sugar, positions on the pentose are given a prime (' )



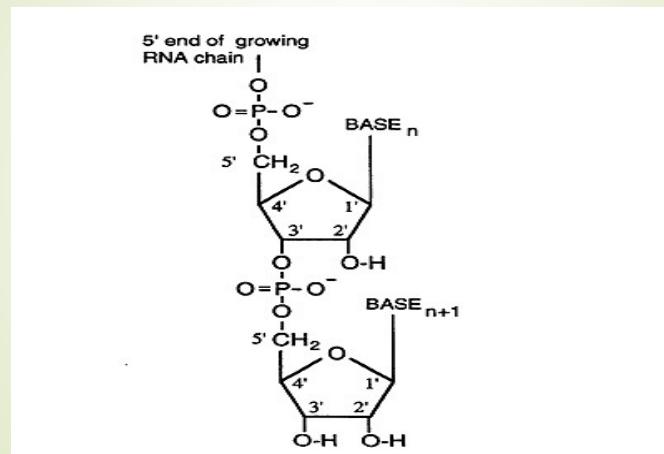
### Phosphate group

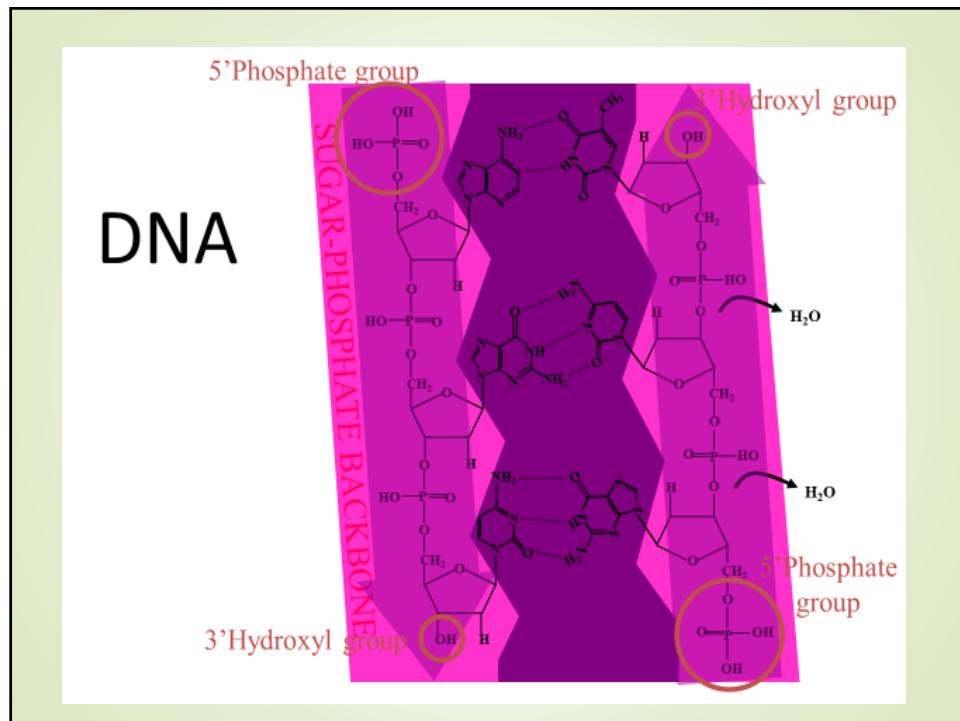
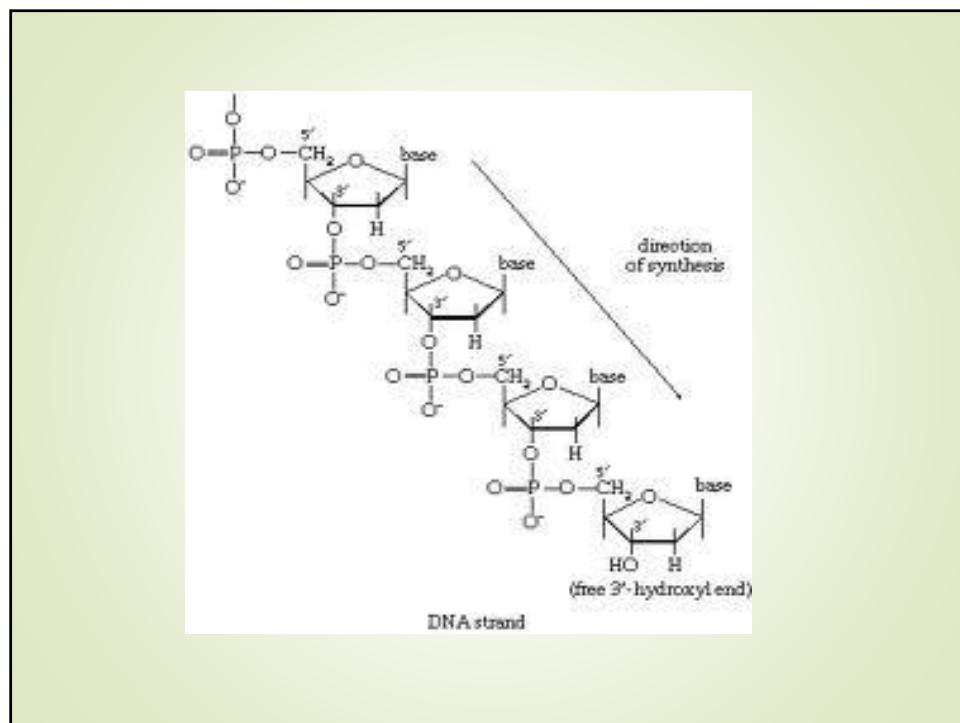
- $\text{HPO}_4$



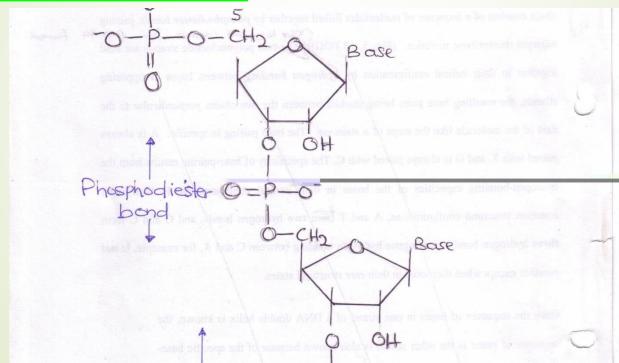


- The 5' position of one pentose ring is connected to the 3' position of the next pentose via a phosphate group as shown below;

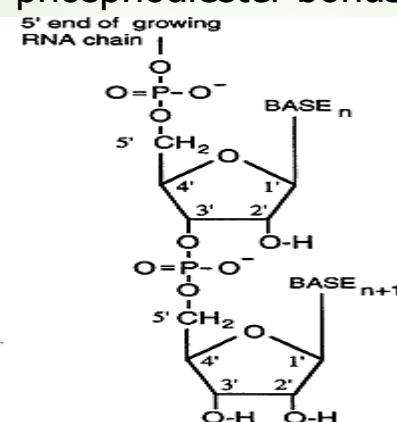




- Thus, the sugar-phosphate backbone is said to consist of 5'- 3' **phosphodiester** bond or linkages.

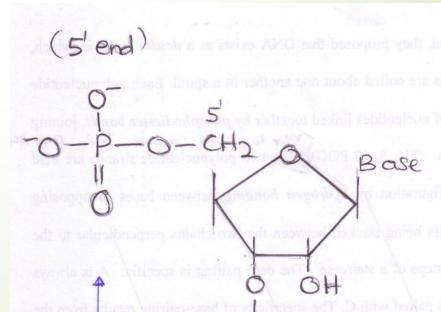
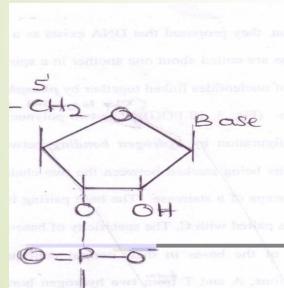


- When DNA (RNA) is broken into its constituent nucleotides, the cleavage may take place on either side of the phosphodiester bonds.



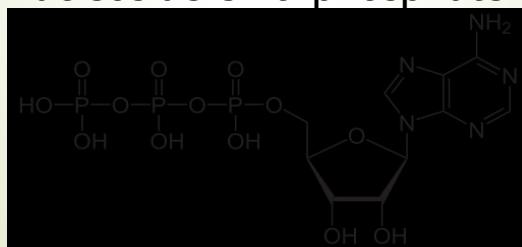
**Thus, the sugar-phosphate backbone is said to consist of 5'- 3' **phosphodiester** bond or linkages**

- The two types of nucleotides released from Nucleic Acids are therefore:
  - (i) nucleoside-3"-monophosphate and
  - (ii) nucleoside-5"-monophosphate.

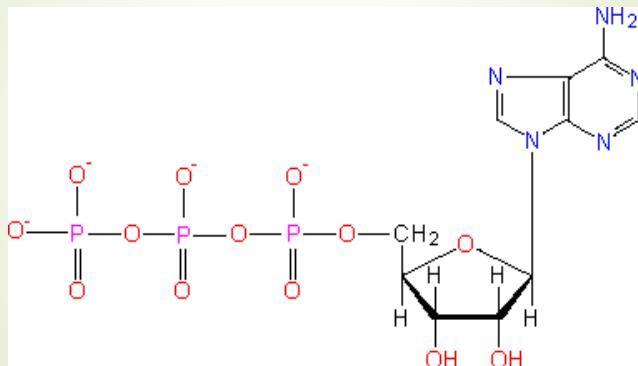


- All the nucleotides can exist in a form in which there is more than one phosphate group linked to the 5' position.

❖ Draw nucleoside-5"-triphosphate

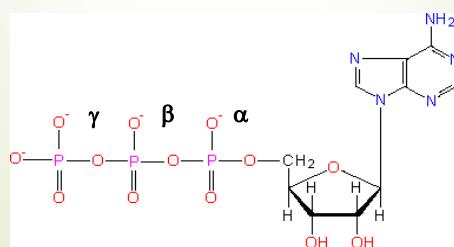


- All the nucleotides can exist in a form in which there is more than one phosphate group linked to the 5'' position.

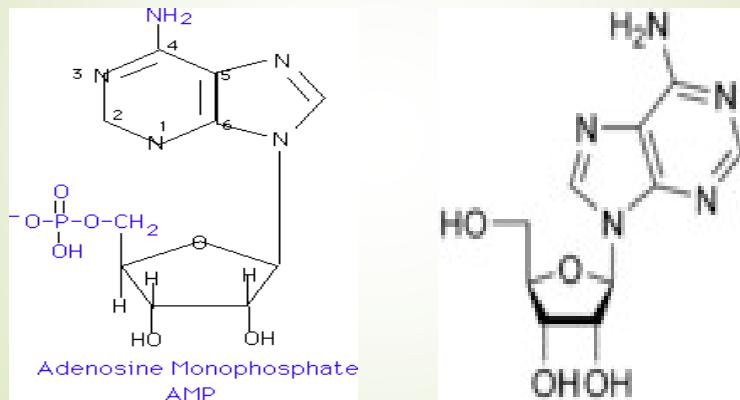


**Adenosine-5'-triphosphate**

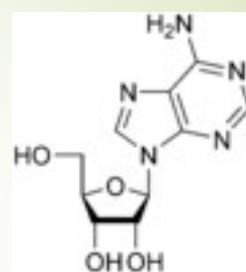
- The bonds between the first ( $\alpha$ ) and second ( $\beta$ ), and between the second ( $\beta$ ) and third ( $\gamma$ ), phosphate groups are **energy-rich** and are used to provide an energy source for various cellular activities.



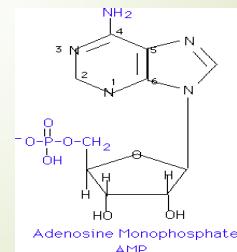
➤ Partial hydrolysis on the other hand yields compounds known as nucleotides and nucleosides.



➤ 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**.



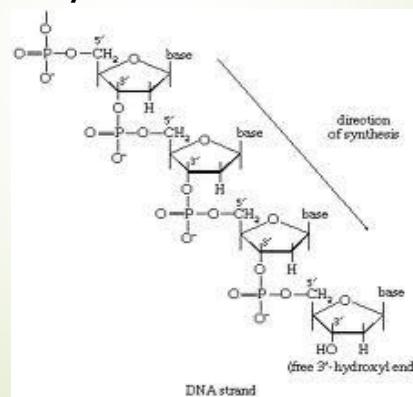
### Assignment:

- 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.

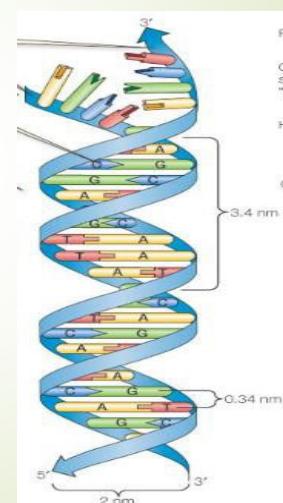


- Currently, the shorthand method of describing two DNA strands has been to have the strand of 5' → 3' polarity on the top line of the sequence with the complementary strand of opposite polarity lying below. For example:

**5'- AGGTC- 3'  
3'- TCCAG- 5'**

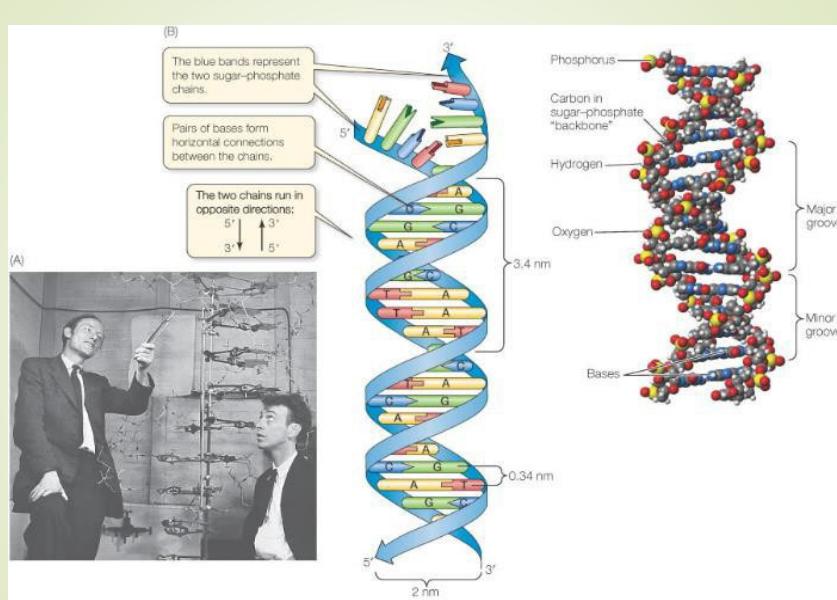
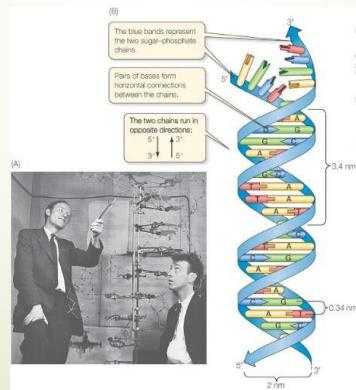
### Some characteristics of the Double-Helix

- Regular Helix has complete turn (pitch) of **34 Å** or (3.4nm)
- Has a diameter of  
**≈ 20 Å** or (**2nm**)



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

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



- 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 5'→3' 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.**

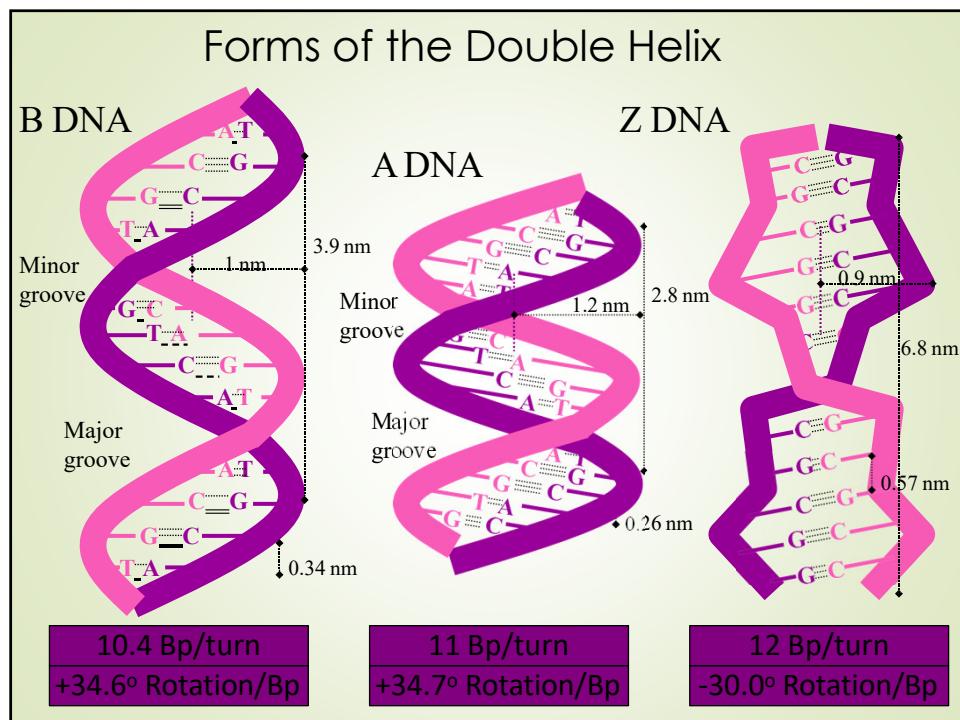
❖ *Due to the increased number of hydrogen bonds holding together a G:C nucleotide pair, regions of DNA rich in G + C are more stable than regions rich in A + T.*

❖ *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.



### 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° angle of inclination to the horizontal.

## OTHER FORMS OF DNA

- **C-DNA:**

- Exists only under high dehydration conditions
- 9.3 bp/turn, 0.19 nm diameter and tilted bases

- **D-DNA:**

- Occurs in helices lacking guanine
- 8 bp/turn

- **E-DNA:**

- Like D-DNA lack guanine
- 7.5 bp/turn

- **P-DNA:**

- Artificially stretched DNA with phosphate groups found inside the long thin molecule and bases closer to the outside surface of the helix
- 2.62 bp/turn

B-DNA appears to be the most common form *in vivo*.

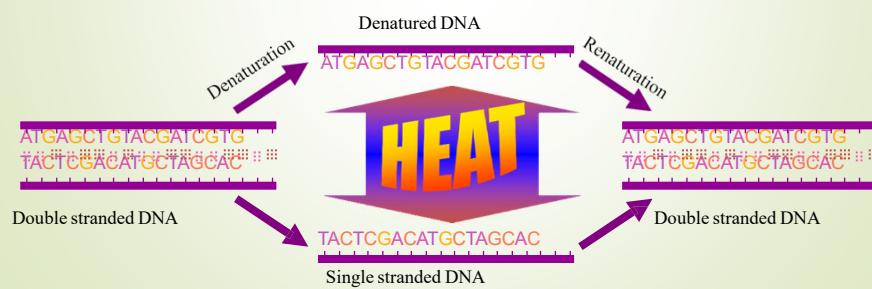
However, under some circumstances, alternative forms of DNA may play a biologically significant role.

## Supercoiling of DNA

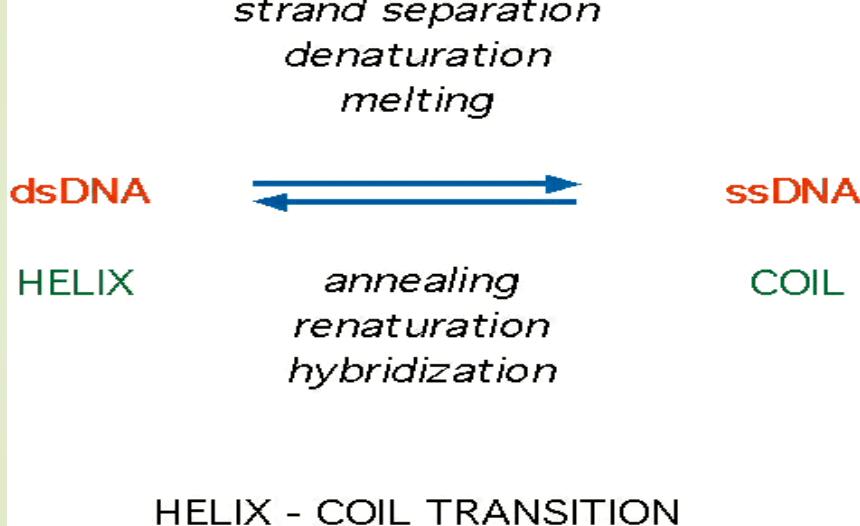
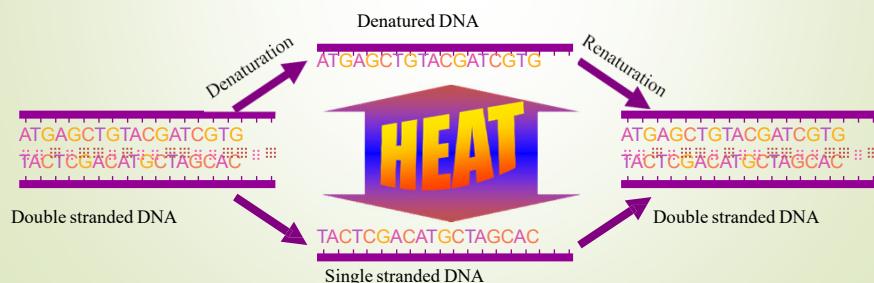
- Both linear and circular DNA may be Supercoiled
- Over- or under-wound double helix based on expected 10 bp/turn
- Supercoiled DNA stores torsional energy

## 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.

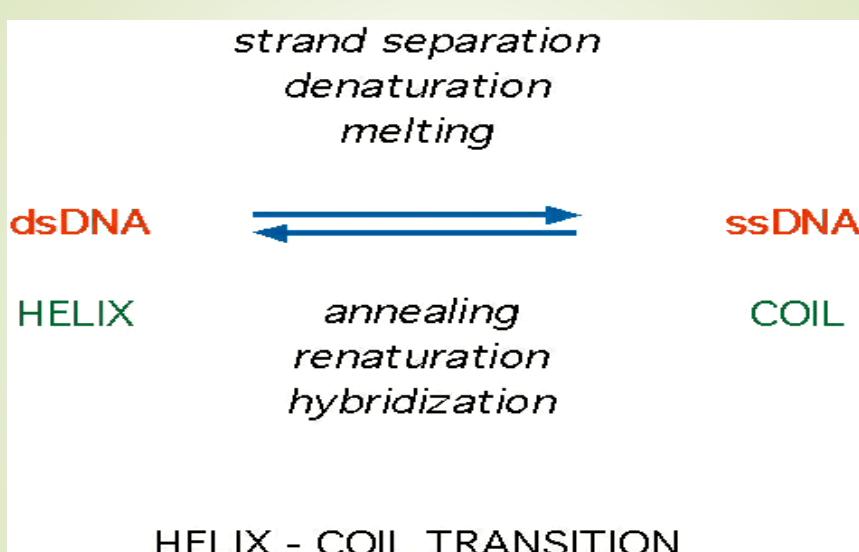


➤ The DNA is said to denature and changes from a double helix to a single stranded DNA random coil.



## Melting DNA

- Heat - kinetic energy overcomes bond strength (strength measured in terms of temperature)
- pH extremes - ionizing interrupts hydrogen bonding
- salt extremes
- “denaturing agents” - formamide, urea



➤ 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.

## Determining Melting Point (Tm)

- DNA is dissolved in standard saline solution near neutral pH
- Solution placed in quartz cuvette in Spectrophotometer
- Cuvette is heated slowly
- A<sub>260</sub> is monitored and plotted vs. temp.

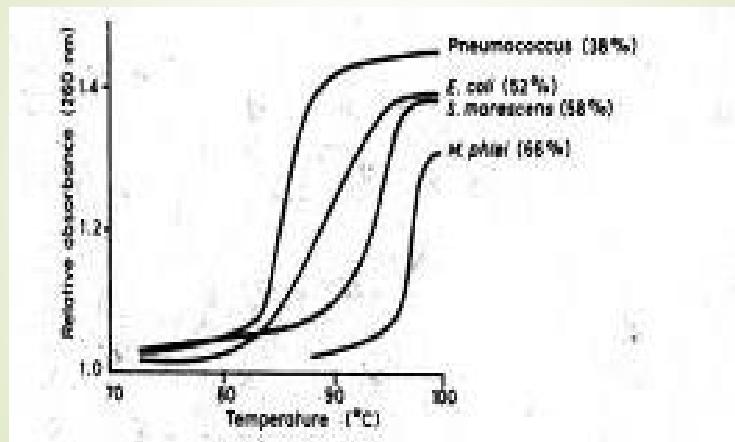
➤ The nature of the melting transition is affected by several factors:

- (i) G + C content
- (ii) The nature of the solvent
- (iii) The nature of the 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 DNA from different organisms



Read and make notes on the other factors

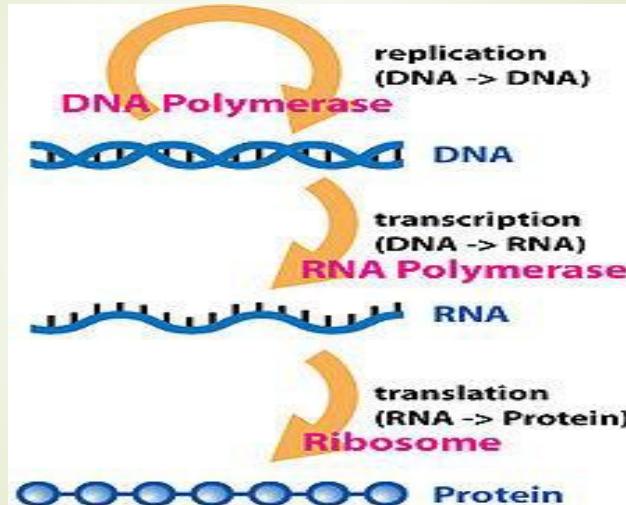
□(ii) The nature of the solvent

□(iii) The nature of the DNA

### Functions of DNA

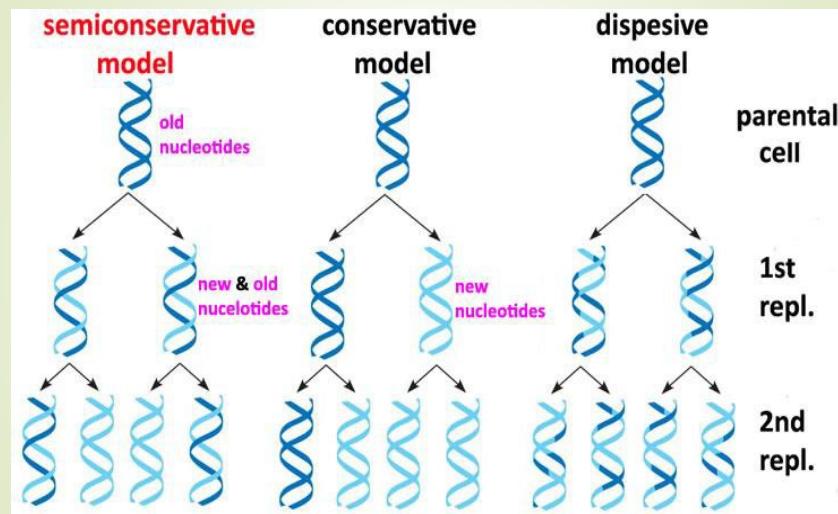
1. Storage of genetic information
  2. **Self-duplication (replication) & inheritance.**
  3. Expression of the genetic message.
- ✓ **DNA's major function is to code for proteins.**

## The Central Dogma of Gene Expression or Genetics



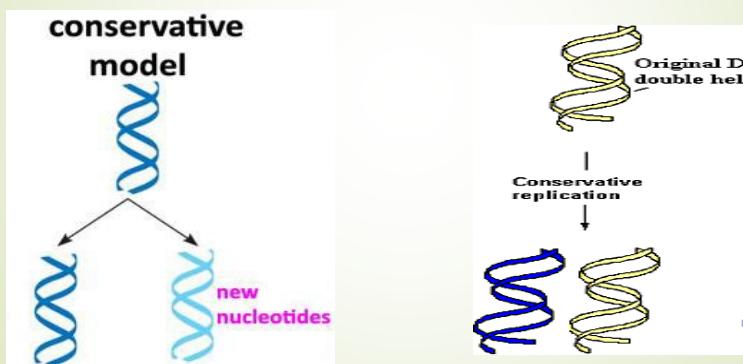
### Replication

- 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.



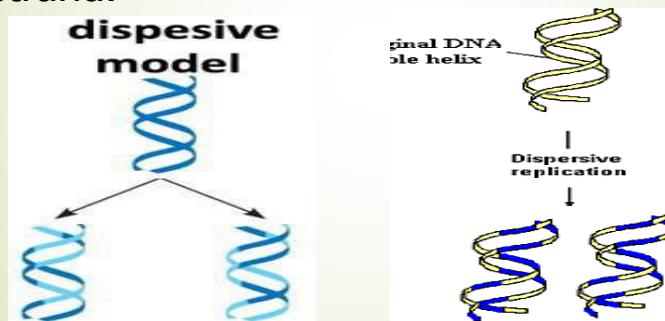
### Conservative Replication

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



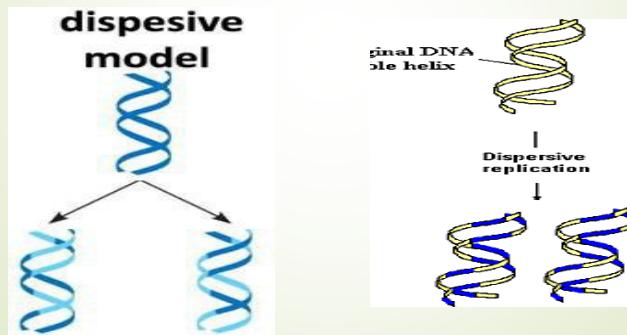
## Dispersive Replication

- 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

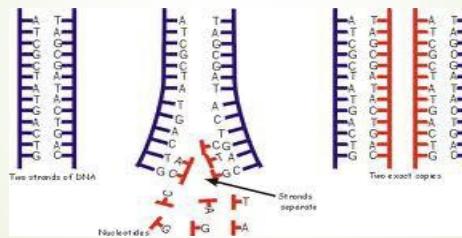


### Semiconservative Replication

- 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.

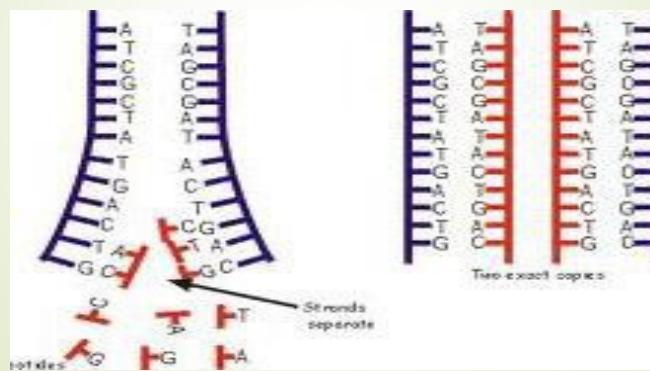


- Each strand acts as a **template** or **guide** for the synthesis of a new DNA molecule by the sequential addition of complementary base pairs.



- 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.



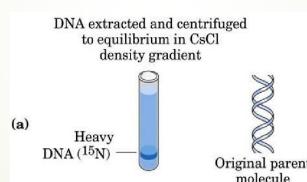
## Evidence for Semi Conservative Replication

- 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 **<sup>14</sup>N**.
- A relatively rare isotope **<sup>15</sup>N** has an atomic weight of 15.

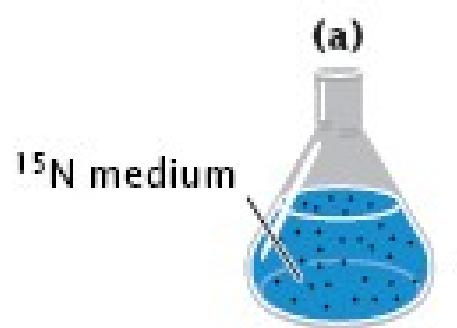
- Meselson and Stahl found that if bacteria are grown in a medium enriched in <sup>15</sup>N, they incorporate the heavy isotope into their DNA, which becomes denser than normal.



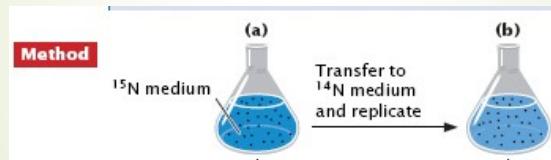
- This labeled DNA **clearly** separates from ordinary DNA in gradient of Cesium Chloride (CsCl) spun in an ultracentrifuge.

- 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  $^{15}\text{N}$  labeled bacteria in  $^{15}\text{N}$ -medium and then to look at the density of the DNA products.



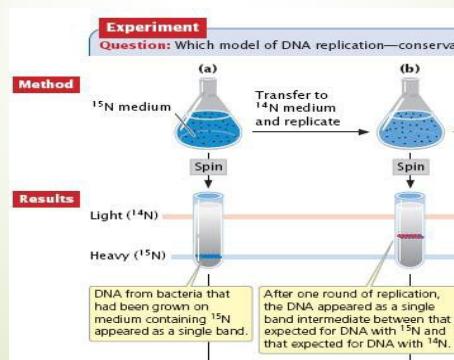
- That is, *E. coli* cells with only  $^{15}\text{N}$  in their DNA were transferred to a  $^{14}\text{N}$  medium and were allowed to divide or replicate;



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

## Experimental Results

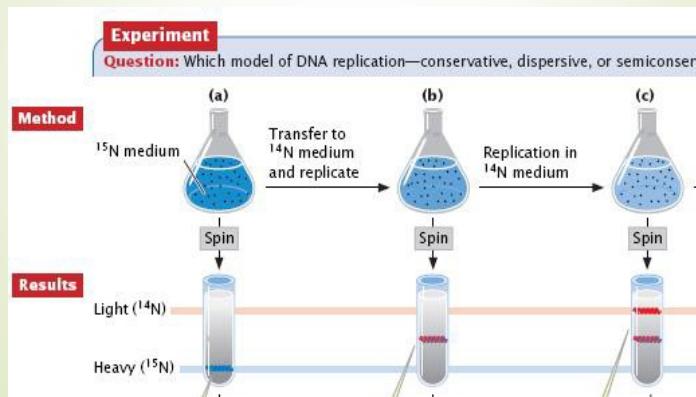
- DNA was extracted periodically and was compared to pure  $^{14}\text{N}$  DNA and  $^{15}\text{N}$  DNA.
- After one replication, the DNA was found to be close to the **intermediate density**.



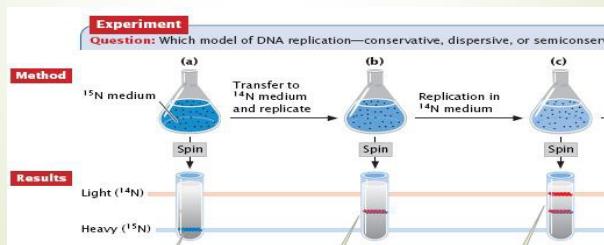
- Based on these findings, the scientists were immediately able to **exclude the conservative model of replication as a possibility.**
- After all, if DNA replicated conservatively, there should have been two distinct bands after a single round of replication

- 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.

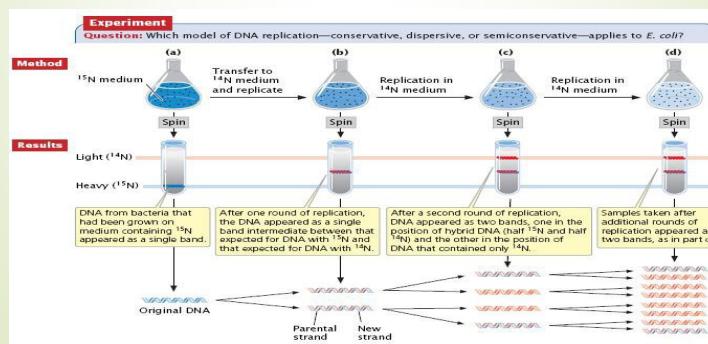


- One corresponding to the intermediate density of DNA of cells grown for only one division in  $^{14}\text{N}$  medium, the other corresponding to DNA from cells grown exclusively in  $^{14}\text{N}$  medium.

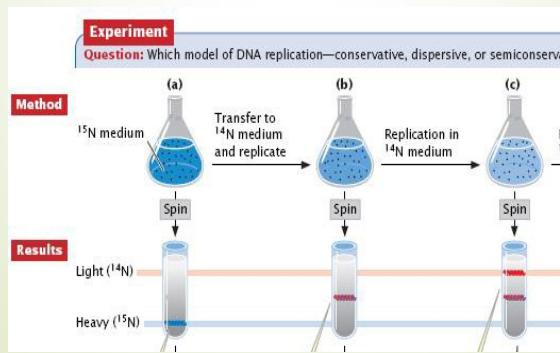


- This was inconsistent with dispersive replication.
- After all, if the dispersive model were the correct model, the scientists would have continued to observe only a single band after every round of replication.

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

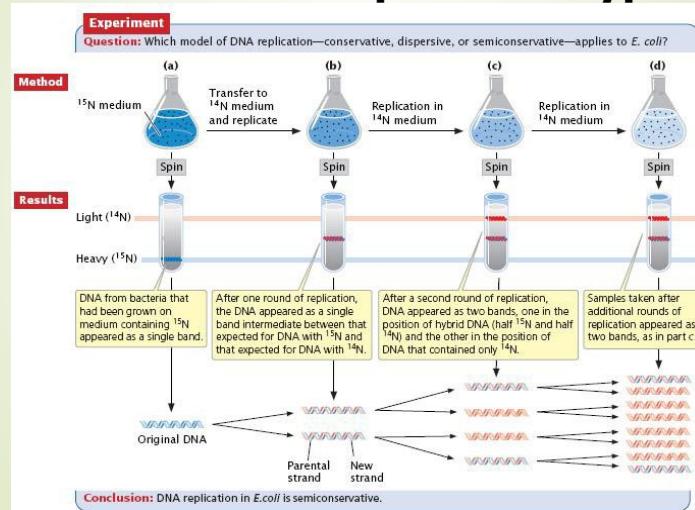


- Semiconservative replication would result in double-stranded DNA with one strand of  $^{15}\text{N}$  DNA, and one of  $^{14}\text{N}$  DNA.
- This will result in equal amounts of DNA with two different densities.



- 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





# **CELL DIVISION & REPLICATION OF DNA**

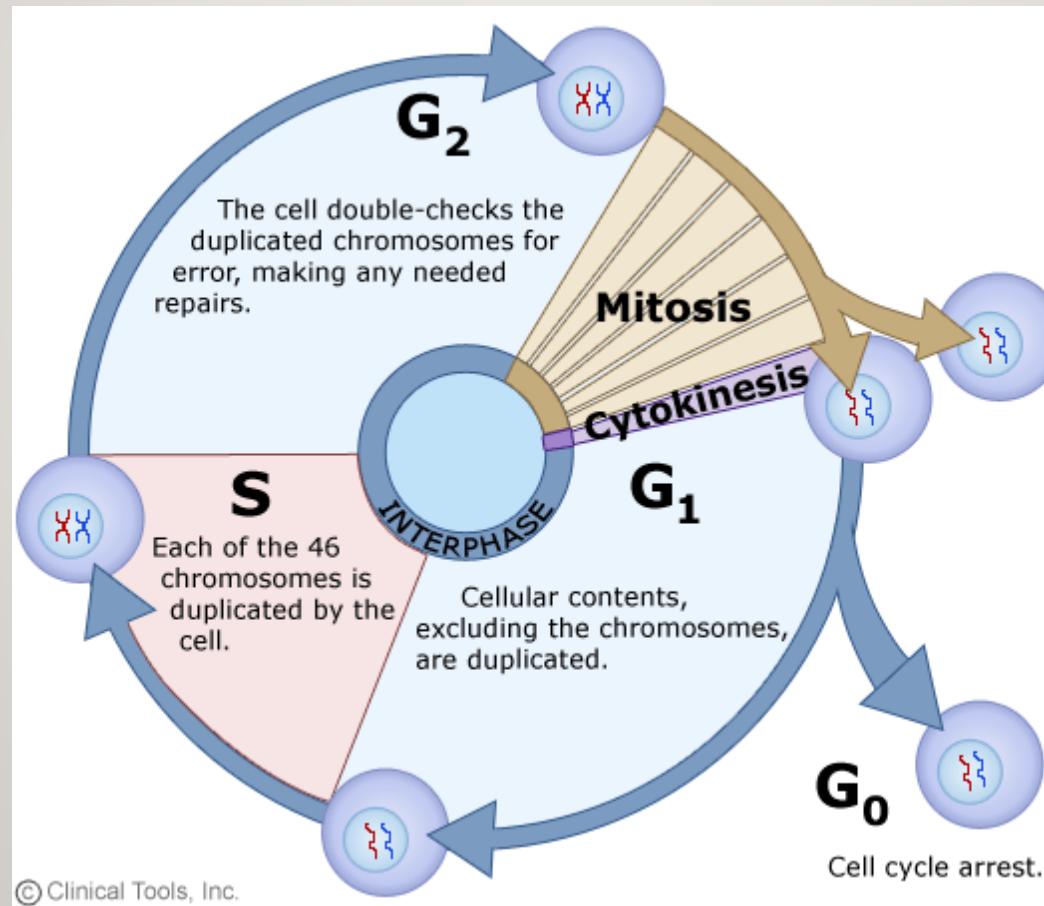
- Cells divide;  
Growth, repair and replacement
- Before cells divide they have to double cell structures, organelles and their genetic information

**DNA replication is the process where an entire double-stranded DNA is copied to produce a second, identical DNA double helix.**

# REPLICATION OF DNA

Why does DNA replicate?

During which phase of the cell cycle does DNA replicate?



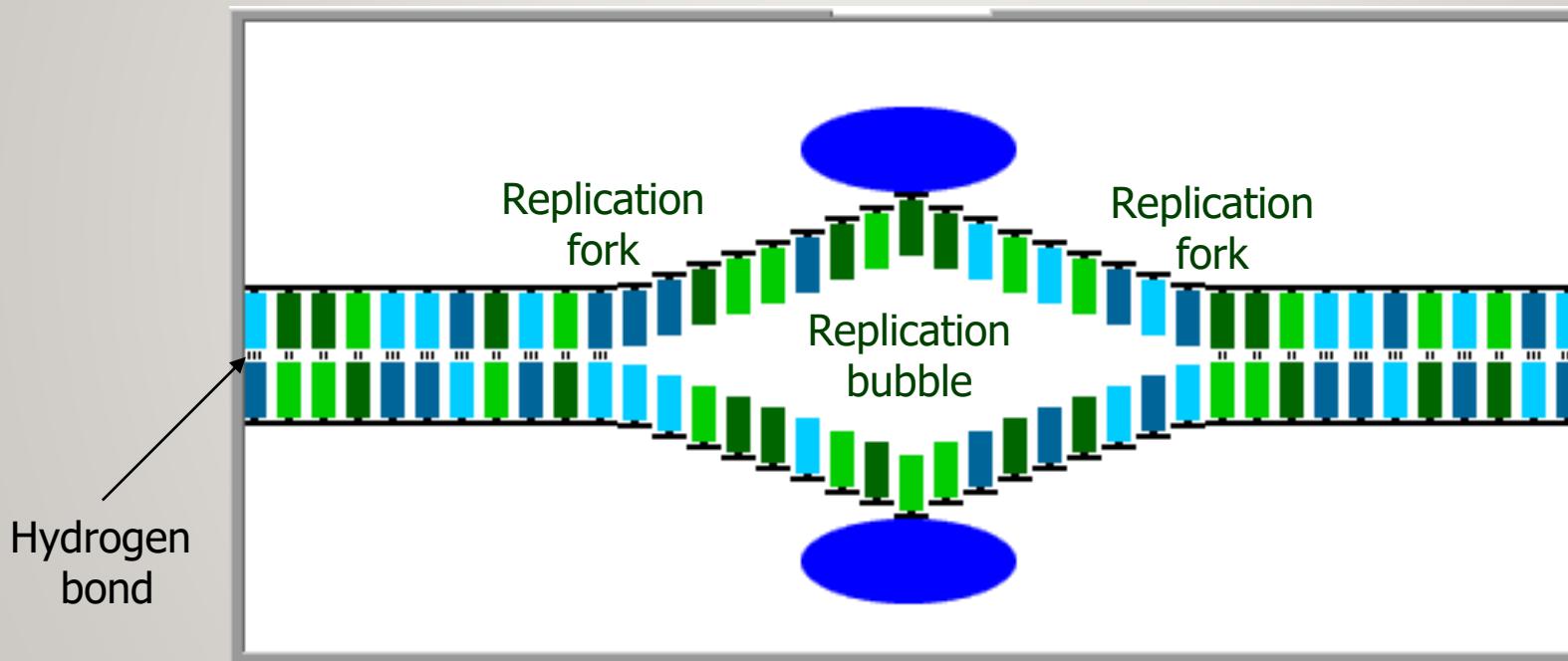
# REPLICATION OF EUKARYOTIC CHROMOSOMES

- Before a cell can divide, it must duplicate or replicate all its DNA.
- In eukaryotes, this occurs during the 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 whether in bacteria or in eukaryotes require many proteins or enzymes acting 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 required enzymes available there.

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

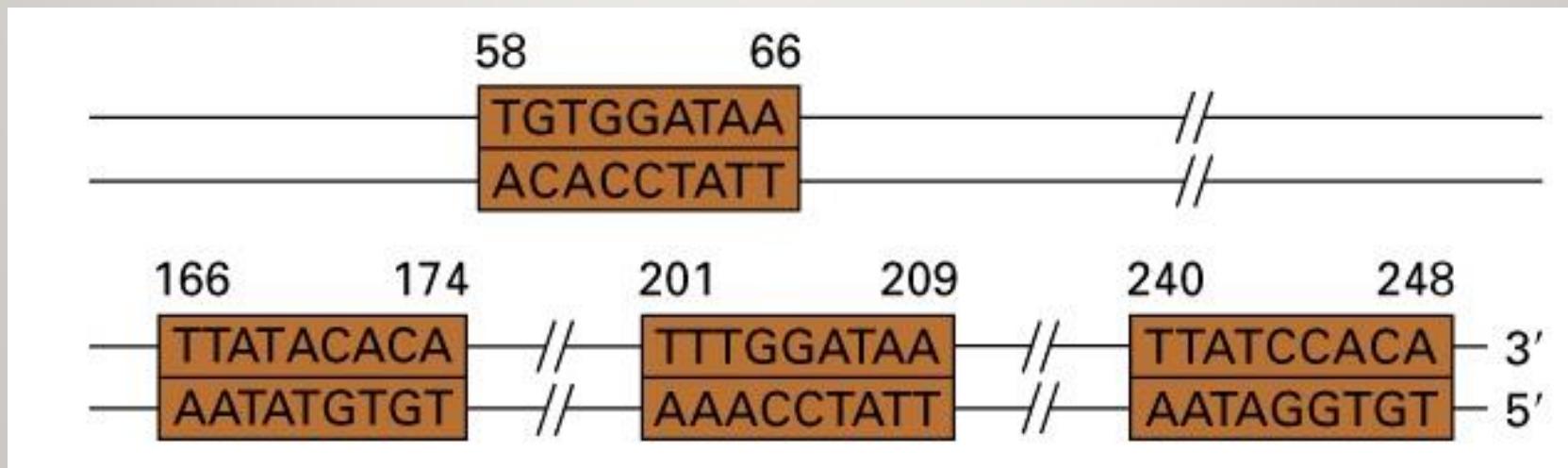


- These sites are recognized by certain proteins in the cell.

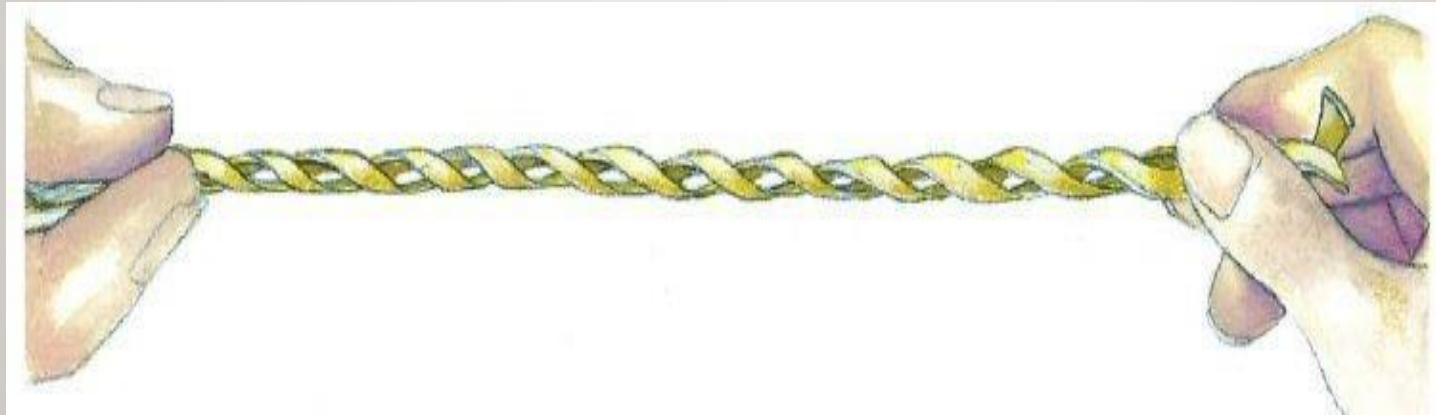
# Consensus sequence of replication origins

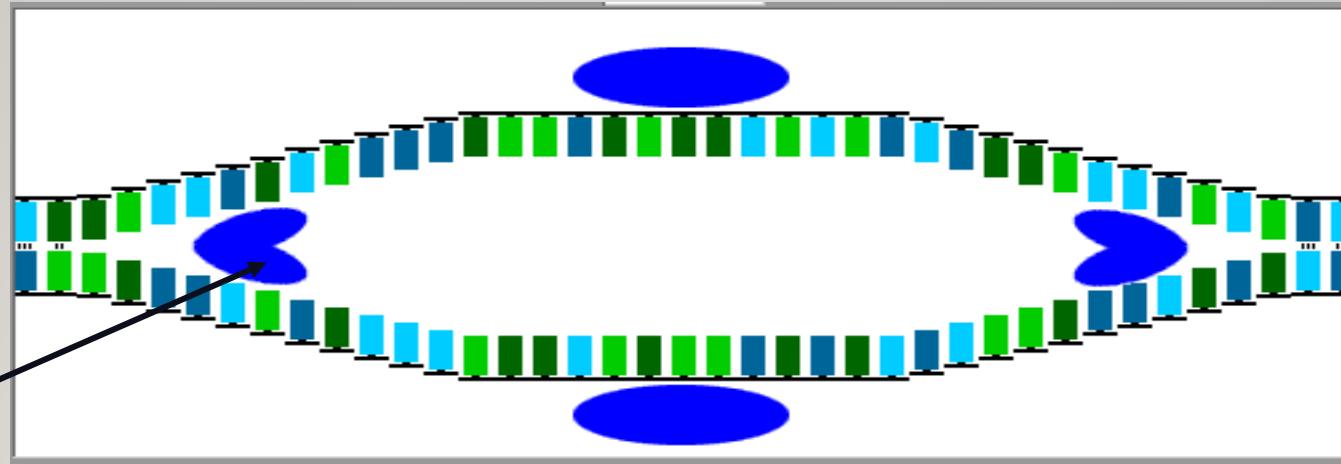
Replication origins, regardless of organism, are

- i. unique DNA segments with multiple short repeats
- ii. usually contain an A-T rich stretch, as breaking two hydrogen bonds between the A-T is easier than breaking the triple hydrogen bonds between C-G.
- iii. recognized by origin-binding proteins



- 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.



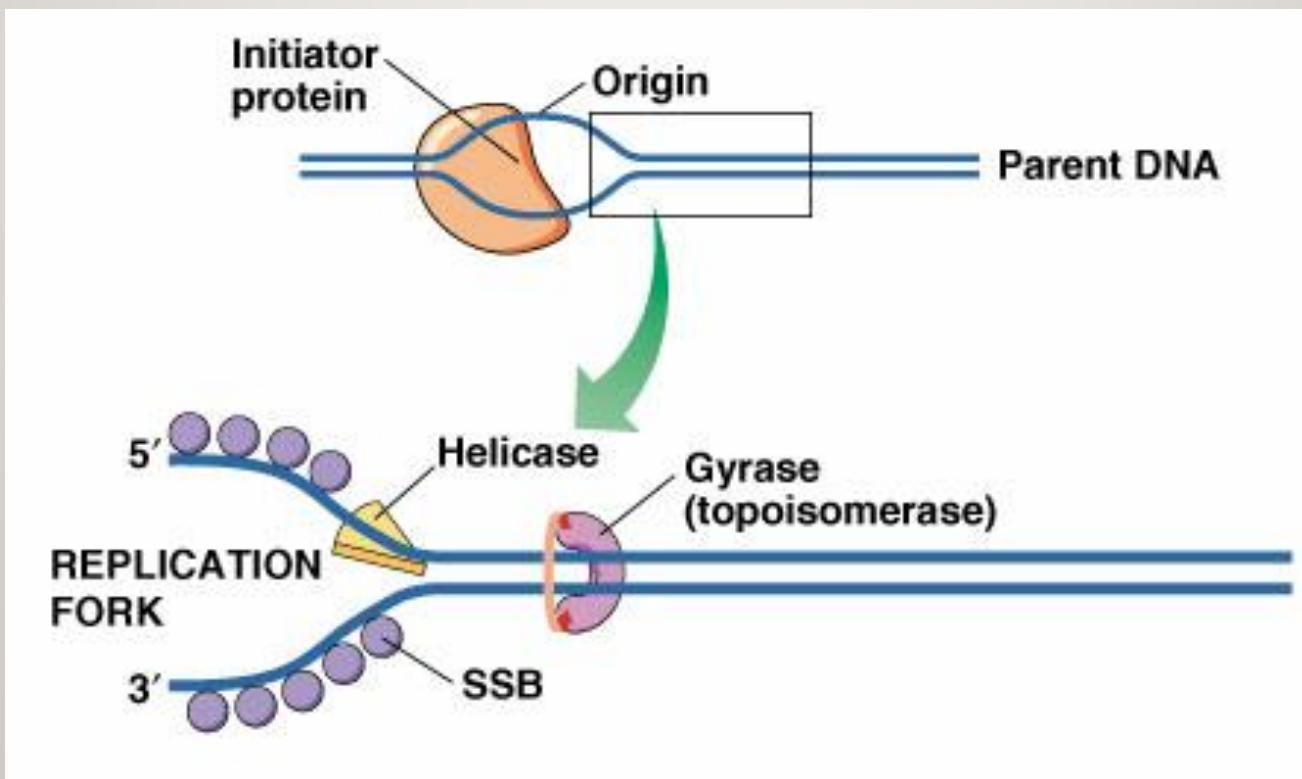


- The two strands separate as the hydrogen bonds between base pairs are broken by the **Helicase**.
  - **Gyrases** unwind and relax the double helix starting at a replication bubble.
  - **SSBs** get attached to the strands holding them apart from each other so each strand acts as template.
- Two replication forks form and the DNA is unwound in opposite directions.**

# Requirements for DNA replication

## Initiation

Initiator protein (DnaA) binds to the origin and separates strands



# Helicase and SSBs

## Helicase

Separates or “melts” duplex DNA

- ▶ Requires energy

## SSBs

- ▶ Single-stranded binding proteins
- ▶ Helps keep the strands from re-annealing

## Gyrase

- ▶ Eliminates supercoiling that accompanies unwinding

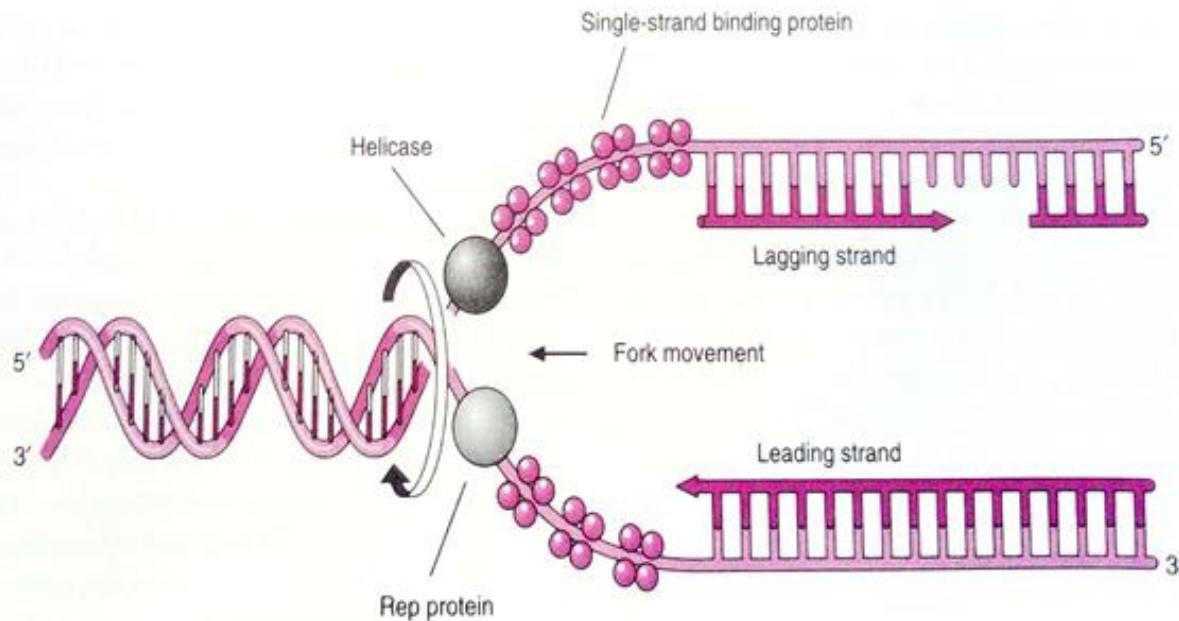
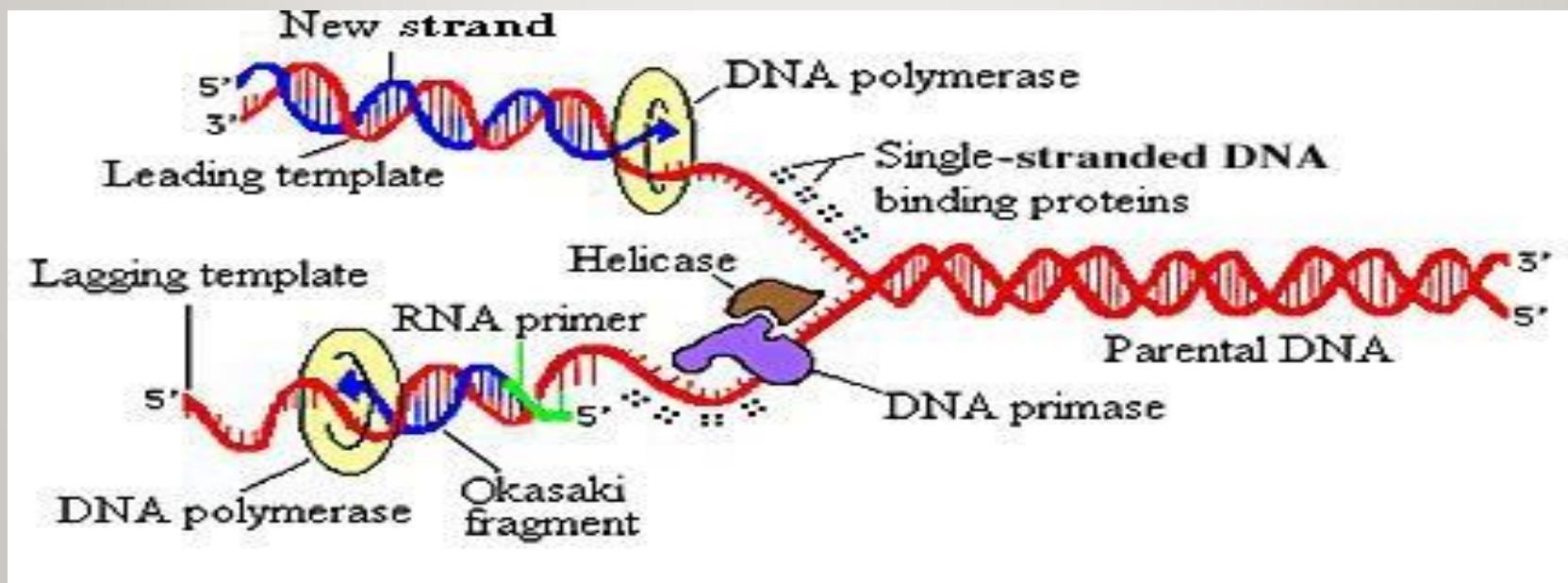


FIGURE 2.4

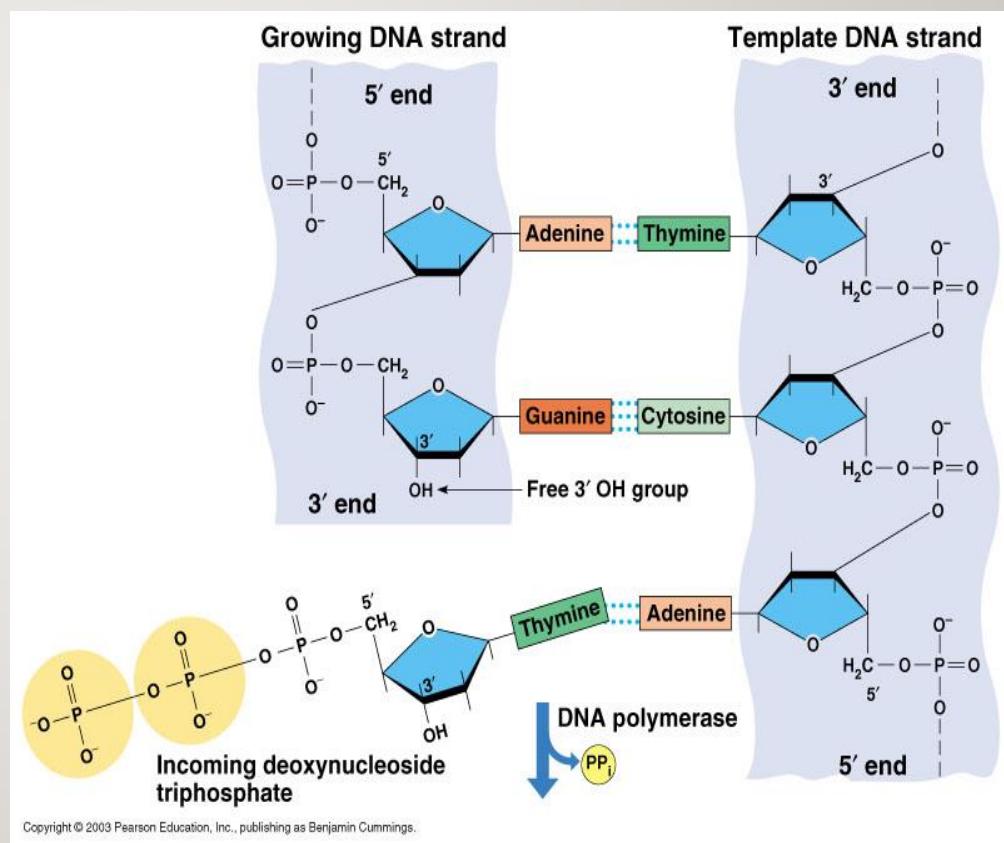
DNA is unwound as Rep protein binds to the leading strand template and helicase II (dnab protein) binds to the lagging-strand template. Separated DNA strands are prevented from reannealing by the binding of SSBs.

- 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.



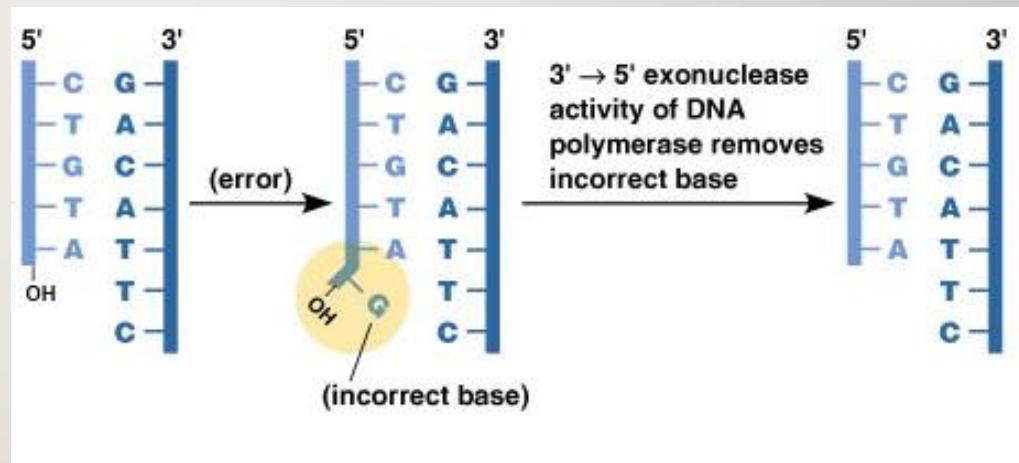
# DNA polymerases

- Add nucleotides against a DNA template to the 3' end of the growing strand (5' → 3' growth)
- Requirements
  - i. A template
  - ii. Deoxyribonucleoside triphosphates (which serve both as the source for the nucleotide *and* as the energy source)
  - iii. A primer

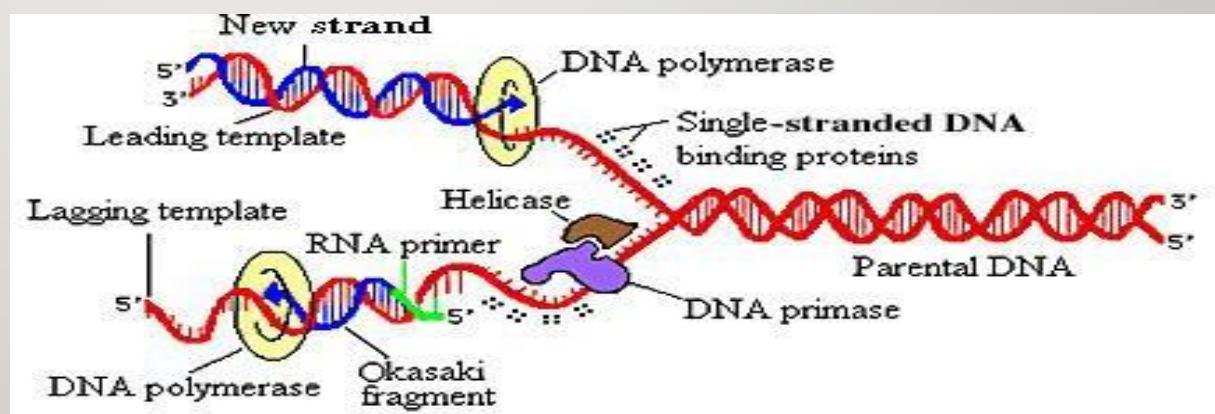


# DNA polymerase III ( $\delta$ in eukaryotes)

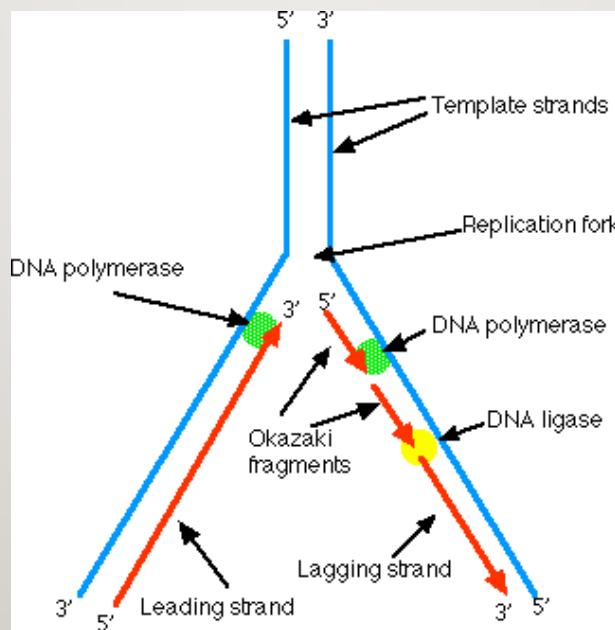
- ▶ The main polymerase in bacteria
- ▶ Consists of 10 peptide subunits
- ▶  $5' \rightarrow 3'$  polymerase
- ▶  $3' \rightarrow 5'$  exonuclease
  - for proofreading
  - excising incorrect nucleotides as it polymerizes
  - exonuclease versus endonuclease



- 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.

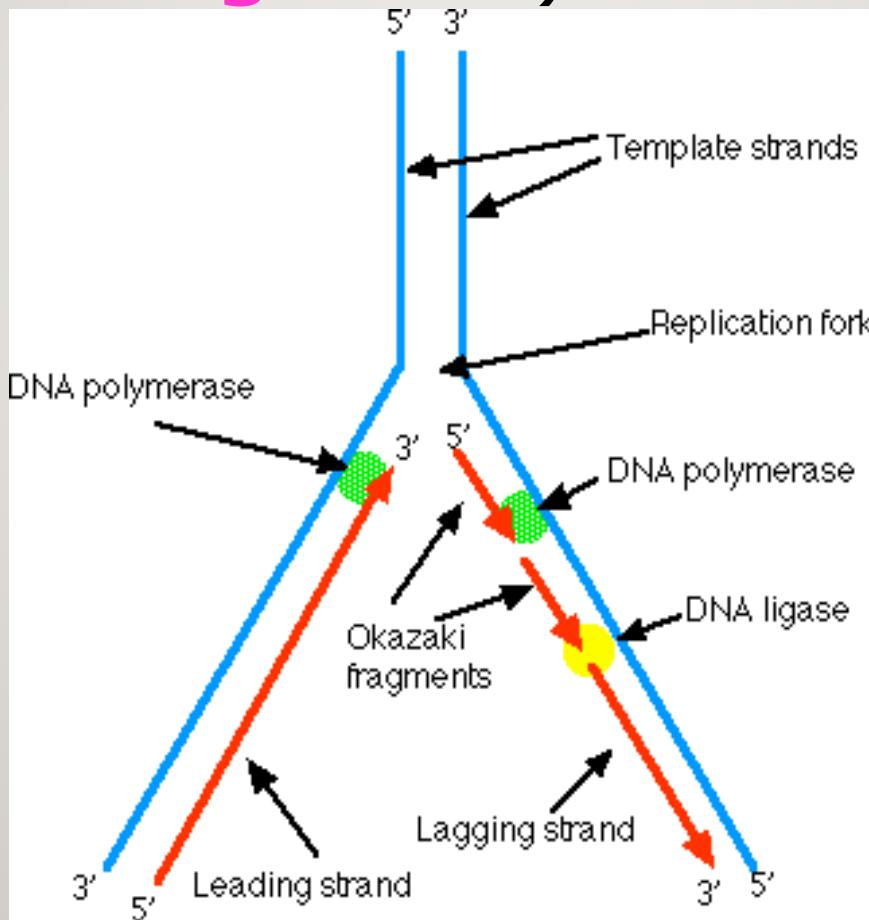


➤ An enzyme called **DNA polymerase** binds to one strand of the DNA and begins moving along it in the 3' to 5' direction, using it as a template for assembling a **leading strand** of nucleotides and reforming a double helix.

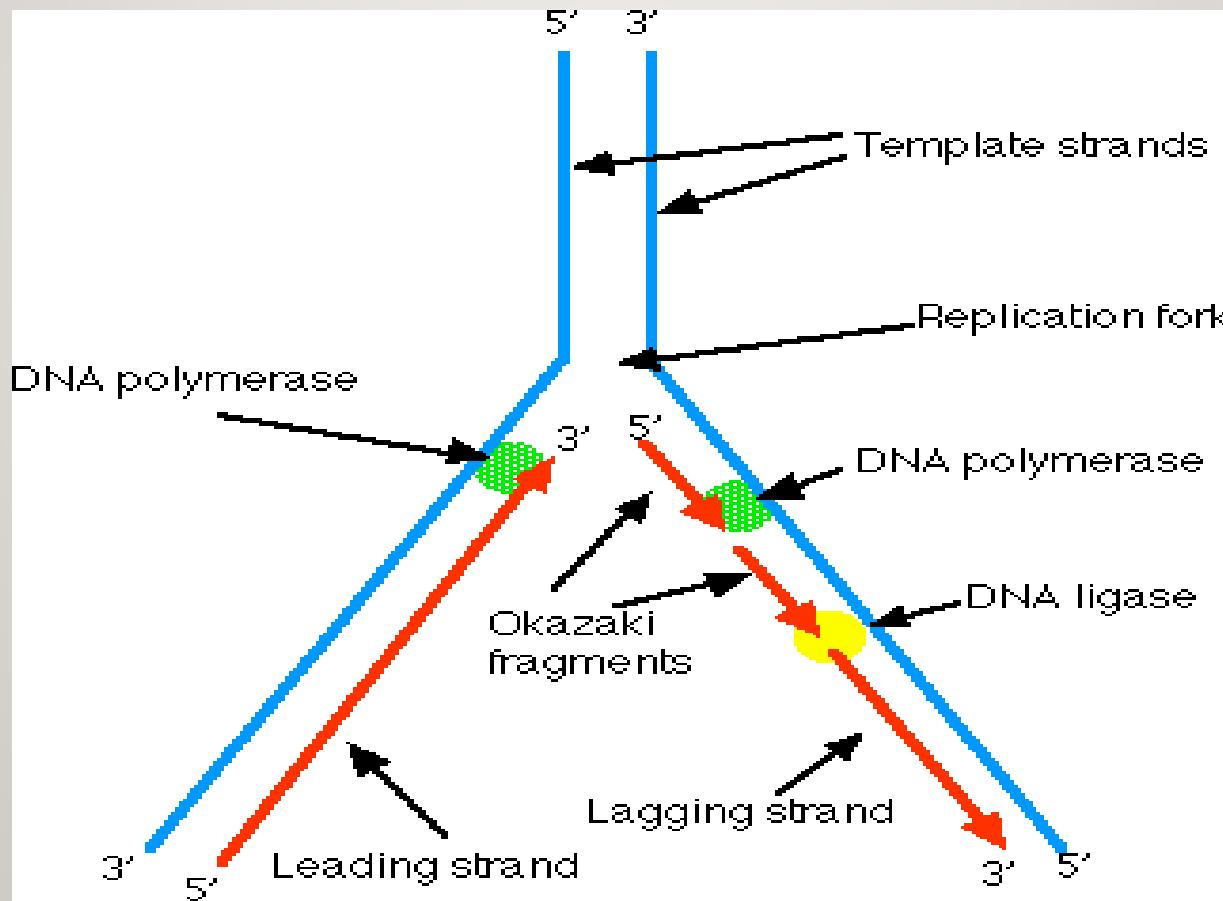


- In eukaryotes, this molecule is called **DNA polymerase delta ( $\delta$ )**
- Because DNA synthesis can only occur in 5' to 3' direction, a molecule of a second type of DNA polymerase (**epsilon,  $\epsilon$ , in eukaryotes**) binds to the other template strand as the double helix opens as shown below.

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



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

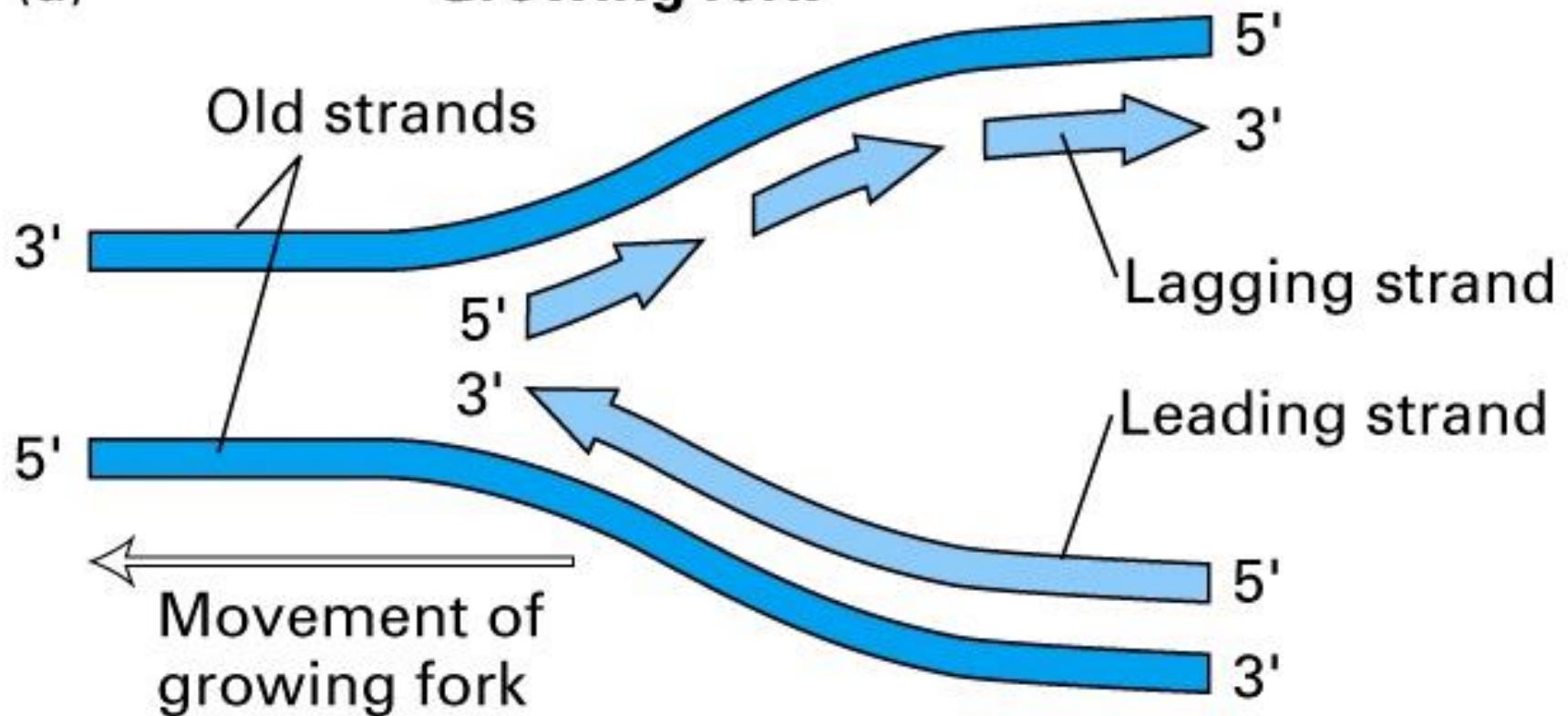


- The original DNA strand is used as a template to synthesize the DNA strand in the  $5' \rightarrow 3'$  direction with the help of an extension formed by an **RNA primer**.
- DNA polymerase can synthesize the strand in  **$5' \rightarrow 3'$  direction only**.

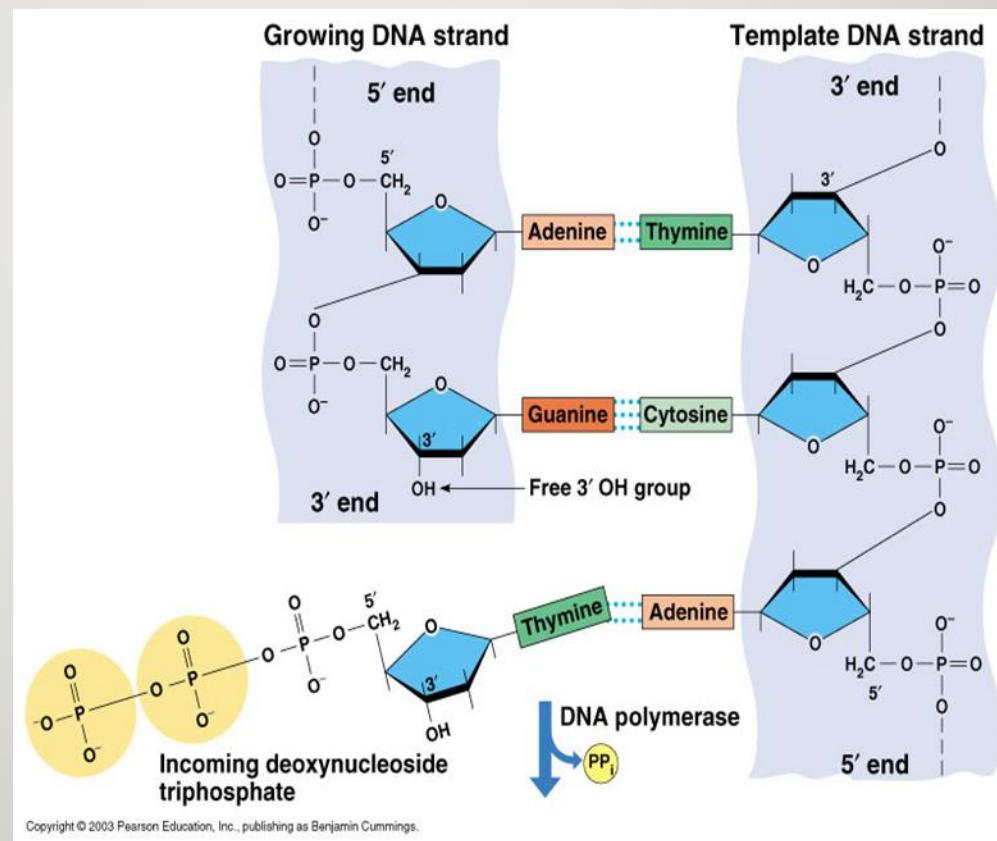
# Leading and lagging strands

(a)

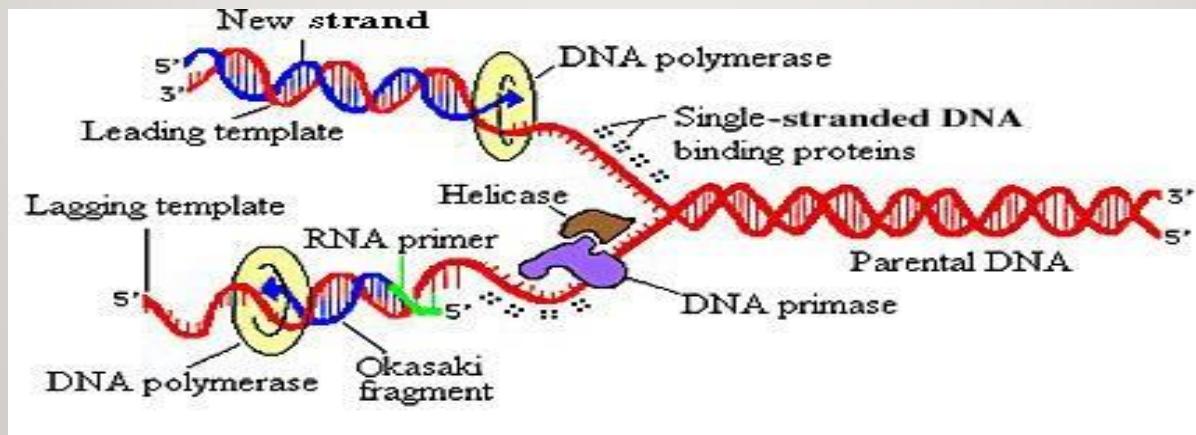
## Growing fork



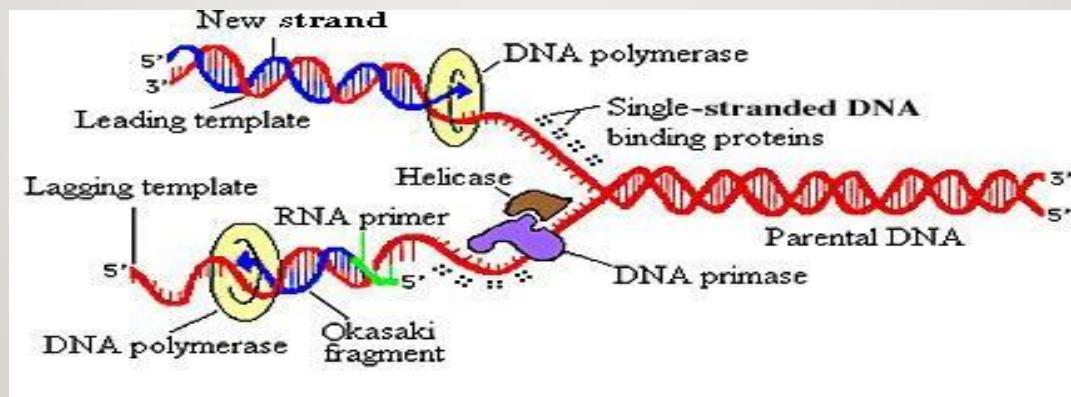
- It hooks the 5' phosphate group of an incoming nucleotide onto the 3' hydroxyl group at the end of the growing nucleic acid chain.



➤ 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.

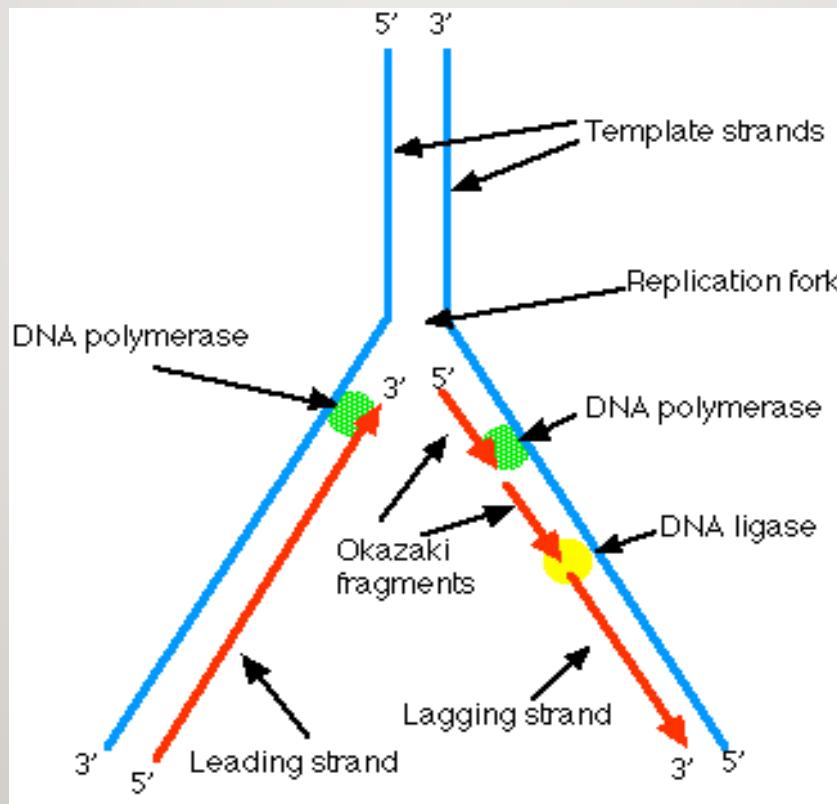


- It is also referred to as the **Okazaki Fragments**.

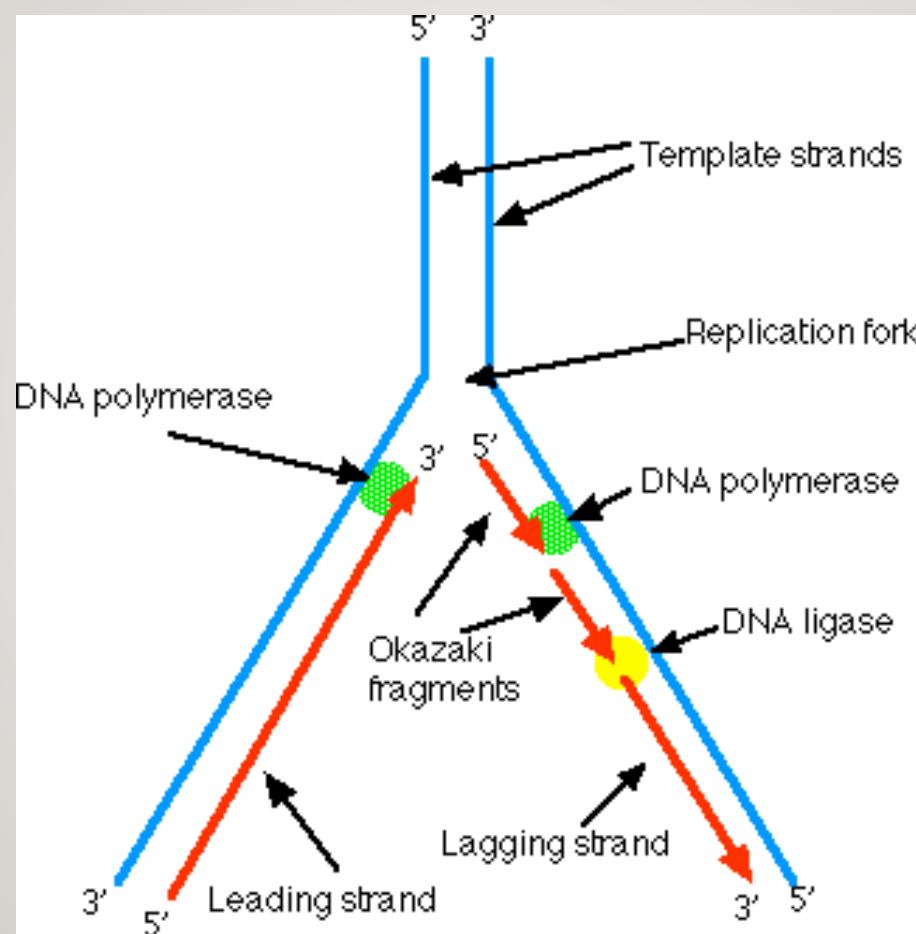
- DNA polymerase cannot begin synthesizing the DNA strand initially.
- It needs a nucleic acid chain in the beginning to begin copying the strand.

- 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.



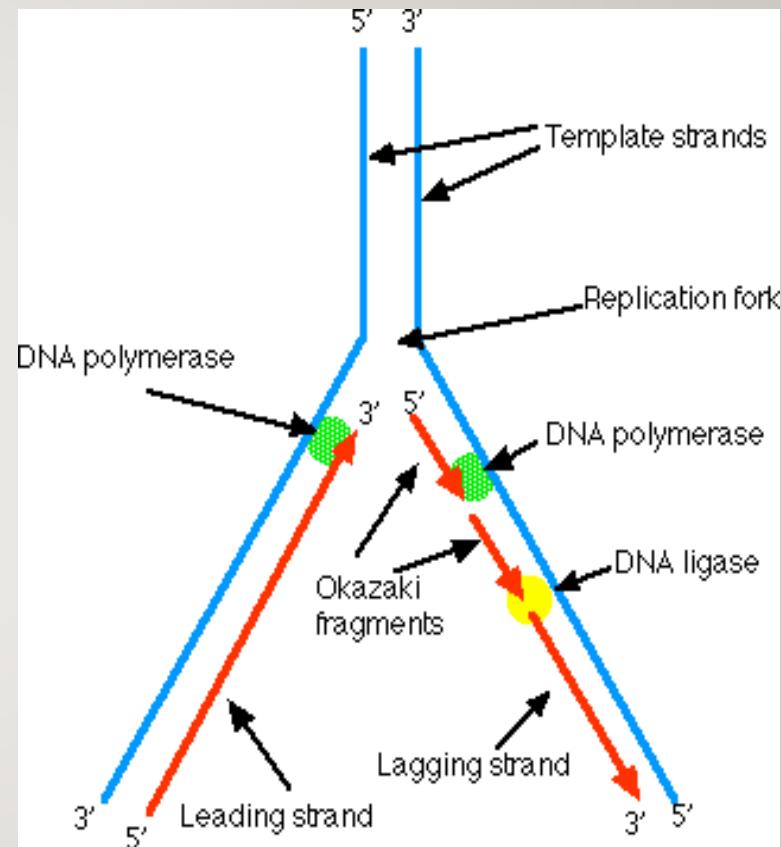
- During the synthesis, only one polymerase remains on the DNA template and copies the DNA in a continuous strand.
- The other polymerase copies only a short stretch of DNA, before running into the primer of the initially sequenced fragment.



- 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

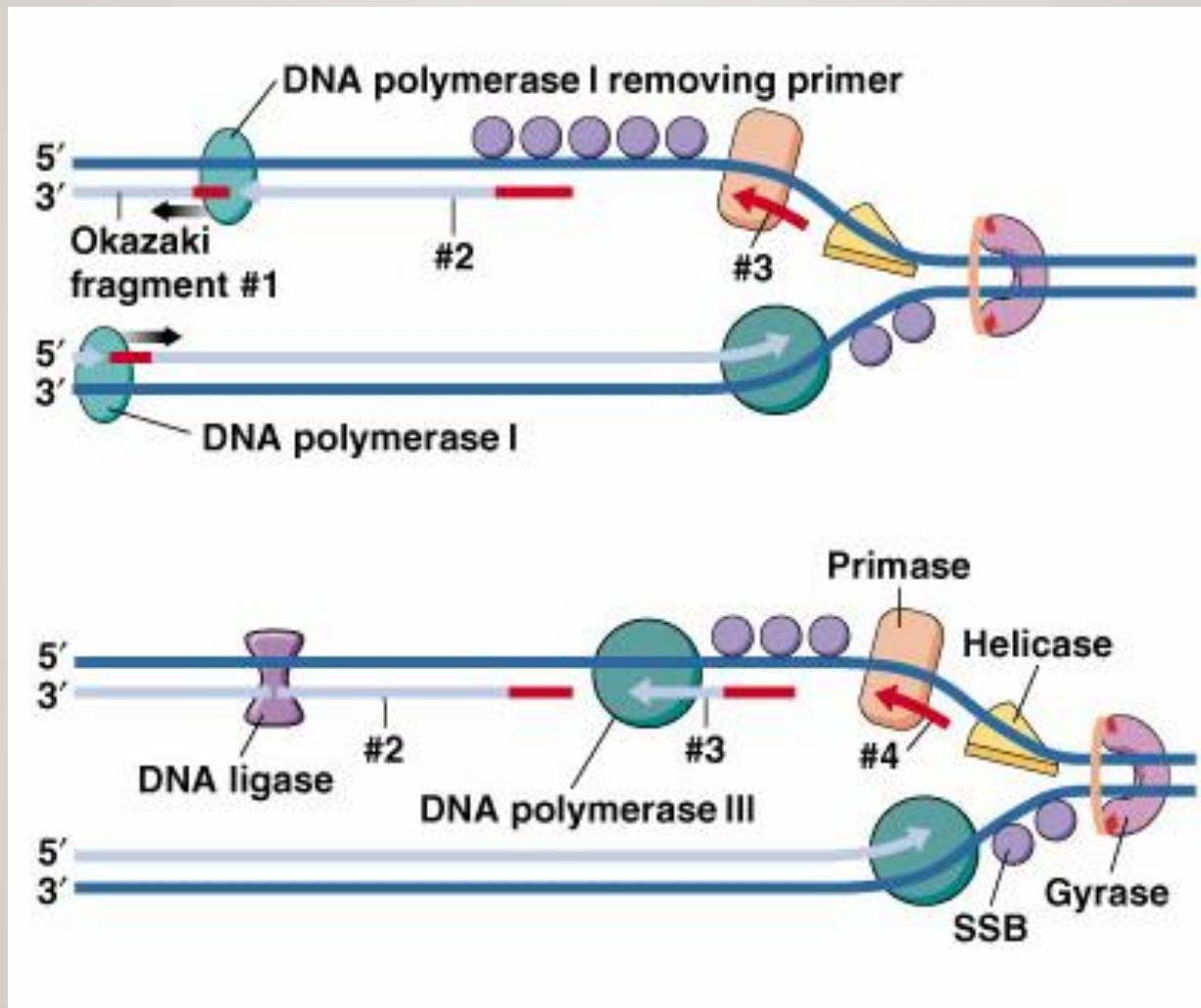
➤ The DNA strand that is read in the  $3' \rightarrow 5'$  and synthesized in the  $5' \rightarrow 3'$  direction continuously, is known as the leading strand.



- **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.

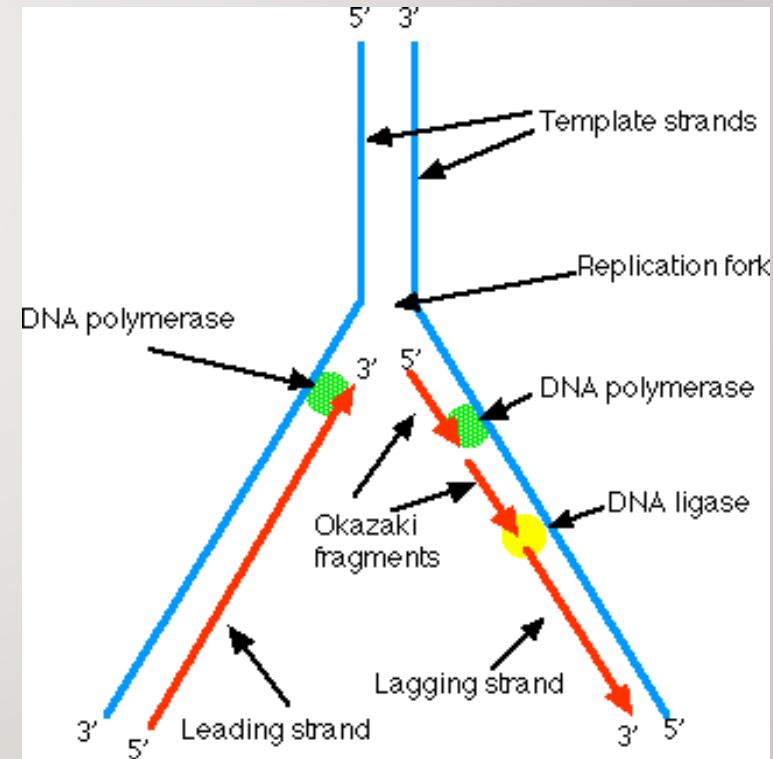
# Ligation and Primer removal



❖ **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  $5' \rightarrow 3'$  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  $5' \rightarrow 3'$  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.

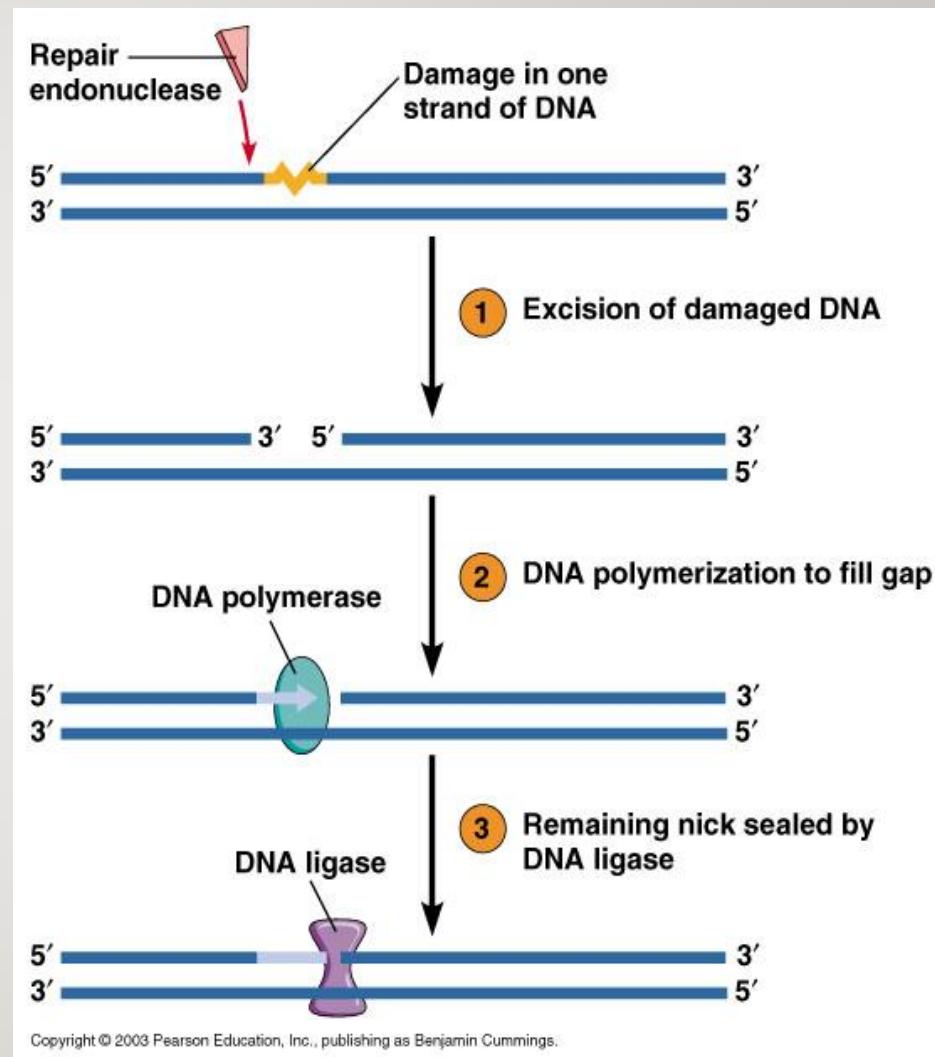
# ALL TOGEHTER

1. An **initiator protein** binds to and separates the strands at the replication origin
2. **Helicase** separates the strands at the replication fork
3. **SSB proteins** hold the strands apart
4. **Gyrase** relieves supercoiling as it develops
5. **Primase** creates a short RNA primer
6. **DNA polymerase III** extends the new strand
7. **DNA polymerase I** replaces the RNA primer with DNA
8. **Ligase** repairs/joins missing phosphodiester bonds between fragments

- 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**.

# Generalized repair mechanism

- ▶ Repair endonucleases recognize errors, perhaps through distortions in the helix.
- ▶ The damaged region is removed, sometimes with the help of helicase
- ▶ Polymerase fills the gap
- ▶ Ligase repairs the backbone



## RATE OF REPLICATION

- The single molecule of DNA, that is the *E. coli* genome contains  $4.7 \times 10^6$  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 ( $4.7 \times 10^6$ ) is copied without error!

- The Genome of complex eukaryotes is huge as compared to prokaryotes.
- 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 same time (multiple initiation sites)

# BACTERIAL AND VIRAL GENETICS

## ❖ BACTERIAL AND VIRAL GENETICS

## ❖ WHY DO WE STUDY BACTERIAL AND VIRAL GENETICS?

- Since the 1940s, the genetic systems of bacteria and viruses have contributed to the discovery of many important concepts in genetics.
- The study of molecular genetics initially focused almost entirely on their genes.

- Today, bacteria and viruses are still essential tools for probing the nature of genes in more complex organisms;
- Partly because they possess a number of characteristics that make them suitable for genetic studies.

- The genetic systems of bacteria and viruses are also studied because these organisms play important roles in human society.
- They have been exploited to produce a number of economically important substances, and they are of immense medical significance, causing many human diseases.

### Advantages of using bacteria and viruses for genetic studies

1. Reproduction is rapid.
2. Many progeny are produced.
3. Growth in the laboratory is easy and requires little space.
4. Genomes are small (About 100-fold less than of eucaryotic cells).

5. Techniques are available for isolating and manipulating their genes.
6. They have medical importance.
7. They can be genetically engineered to produce and transfer genes of interest in large amounts.

- Genetic analysis in bacteria and viruses are different than that in eukaryotes.
- This is because bacteria and viruses have special genome organization, therefore different techniques and methods are used to analyze their genes and mutations.
-

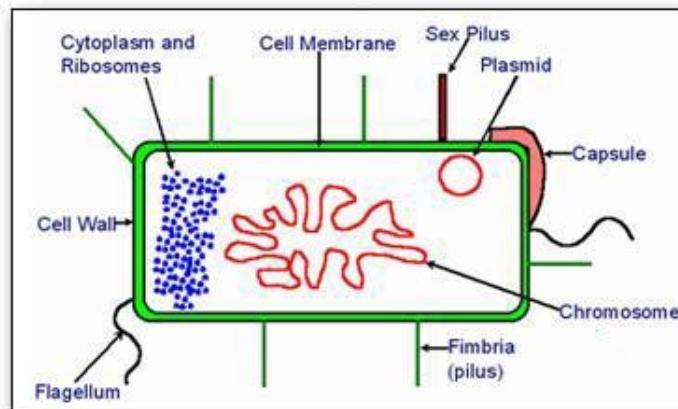
- Because they grow rapidly and also make their DNA rapidly, they are often used as host cells or vectors in recombinant DNA technology.

### The Bacterial Chromosome

- Bacterial chromosomes are highly compacted structures and share many properties with their eukaryote counterparts, despite not being contained within a cell nucleus.

- While eukaryotes have two or more chromosomes, prokaryotes such as bacteria possess a single chromosome composed of double-stranded DNA in a loop or
- The DNA is in the form of a double helix which forms a closed ring or circle with no free ends.

### Bacterial Chromosome

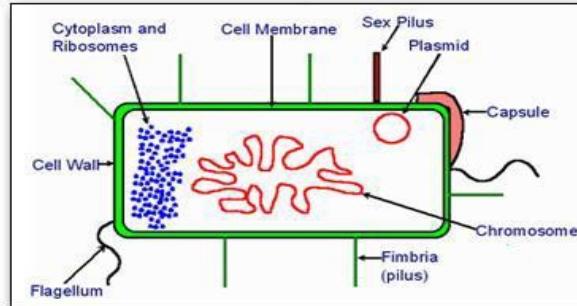


- The bacterial chromosome must be tightly packed to fit into the small volume of the bacterial cell.
- Compacting the DNA involves supercoiling, or further twisting of the twisted chromosome.

- Bacteria lack the histone proteins that are found bound to the DNA and that form the nucleosomes of eukaryotic chromosomes.
- However, it is believed that polyamines (organic molecules with multiple NH<sub>2</sub> or amine groups) such as spermidine, as well as some basic proteins, aid in compacting the bacterial chromosome.

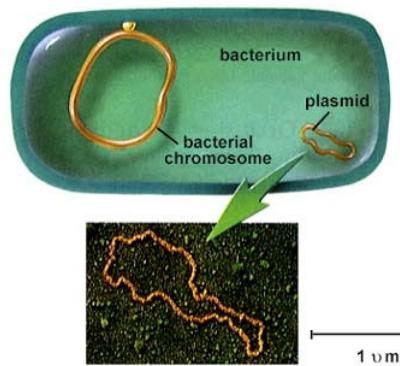
- These basic proteins have a net positive charge that bind them to the negative charge of the phosphates in the DNA backbone.
- Replication of the circular chromosome begins at a single point, called OriC, and proceeds in both directions around the circle, until the two replication forks meet up.

- The bacterial chromosome lacks a protein coat and it is in direct contact with the cytoplasm, since a nuclear membrane is absent and it is called **nucleoid**.
- In addition to the nucleoid, a bacterial cell may show the presence of extra chromosomal DNA molecules called plasmids.



- Like the bacterial chromosome, plasmids are double stranded circular DNA molecules which can replicate and function independently.

Typical Prokaryotic Plasmid:  
Circular, small, and code for properties that  
are useful to the prokaryote, but not  
necessary.



- The plasmid has its own replication origin and the replication of plasmids is independent of the replication of chromosome.

- The plasmids mainly carry genes responsible for characteristics like **fertility**, **antibiotic resistance** and production of **bacteriocin** (a protein that kills closely related bacteria).
- The plasmids can be easily isolated from or introduced into the bacterial cells.

- They are small, circular DNA molecules
- Autonomous, extrachromosomal genetic elements
- Usually not essential to bacterial function but can be.

- Many of the plasmids first isolated and characterized carried genes for antibiotic resistance
- Plasmids control their own replication
- Episomes, such as the F (fertility) factor, can either exist as freely-replicating plasmids or by integrating into the chromosome.

## FEATURES OF PLASMIDS

- Non-chromosomal DNA (1- > 400 kilobase pairs)
- Small (less than 1% of chromosome- 5-100 genes)
- Circular
- Non viral
- Sometimes selectively advantageous
- Can propagate themselves independently
- May be exchanged with other bacteria



Plasmids are small circles of DNA found naturally in the cells of some organisms. A plasmid can replicate itself as well as any other DNA inserted into it. For this reason, plasmids make excellent cloning vectors—structures that carry DNA from cells of one species into the cells of another.

## TYPES OF PLASMIDS

- Naming usually depends on genes they carry
  - Colicine plasmids
  - R – factors
  - Ti plasmids
  - F - plasmid

## Plasmids give the bacteria extra properties

They can code for toxins

Anthrax toxin is caused by a plasmid

Botulism is caused by a plasmid.

Tetanus is caused by a plasmid.



## Plasmid's other properties

They can perform nitrogen-fixation (take nitrogen from the air, and use it.)

*Klebsiella pneumoniae*

Some plasmids make plants grow tumors

This plasmid is known as a Ti (tumour inducing) plasmid



## Antibiotic resistance

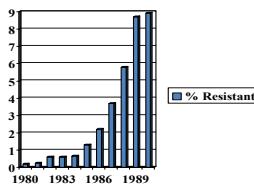
### Plasmids can code for antibiotics

1. Some bacteria become immune to antibiotics because they have plasmids that make them immune. They can also give their plasmids to other bacteria through transformation.
  - a. ampicillin resistance
  - b. kanamycin resistance
  - c. chloramphenicol resistance
  - d. B-galactosidase resistance
  - e. Gentamycin resistance



## Another example antibiotic resistance

- Note Gonorrhea
  - 1989 Resistance went from <0.2% to almost 9% in 10 years!
  - 1999, resistance was reported to be widespread in the US.
  - 2000 The Centers for Disease Control found resistance to Cipro – our ‘big gun’ and recommended a whole regimen of treatments:



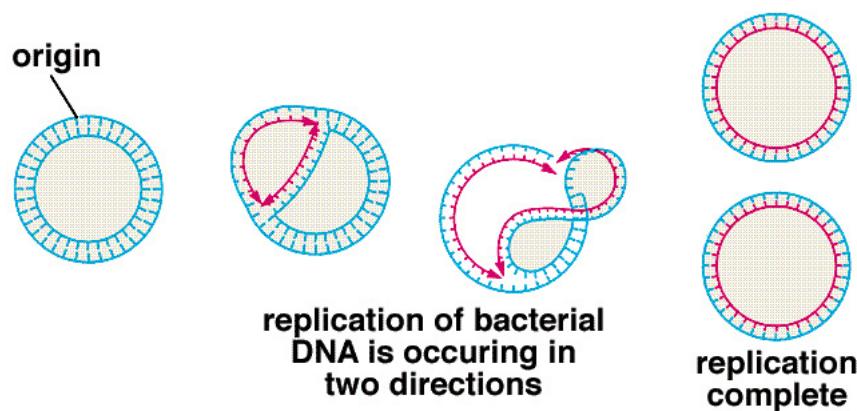
500mg single-dose [ciprofloxacin](#) and 400mg [ofloxacin](#) as broad-spectrum [fluoroquinolones](#) and [cephalosporins](#), respectively, to treat uncomplicated gonorrhea ([CDC, 2000](#)).

## PLASMID REPLICATION AND COPY NUMBERS

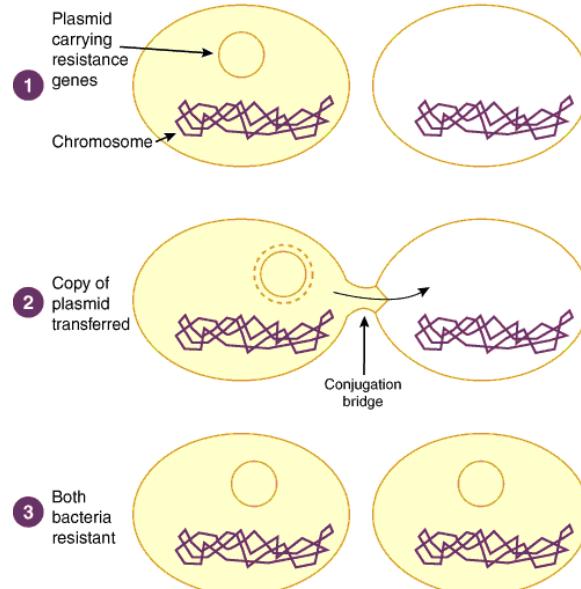
- Plasmid copy numbers differ per cell with different plasmids
- Plasmid replication is generally regulated
  - Involves both plasmid and chromosomal genes

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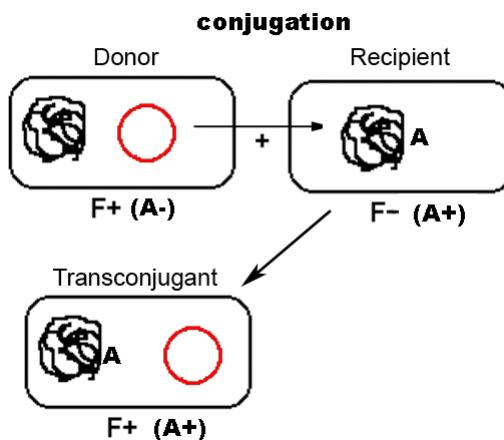
### DNA replicates in prokaryotes



## How Plasmids get transferred from one cell to another.



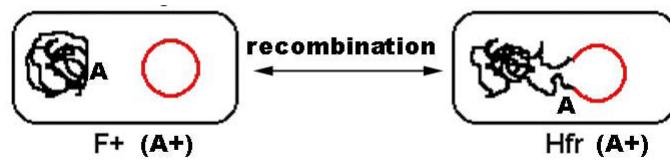
**Simple conjugation – The donor F+ plasmid produces a pilus that makes contact with the recipient F- cell. The F plasmid is copied into the recipient cell.**



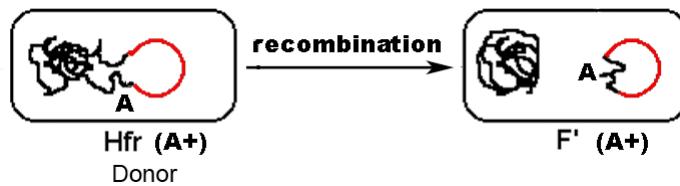
The recipient cell is now called a transconjugant and has converted from F- to F+

## Formation of an Hfr Strain

Very rarely the F plasmid can integrate into the E. coli chromosome. The cell with the integrated F plasmid is called “Hfr”.

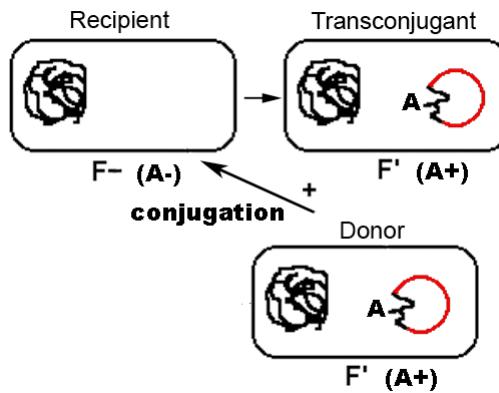


The integrated F plasmid usually excises itself precisely resulting in a switch back to simple F+.



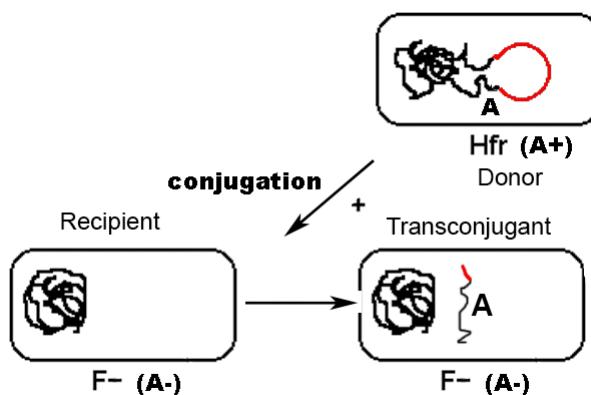
This time, when the F plasmid excises itself, it accidentally drags along a piece of the adjacent chromosomal sequences – the A gene. This recombinant F plasmid is called an F' (F prime). Since the A gene is still in the cell, the cell is still A+.

**Now, when the F' plasmid conjugates into an F- recipient, it contains the A gene, Thus the recipient cell which is A- becomes A+ .**



**The F plasmid is stable in the recipient cell.**

**Even though the F plasmid is integrated in the chromosome, it still tries to move itself by conjugation.**



**Transconjugant has a small piece of the F plasmid along with the A gene. It does not reform a circular plasmid; it is a linear piece of DNA. Bacteria don't like linear DNA (thinks it's a bacteriophage), so they try to degrade it with enzymes**

The linear piece of DNA with the A gene must be rescued by recombining into a site of the recipient cell that shares homology with the A gene. If it happens the cell becomes A+. If the conjugated fragment doesn't recombine, it will be lost, and the recipient cell will be unchanged.



The end result is that the original Hfr donor cell has converted the chromosome of the recipient.

### Why plasmids are used in genetic engineering

They are easy to put new genes onto: cut the plasmid and the gene you want with the same restriction enzyme...they will 'match' and line up... sew them together with ligase enzyme.

Plasmids and bacteria replicate quickly... You get lots of product..

## Genetics of Viruses

- Viruses are a group of simple, non-cellular, microorganisms which consist minimally of protein and nucleic acids; DNA or RNA but not both and which can replicate only within particular cells (bacteria, animal, protozoa, algae, fungi and plants).
- A virus particle, also known as a virion, is essentially a nucleic acid (DNA or RNA) enclosed in a protein shell or protective coat.

- Viruses are extremely small, approximately 15 - 25 nanometers in diameter.
- Viral genomes are much different than prokaryotes and eukaryotes:
  - - may be **double-stranded DNA, single-stranded DNA, double-stranded RNA** or **single-stranded RNA**
  - - organized as single nucleic acid molecules in linear or circular arrangements

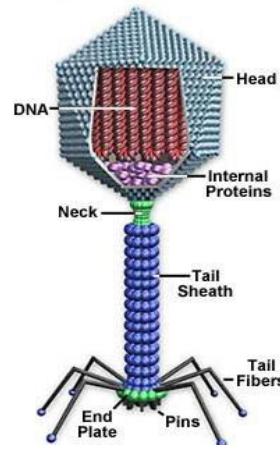
- The viral genome can consist of a very small number of genes or up to hundreds of genes depending on the type of virus
- The type of genetic material found in a particular virus depends on the nature and function of the specific virus.
- .

### VIRUS-HOST RANGE.

- The host range of a virus is the spectrum of host cells the virus can infect.
- Some viruses have broad host ranges which may include several species(e.g. swine flu and rabies).
- Some viruses have host ranges so narrow that they can infect;
  - only one species(e.g. phages of E.coli)
  - only a single tissue type of one species

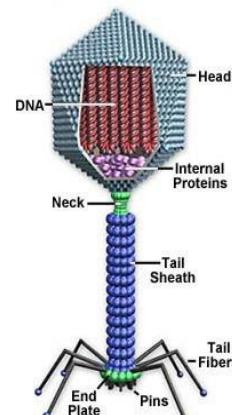
## Viral Structure

- The protein coat that envelopes viral genetic material is known as a **capsid**.



- A capsid is composed of protein subunits called **capsomeres**.

- Capsids can have several shapes:
  - Icosahedral,
  - Helical or
  - complex.



## Functions of the Capsid

- The capsid has three functions:
  - (1) it protects the nucleic acid from digestion by enzymes,
  - (2) contains special sites on its surface that allow the virion to attach to a host cell, and
  - (3) provides proteins that enable the virion to penetrate the host cell membrane and, in some cases, to inject the infectious nucleic acid into the cell's cytoplasm.
- In addition to the protein coat, some viruses have specialized structures. For example, the flu virus has a membrane-like envelope around its capsid.

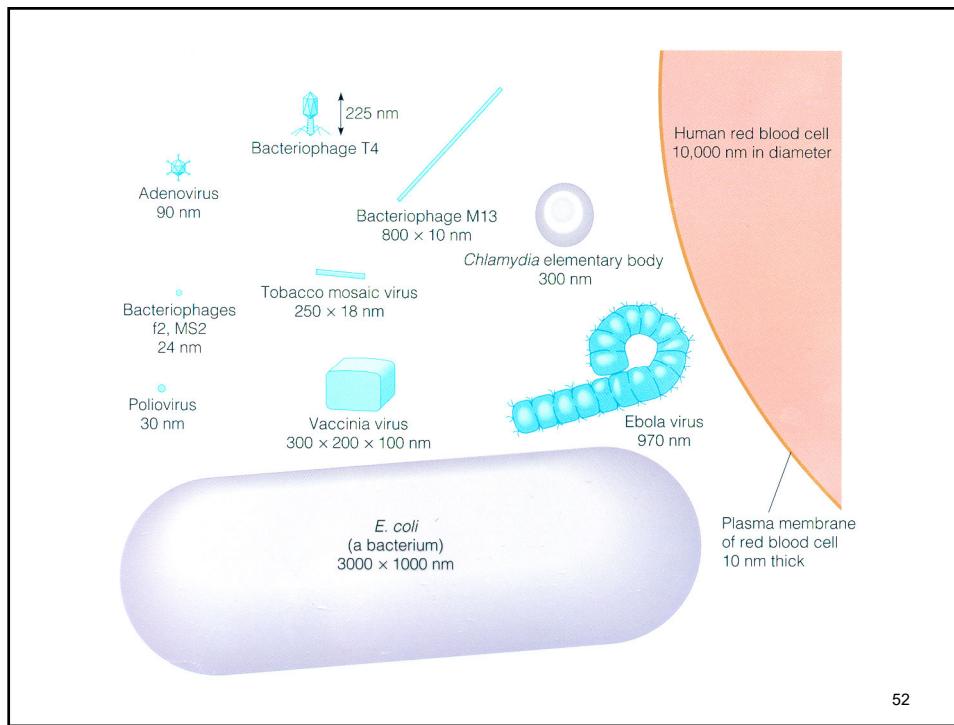
- **Envelope** - Many types of virus have a glycoprotein envelope surrounding the nucleocapsid.
- The envelope is composed of two lipid layers interspersed with protein molecules (lipoprotein bilayer)
- Derived from host cell membrane which is usually virus-modified and contains proteins and glycoproteins of viral origin
- Helps viruses infect their host.

- Without a host cell, viruses cannot carry out their life-sustaining functions or reproduce.
- They cannot synthesize proteins, because they lack ribosomes and must use the ribosomes of their host cells to translate viral messenger RNA into viral proteins.

- **Nucleic Acid** - Just as in cells, the nucleic acid of each virus encodes the genetic information for the synthesis of all proteins.
- While the double-stranded DNA is responsible for this in prokaryotic and eukaryotic cells, only a few groups of viruses use DNA.

- Viruses cannot generate or store energy in the form of adenosine triphosphate (ATP), but have to derive their energy, and all other metabolic functions, from the host cell.
- They also parasitize the cell for basic building materials, such as amino acids, nucleotides, and lipids (fats).

- Viruses can reproduce only within a host cell
- They are obligate intracellular parasites –i.e. can only express genes from living cells
- Viruses have specific host range, or a limited number of host cells that they can infect



## Bacteriophage

- Definition

- Obligate intracellular parasites that multiply inside of bacteria by using some or all of the host biosynthetic machinery

- T4 Phage

- One of the largest phage
  - Specifically infect on *E. coli B* strain

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## Composition

- Bacteriophages contain Nucleic Acid and Protein.
- Nucleic Acid can be DNA or RNA, depending upon the phage
- Proteins function in infection and to protect the nucleic acid from nuclease in the environment.

54

## Structure of (T4) Phage

### Bacteriophage Structure

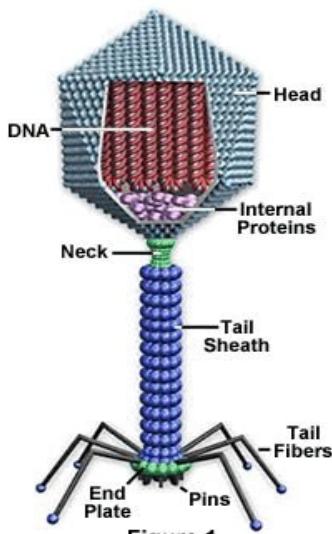


Figure 1

- Size
  - Approximately 200 nm long and 80-100nm wide
- Head or Capsid
  - Icosahedral
  - Protective covering for the nucleic acid
- Tail
  - A hollow tube through which the nucleic acid passes during infection.
  - Surrounded by a contractile sheath.
  - Base Plates
  - Tail fibers

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## Viral Replication

- A single virus particle or virion in itself is essentially inactive/inert.
- It lacks needed components that cells have to reproduce.
- When a virus infects a cell (Host), it marshals the cell's ribosomes, enzymes and much of the cellular machinery to replicate.

- Viral replication produces many progeny, that when complete, leave the infected host cell to infect other cells in the organism.
- The exact nature of what happens after a host is infected varies depending on the nature of the virus.
- The process for viral replication be it dsDNA, ssDNA or RNA will differ.

- For example, double-stranded DNA viruses typically must enter the host cell's nucleus before they can replicate.
- Single-stranded RNA viruses however, replicate mainly in the host cell's cytoplasm.

- Once a virus infects its host and the viral progeny components are produced by the host's cellular machinery, the assembly of the viral capsid is a non-enzymatic process.
- It is usually spontaneous.

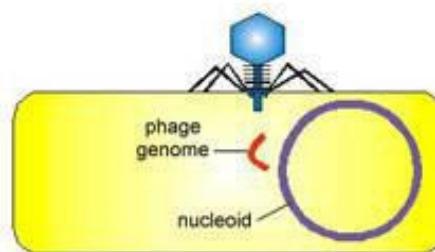
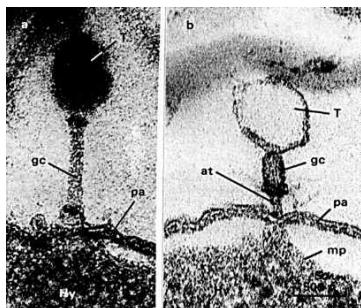
### Infection of Host cells by Viruses

❖ The basic process of viral infection and virus replication occurs in 6 main steps.

- **Adsorption/Attachment** - virus is attached to specific receptors on the host cell.

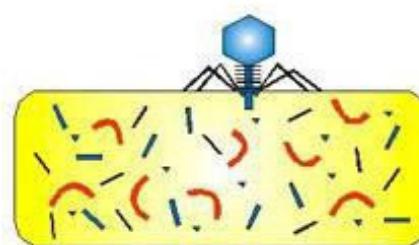


➤ **Penetration** - virus injects its genome into host cell. At this point, the virus can no longer be recovered from the intact cell.

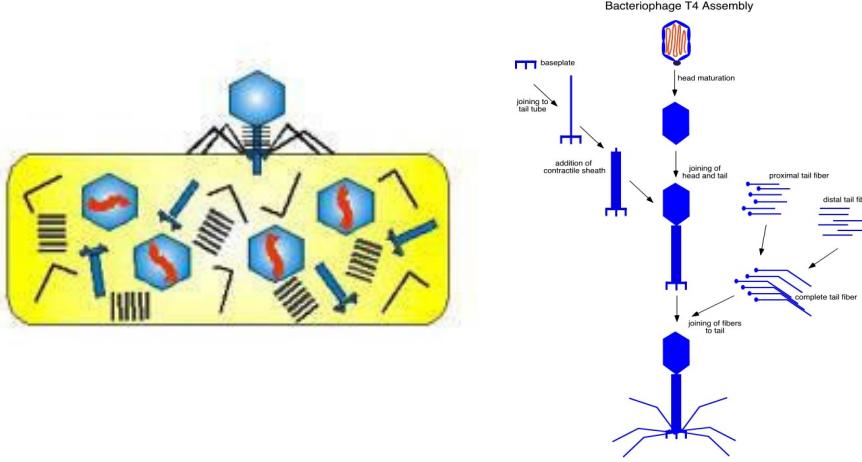


Sheath Contraction

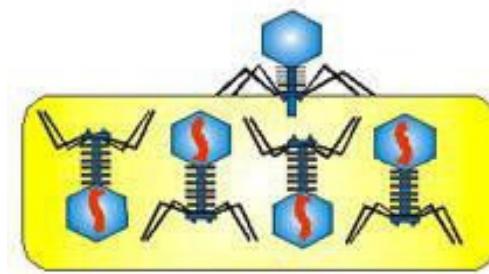
➤ **Replication or Synthesis** – The viral genome replicates using the host's cellular machinery.



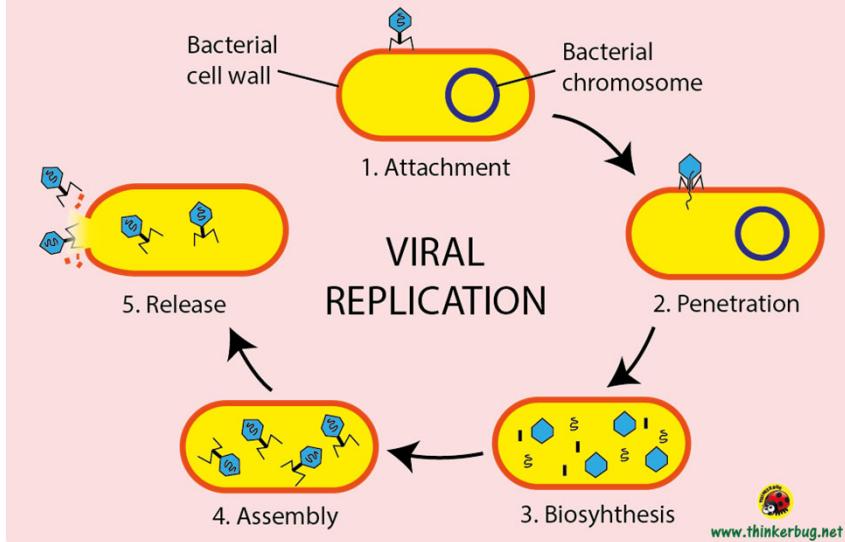
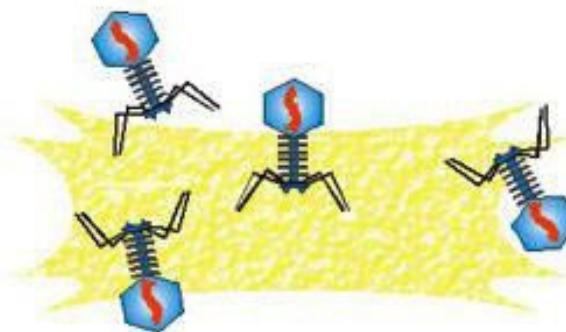
➤ **Assembly** - viral components and enzymes are produced and begin to assemble.



➤ **Maturation** - viral components assemble and viruses fully develop.



➤ **Release** - newly produced viruses are released from the host cell. The virus releases enzymes to break the cell wall and the cell wall bursts.



## General Viral Life Cycle

➤ A virus undergoes lytic and/or lysogenic cycle to reproduce.

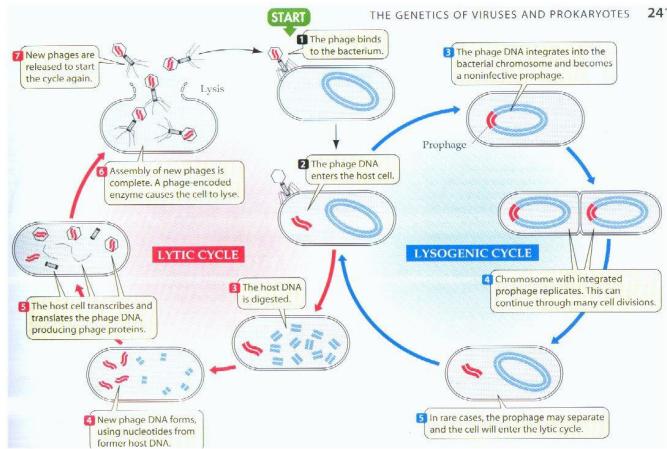
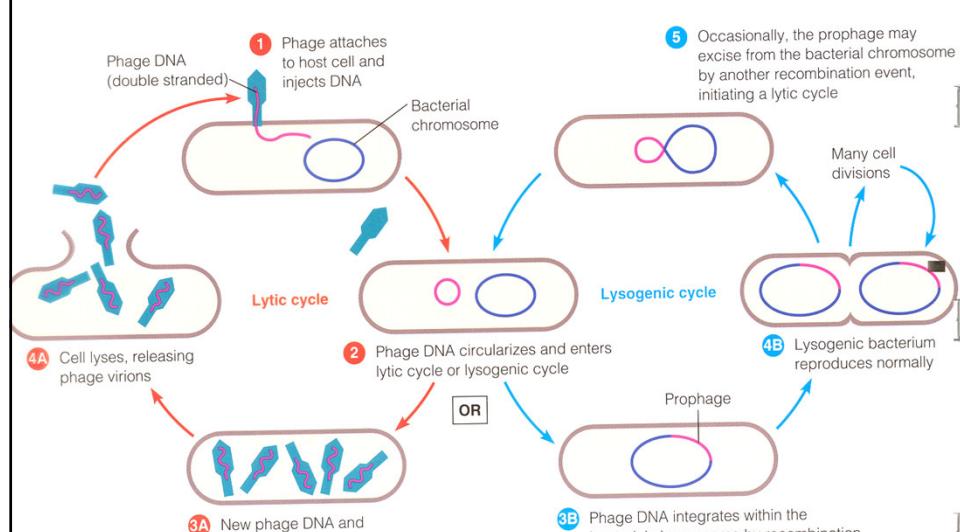


Figure 13.12: The lysogenic cycle of bacteriophage  $\lambda$  in *E. coli*.



## The Lytic cycle

- The lytic cycle is thought to be the major method of viral replication as it results in total cell lyses (that is, cell destruction of the infected bacterium or host cell).
- The viruses that undergo lytic cycle are called **virulent viruses**.
- This begins with the six stage cycle that we have discussed above.

- This begins with the six stage cycle that we have discussed above.
- The virus injects its nucleic acids into the host cell that form a circle in the center of the cycle.
- The host cell is then “directed” into replicating the viral nucleic acid instead of its own nucleic acids.

## Induction

- The process by which viral DNA is switched from the lysogenic cycle to the lytic cycle.
- UV radiation or ionizing radiations
- hydrogen peroxide
- nitrogen mustard

### O\WIF#D Q G #D\ VR J HQ IF#F\ FOHV

- |  |   |
|--|---|
| <ul style="list-style-type: none"><li>• Lytic cycle:</li><li>• Results in the death or lysis of the host cell.</li><li>• Bacteriophage takes over the machinery of the cell, so viral replication and release occur</li><li>• Virulent bacteriophage reproduce by a lytic replication cycle, that lyse their host cells.</li></ul> | <ul style="list-style-type: none"><li>• Lysogenic cycle:</li><li>• Involves the incorporation of the viral genome into the host cell genome.</li><li>• Phage becomes a prophage, integrated into the host genome.</li><li>• Later, the phage may reenter the lytic cycle and replicate itself.</li><li>• Temperate viruses integrate and remain latent.</li></ul> |
|--|---|



- **MUTATIONS**

## MUTATIONS

- A mutation is any change in the sequence of DNA in a genome, OR
- A mutation is a permanent change in the DNA sequence of a gene
- Mutations in a gene's DNA sequence can alter the amino acid sequence of the protein encoded by the gene.

- Mutations range in size from a single DNA building block (DNA base) to a large segment of a chromosome.

- Mutations can be beneficial, neutral, or harmful for the organism, but mutations do not “try” to supply what the organism “needs.”
- In this respect, mutations are random—whether a particular mutation happens or not is unrelated to how useful that mutation would be.

### How does mutation happens?

- Like words in a sentence, the DNA sequence of each gene determines the amino acid sequence for the protein it encodes.
- The DNA sequence is interpreted in groups of three nucleotide bases, called **codons**.
- Each codon specifies a single amino acid in a protein.

### THE GENETIC CODE

- The set of rules that determine how a nucleotide sequence is converted into the amino acid sequence of proteins.

OR
- The rules by which the nucleotide sequence of a gene is translated into the amino acid sequence of a protein.

THE GENETIC CODE							
		Second letter					
First letter	U	UUU } Phe UUC UUA } Leu UUG }	UCU } Ser UCC UCA UCG }	UAU } Tyr UAC UAA Stop UAG Stop	UGU } Cys UGC UGA Stop UGG Trp }	U C A G	Third letter
	C	CUU } Leu CUC CUA CUG }	CCU } Pro CCC CCA CCG }	CAU } His CAC CAA Gln CAG }	CGU } Arg CGC CGA CGG }	U C A G	
	A	AUU } Ile AUC AUA AUG Met	ACU } Thr ACC ACA ACG }	AAU } Asn AAC AAA Lys AAG }	AGU } Ser AGC AGA Arg AGG }	U C A G	
	G	GUU } Val GUC GUA GUG }	GCU } Ala GCC GCA GCG }	GAU } Asp GAC GAA Glu GAG }	GGU } Gly GGC GGA GGG }	U C A G	

THE GENETIC CODE							
		Second Letter					
First letter	T	TTT } Phe TTC TTA } Leu TTG }	TCT } Ser TCC TCA TCG }	TAT } Tyr TAC TAA Stop TAG Stop	TGT } Cys TGC TGA Stop TGG Trp }	T C A G	Third Letter
	C	CTT } Leu CTC CTA CTG }	CCT } Pro CCC CCA CCG }	CAT } His CAC CAA Gln CAG }	CGT } Arg CGC CGA CGG }	T C A G	
	A	ATT } Ile ATC ATA ATG Met	ACT } Thr ACC ACA ACG }	AAT } Asn AAC AAA Lys AAG }	AGT } Ser AGC AGA Arg AGG }	T C A G	
	G	GTT } Val GTC GTA GTG }	GCT } Ala GCC GCA GCG }	GAT } Asp GAC GAA Glu GAG }	GGT } Gly GGC GGA GGG }	T C A G	

## Mutate a sentence

- We can think about the DNA sequence of a gene as a sentence made up of entirely three-letter words.

- In the sequence, each three-letter word is a codon, specifying a single amino acid in a protein.

- ❖ Considering a sentence like:

**The sun was hot but the old man did not get his hat.**

- If you were to split this sentence into individual three-letter words, you would probably read it like this:

**The sun was hot but the old man did  
not get his hat.**

This sentence represents a gene.

**The sun was hot but the old man did  
not get his hat.**

- Each letter corresponds to a nucleotide base, and each word represents a codon.

What will happen if you shifted the three-letter "reading frame?"

**We will end having the following:**

**T hes unw ash otb utt heo ldm and idn  
otg eth ish at.**

**Or**

**Th esu nwa sho tbu tth eol dma ndi  
dno tge thi sha t.**

➤ From the three “reading frames” shown above, ONLY ONE can be translated into an understandable sentence i.e.,

**The sun was hot but the old man did  
not get his hat.**

➤ In the same way, only one three-letter reading frame within a gene codes or specify for the correct protein.

- Now, back to the original sentence of:

**The sun was hot but the old man did not get his hat.**

- Let us mutate the reading frame of this sentence by inserting or deleting letters within the sentence.

**What happens?**

### Generation of Mutations (Mutagenesis)

- In general, the appearance of a new mutation is a rare event.
- Most mutations that were originally studied occurred spontaneously i.e., they are historically recognized in nature from an unknown source.
- This class of mutation is termed **spontaneous mutations**.

- This class of mutations represent only a small number of all possible mutations.
- But to understand biological systems further, geneticists/scientists can create new mutations by treating organisms with a mutagenizing agent or a **mutagen**.

- These mutations are called **induced mutations**.
- Mutations can be induced by several methods.
- Three general approaches are used to generate mutations are **radiation**, **chemical** and **transposon insertion**.

- The first induced mutations were created by treating Drosophila with X-rays.
- In addition to X-rays, gamma rays and fast neutron bombardment have also been used.
- These treatments can induce **point mutations** (changes in a single nucleotide) or deletions (loss of a chromosomal segment).

- Chemical mutagens work mostly by inducing **point mutations**.
- Point mutations occur when a single base pair of a gene is changed.
- These changes are classified as **transitions** or **transversions**.

- Transitions occur when a purine is converted to a purine (**A to G or G to A**) or a pyrimidine is converted to a pyrimidine (**T to C or C to T**).
- A transversion results when a purine is converted to a pyrimidine or a pyrimidine is converted to a purine

- Two major classes of chemical mutagens which are routinely used are;
- **alkylating agents** and **base analogs**.  
Each has a specific effect on DNA.
- Alkylating agents [such as ethyl methane sulphonate (EMS) and ethyl ethane sulphonate (EES)] can mutate both replicating and non-replicating DNA

- By contrast, a base analog (e.g., 5-bromouracil) only mutate DNA when the analog is incorporated into replicating DNA.
- Each class of chemical mutagen has specific effects that can lead to transitions, transversions or deletions.

- Scientists are now using the power of transposable elements to create new mutations.
- Transposable elements are mobile pieces of DNA that can move from one location in a genome to another.
- Often when they move to a new location, the result is a new mutant.

- The mutant arises because the presence of a piece of DNA in a wild type gene disrupts the normal function of that gene

- Among the mutations that affect the function of a protein (gene), some allow the protein to be **active** at the organism's **normal temperature** but **inactive** at either **higher** or **lower** temperatures.
- The former are **temperature-sensitive** (**Ts**) mutations and the latter are **cold-sensitive** mutations

- Usually, a gene fails to function at a higher temperature, but functions normally at a low temperature.
- Such mutations are called temperature-sensitive (*heat-sensitive*).
- *Cold-sensitive* mutants on the other hand function normally at higher temperatures, but fail to function at a reduced temperature.

- Not all mutations in DNA lead to a detectable change in the phenotype.
- Mutations without any apparent effect are called silent mutations.

## Classes of Mutations

- We can divide mutations into two general classes.
- These are **Point mutations** and **Rearrangement mutations** or **Chromosomal mutations**.

- This causes a corresponding change in the protein that the gene produces.
- A rearrangement mutation on the other hand affects a large region.
- The simplest type of rearrangements are **insertions** of additional material or **deletions** of a stretch of the gene.

## Point Mutation

- When a single base in the nucleotide sequence is replaced by another, then it is known as point mutation.
- Point mutations also include insertion and/or deletion of a single base in the DNA strand.
- Usually, they are caused due to error in DNA replication.
- 

- At times, it occurs after exposure to mutagens like heat and radiation.
- Point mutations can be either **transitions** or **transversions**.
- In the former case, a purine base (adenine or guanine) is substituted by another purine or

- A pyrimidine base (cytosine or thymine) is replaced by another pyrimidine.
- In transversion type of point mutations, purine is substituted by pyrimidine or vice versa.
- Transition point mutation is more common than transversion type.

- The effects of point mutation can vary depending upon the site of mutation on the gene.
- If point mutation occurs in the coding sequence of DNA or exon, then the protein coded by the altered gene is changed.

- A point mutation can be reversed by another point mutation, in which the nucleotide is changed back to its original state
- Point mutations that occur within the protein coding region of a gene may be classified into **three** kinds, depending upon what the erroneous codon codes for:

- **Silent mutations**: which code for the same (or a sufficiently similar) **amino acid**.
- **Missense mutations**: which code for a different amino acid.
- **Nonsense mutations**: which code for a stop and can truncate the protein.

## Silent Mutation

### Silent mutation

Wild Type DNA TAC GGG AAA GTC CGT GGC

Wild Type mRNA AUG CCC UUU CAG GCA CCG

Amino acids Met -Pro- Phe- Gln- Ala- Pro

Mutated DNA TAC GGG AAG GTC CGT GGC

Mutated mRNA AUG CCC UUC CAG GCA CCG

Amino acids Met -Pro- Phe- Gln- Ala- Pro

THE GENETIC CODE  
Second letter

	U	C	A	G		
U	UUU } Phe UUC } UUA } Leu UUG }	UCU } Ser UCC UCA UCG }	UAU } Tyr UAC } UAA Stop UAG Stop	UGU } Cys UGC } UGA Stop UGG Trp	U C A G	
C	CUU } CUC } Leu CUA CUG }	CCU } CCC } Pro CCA CCG }	CAU } His CAC } CAA } Gln CAG }	CGU } CGC } Arg CGA CGG }	U C A G	
A	AUU } AUC } Ile AUA AUG Met	ACU } ACC } Thr ACA ACG }	AAU } Asn AAC } AAA } Lys AAG }	AGU } Ser AGC } AGA } Arg AGG }	U C A G	Third letter
G	GUU } GUC } Val GUA GUG }	GCU } GCC } Ala GCA GCG }	GAU } Asp GAC } GAA } Glu GAG }	GGU } GGC } Gly GGA GGG }	U C A G	

- Such mutations are said to be silent because they cause no change in their product and cannot be detected without sequencing the gene (or its mRNA)

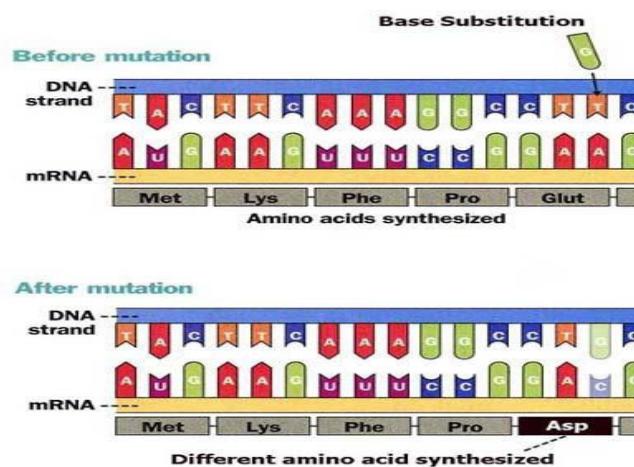
**Silent mutation**

Wild Type DNA	TAC GGG AAA GTC CGT GGC
Wild Type mRNA	AUG CCC UUU CAG GCA CCG
Amino acids	Met -Pro- Phe- Gln- Ala- Pro
Mutated DNA	TAC GGG AAG GTC CGT GGC
Mutated mRNA	AUG CCC UUC CAG GCA CCG
Amino acids	Met -Pro- Phe- Gln- Ala- Pro

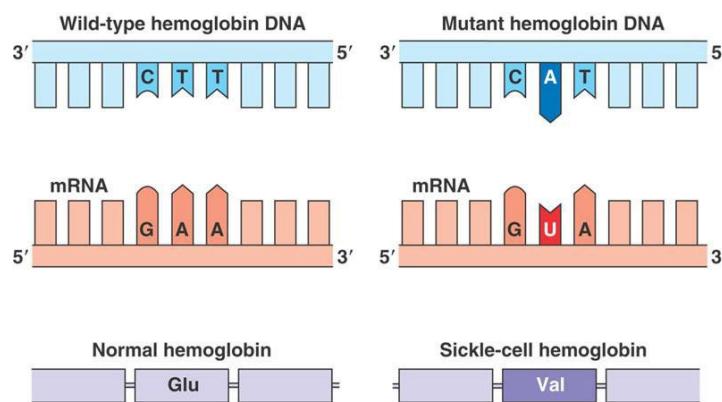
### Missense mutations

- Missense mutation is a genetic change that results in the substitution of one amino acid in protein for another. Or
- It is a mutation in which a codon specifying one amino acid is altered so as to specify a different amino acid.
- It is missense because the resulting codon has the "wrong sense" for an amino acid.)

- A missense mutation is a "readable" genetic message although its "sense" (its meaning) is changed.
- This is in contrast to a nonsense mutation which has no meaning except to halt the reading of the genetic message.



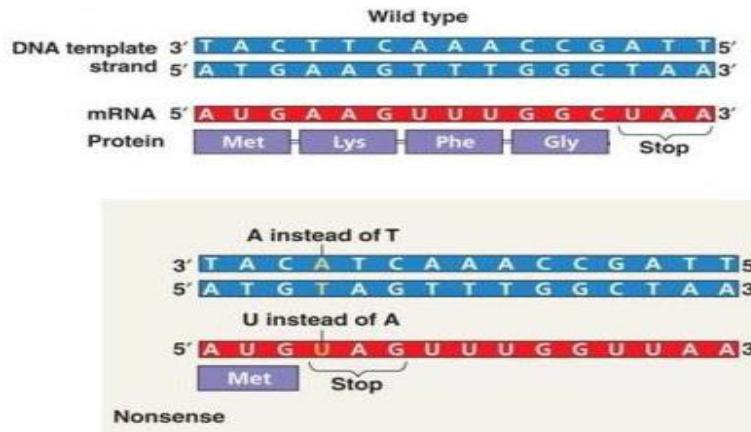
- The first missense mutation discovered in humans was found to be responsible for sickle hemoglobin, the molecular basis of sickle cell trait and sickle cell anemia.
- The mutation causes an amino acid change from **glutamic acid** to **valine**, converting normal adult hemoglobin (hemoglobin A) to sickle hemoglobin (hemoglobin S) as shown below.



THE GENETIC CODE												
Second letter												
First letter	U	UUU UUC UUA UUG	Phe Ser Leu Leu	C	UCU UCC UCA UCG	Ser	G	UAU UAC UAA UAG	Tyr Stop Stop Stop	UGU UGC UGA UGG	Cys Stop Stop Trp	U C A G
	C	CUU CUC CUA CUG	Leu	CCU CCC CCA CCG	Pro	CAU CAC CAA CAG	His Gln	CGU CGC CGA CGG		Arg	U C A G	
	A	AUU AUC AUA AUG	Ile Leu Met	ACU ACC ACA ACG	Thr	AAU AAC AAA AAG	Asn Asn Lys	AGU AGC AGA AGG		Ser Arg	U C A G	
	G	GUU GUC GUA GUG	Val	GCU GCC GCA GCG	Ala	GAU GAC GAA GAG	Asp Asp Glu	GGU GGC GGA GGG		Gly	U C A G	
											Third letter	

## Nonsense Mutations

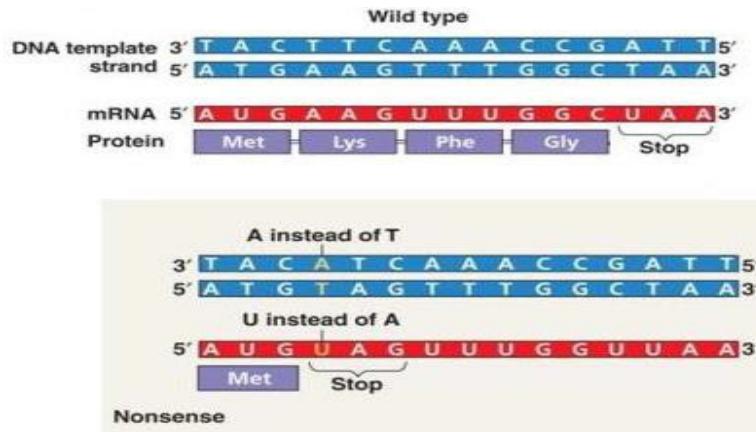
- Nonsense mutation is a change in a base in the DNA that prematurely stops the translation of messenger RNA (mRNA) resulting in a polypeptide (protein) chain that ends prematurely.
- This results in a protein product that is truncated and incomplete and usually nonfunctional.



- The nonsense mutation converts a codon that encodes an amino acid into a **stop codon**, i.e., a that specifies the termination of translation.
- There are three nonsense codons (**UAG**, **UAA**, and **UGA**) in mRNA (see the Genetic Code).
- One of them comes normally at the end of each polypeptide (see above slide).

THE GENETIC CODE						Third letter
		Second letter				
First letter	U	U    UUU UUC UUA UUG	C    UCU UCC UCA UCG	A    UAU UAC UAA UAG	G    UGU UGC UGA UGG	U    C C    A A    G
	C	CUU CUC CUA CUG	CCU CCC CCA CCG	CAU CAC CAA CAG	His Gln	CGU CGC CGA CGG
	A	AUU AUC AUA AUG	Ile Leu Leu Met	ACU ACC ACA ACG	Thr	AGU AGC AGA AGG
	G	GUU GUC GUA GUG	Val	GCU GCC GCA GCG	Ala	Asn Lys
				GAU GAC GAA GAG	Asp Glu	Gly

- Three codons in the genetic code tell the cell to stop adding amino acids to a protein because the end of the gene has been reached.
- In a nonsense mutation, a codon that stands for an amino acid mutates to one of these three stop codons.



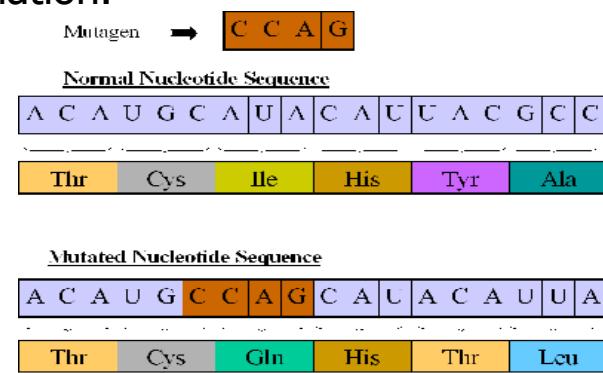
- The term "nonsense mutation" is used because the stop codon has "no sense" for an amino.
- **Cystic fibrosis** is a disease caused by a nonsense mutation.
- It is a genetic disorder that affects most critically the lungs, and also the pancreas, liver, and intestine.

- The signs and symptoms of cystic fibrosis are poor growth and poor weight gain despite a normal food intake, frequent chest infections, and coughing.

### Frameshift Mutation

- A mutation in a DNA chain that occurs when the number of nucleotides inserted or deleted is not a multiple of three; OR
- It is a type of mutation in which a number of nucleotides not divisible by three is inserted into or deleted from a coding sequence.

- This therefore will make every codon beyond the point of insertion or deletion (downstream) read incorrectly during translation.



- A frameshift mutation will in general cause the reading frame of the codons after the mutation to code for different amino acids.
- The frameshift mutation will also alter the first stop codon ("UAA", "UGA" or "UAG") encountered in the sequence.

- The polypeptide which is being created could be abnormally short or abnormally long, and will most likely not be functional.
- Frameshift mutations can be caused by **intercalating agents**.

- These are chemical agents that insert between adjacent base pairs (like inserting between the rungs of a ladder).
- The intercalation causes a conformational change in the double helix, so that when replication occurs, the aberrant conformation causes small deletions or insertions to occur in the newly synthesized DNA.

## Difference between Point and Frameshift Mutations

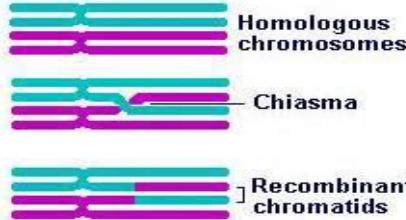
- A **point mutation** is where a **single letter is the only thing changed** in the DNA sequence.
- Lets say your phone number (or DNA code) was 483-183**9** and you mistakenly told someone that your phone number was 483-183**5**.

- that one digit is enough to make that person dial the wrong number (or cause a mutation in DNA.)
- For example suppose your DNA sequence was ACT **GCT**, a point mutation would just be a change in one of those bases (or letters), so it could end up like : ACT **A**CT.

- A **frameshift mutation** on the other hand is generally much more serious and will cause a change all the way down (downstream) a DNA sequence, making each codon a different sequence, not just in one point or base like a point mutation.

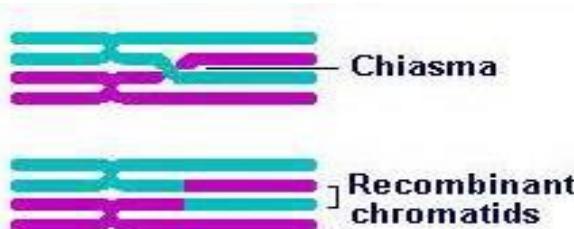
### TRANSFER OF GENETIC MATERIAL

- Sometimes when two pieces of DNA come into contact with each other, sections of each DNA strand will be exchanged.
- This is usually done through a process called crossing over in which the DNA breaks and is attached on the other DNA strand leading to the transfer of genes and possibly the formation of new genes.



- Genetic recombination is the transfer of DNA from one organism to another.
- The transferred donor DNA may then be integrated into the recipient's nucleoid by various mechanisms.

- In the case of homologous recombination, homologous DNA sequences having nearly the same nucleotide sequences are exchanged by means of breakage and reunion of paired DNA segments.



- Genetic information can be transferred from organism to organism through vertical transfer (from a parent to offspring) or through horizontal transfer methods such as **transformation**, **transduction** or **conjugation**.
- Bacterial genes are usually transferred to members of the same species but occasionally transfer to other species can also occur.

### ❖ **Bacterial transformation**