Chapter 16 : The Molecular Basis of Inheritance

over view :

- In **1953**, James Watson and Francis Crick shook the world with an elegant **double-helical model** for the structure of ***deoxyribonucleic acid***(***DNA)*** .

- **Hereditary information** Is encoded in the chemical language of DNA and reproduced in all the cells of your body **.**

  - the DNA program directs the development of many different types of traits .

• Concept 16.1: DNA is the genetic material

a) The search for the genetic material

- until 1940**,** the two chemical components of chromosomes (DNA + protein) were the candidates for the genetic material . scientists weren't know who is responsible for heritance .

-The role of DNA in heredity Was first worked out by studying bacteria and the viruses .
-by scientific inquiry biologists got important evidences that DNA is the genetic material .

1. ***Evidence That DNA Can Transform Bacteria***

Frederick Griffith was studying *Streptococcus pneumoniae* " a bacterium that causes pneumonia in mammals" .
- He worked with two strains of the bacterium a *pathogenic strain* (disease causing) and *a nonpathogenic strain* (harmless) .

>> Look to the next page to see the inquiry (experiment and conclusion)of Griffith ☺

- Griffith found that when he mixed ***heat-killed remains of the pathogenic strain*** With **living cells of the nonpathogenic strain** , the result was some of these living cells became pathogenic .
- Griffith called this phenomenon **Transformation** " the change in genotype and phenotypeof the cell due to the assimilation of external DNA ".

**but this transformation is different from the conversation of normal animal cell to cancerous one , which was discussed in chapter 12) .

Figure 16.2 : can a genetic trait be transferred between different bacterial strains ?

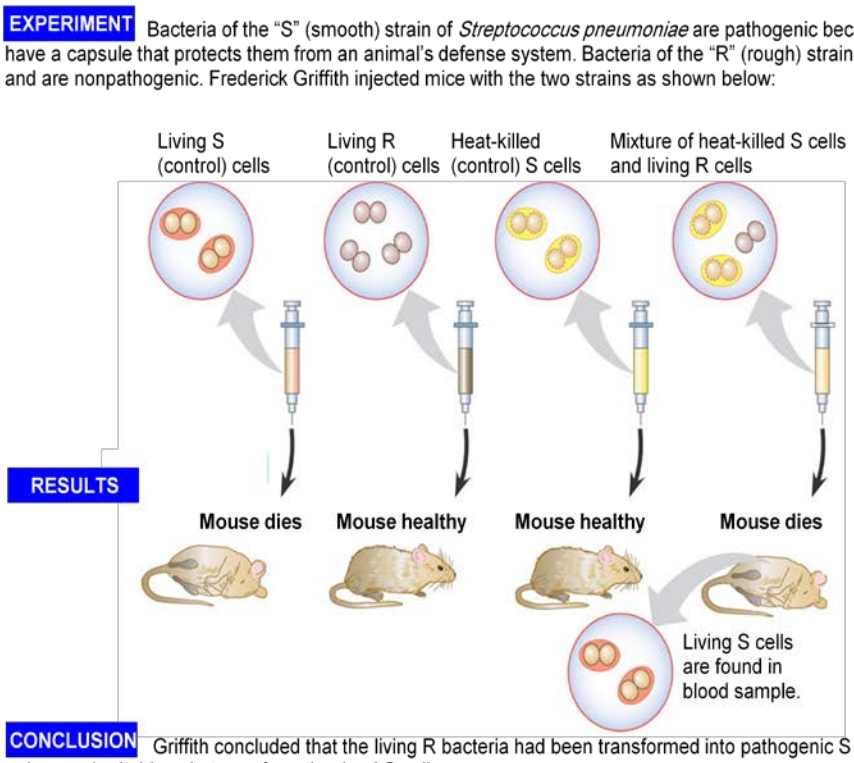


Figure 16.2

- After that , an american bacteriologist Oswald Avery did a search for the identity of the transforming substance in Griffith inquiry . Avery focused on the three main candidates (DNA , RNA, protein)

- Avery used a specific treatment that inactivate one of these 3 candidates then apply the Griffith inquiry many times to test the 3 substances and the result was , **transformation occurred only when the DNA was active** .

  >> so the transforming agent was DNA ☺

---

2. Evidence That Viral DNA Can Program Cells

- This evidence material Came from studies of a virus that infects bacteria .
- Viruses that infect bacteria" **bacteriophages** " Are widely used as tools by researchers in molecular genetics .

Viruses :
- Viruses are much simpler than any cell .

- Virus is a little more than DNA(or sometimes RNA) enclosed by a protective coat(simply protein).

- to reproduction , virus must infect a cell and take over the cells metabolic machinery .

- *Bacterio***phages** "phage" = bacteria **eater**

The Inquiry :

Alfred Hershey and Martha Chase Performed experiments showing that DNA is the genetic.

- They used T2 phage ..
- First of all we know that T2 as another phages is composed of DNA and protein .
- T2 could reprogram its host cell (infected cell) to produce viruses , but which viral component (protein Or DNA) will still in the cell and reprogramming it ?
- ✓ Alferd Hershey and his colleagues answer the question by an experiment showing that DNA is the genetic material of a phage which called T2 .

Hershey and Chase experiment >>

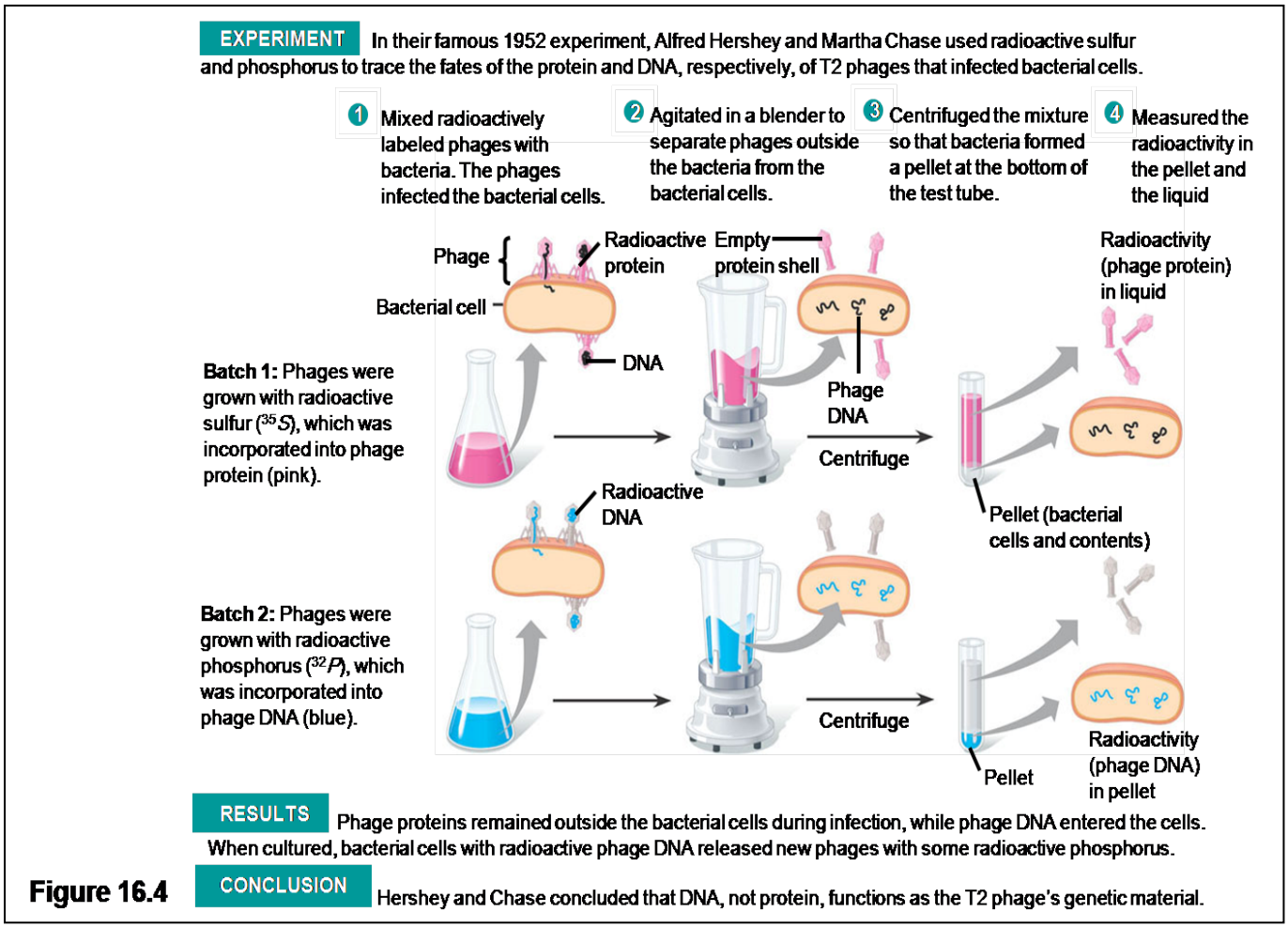


Figure 16.4

- Results are in 2 points :
  1) they found that phage proteins remained outside the bacterial cells during infection , while phage DNA entered the cells .
- 2) when these bacteria were returned to a culture medium , the infection ran its course and the infected cell released phages that contained some radioactive phosphorus (which was used to label the DNA at the beginning of the experiment).

3) ***Additional Evidence That DNA Is the Genetic Materia***l

- Prior to the 1950s, it was already known that **DNA** is <u>a polymer of nucleotides,</u> each consists of three components :

1- nitrogenous base (**A**denine or **T**hymine or **C**ytosine or **G**uanine)

2- sugar (deoxyribose)

3- phosphate group

Figure 16.5 : The structure of DNA strand

- The phosphate of one nucleotide attached to the sugar of the next one resulting in a "backbone" .

-the polynucleotide strand has directionality from the <u>5 end</u> (with the phosphate group) to the <u>3 end</u> (with -OH group) .

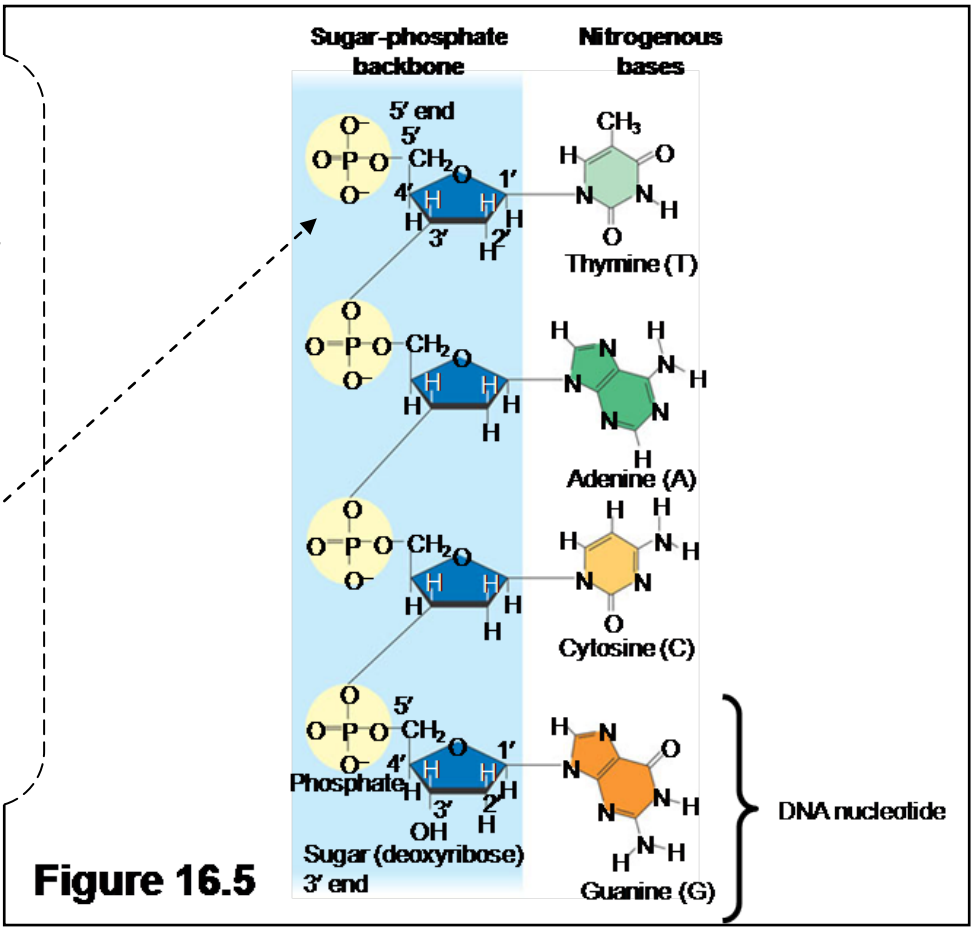


Figure 16.5

the Biochemist Erwin Chargaff noticed >

- The base composition of DNA varies from one species to another ,

for example (human DNA nucleotide contains (A) base > 30.3% , when the DNA of the bacterium E.coil has only 26% (A) base .

- There is an equivalence between the bases types in any given species , (A) approximately equaled to (T) and (c) approximately equaled to (G) < **Chargaff rule**>

**the basis of these rules remained un explained until the discovery of the double helix .

B) Building a Structural Model of DNA

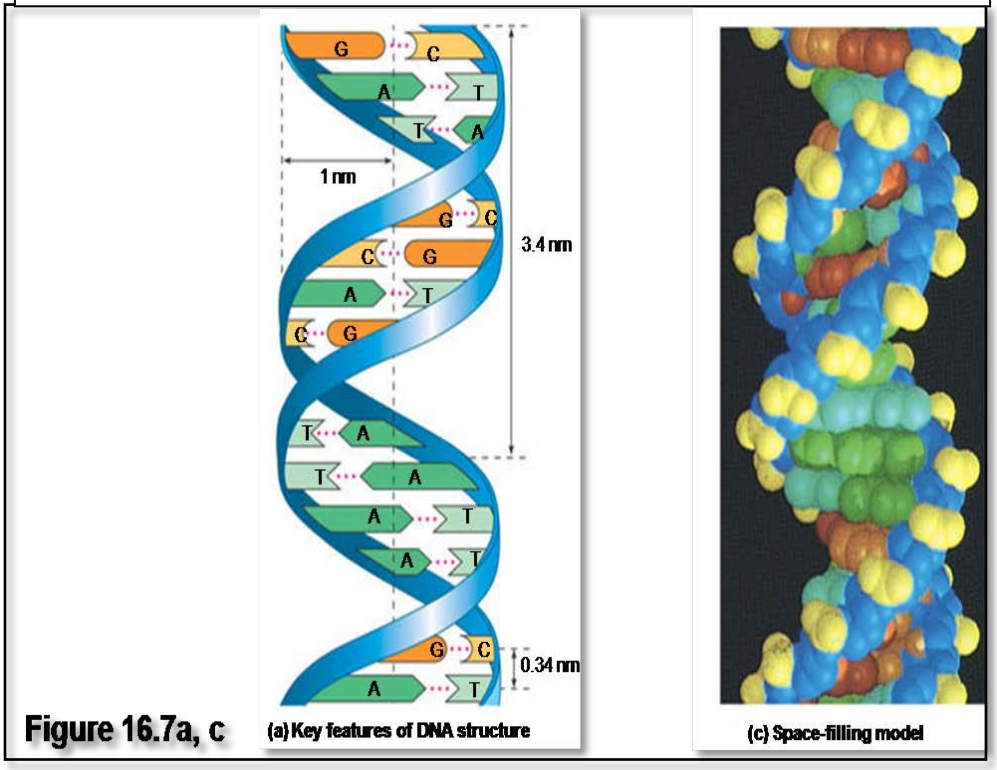
- - Maurice Wilkins and Rosalind Franklin Were using a technique called X-ray crystallography to study molecular structure , Rosalind Franklin Produced a picture of the DNA molecule using this technique .

- James Watson and Frances Crick deduced that DNA was a **double helix** ,Through observations of the X-ray crystallographic images of DNA.

- Franklin had concluded that DNA was composed of two anti-parallel sugar-phosphate backbones, with the nitrogenous bases paired in the molecule's interior .

- The nitrogenous bases are paired in specific combinations: **A**denine with **T**hymine, and **C**ytosine with **G**uanine.

Figure 16.7 : The double helix



-The **ribbons** in this diagram represent the sugar phosphate backbones .

-The 2 strands held together by hydrogen bonds between the nitrogenous bases which are paired in the interior of the double helix .

Note : This arrangement was appealing(مقنع جداً) because it put the relatively hydrophobic nitrogenous bases in the molecules interior and thus away from the surrounding aqueous solution .

Figure 16.7 : the double helix

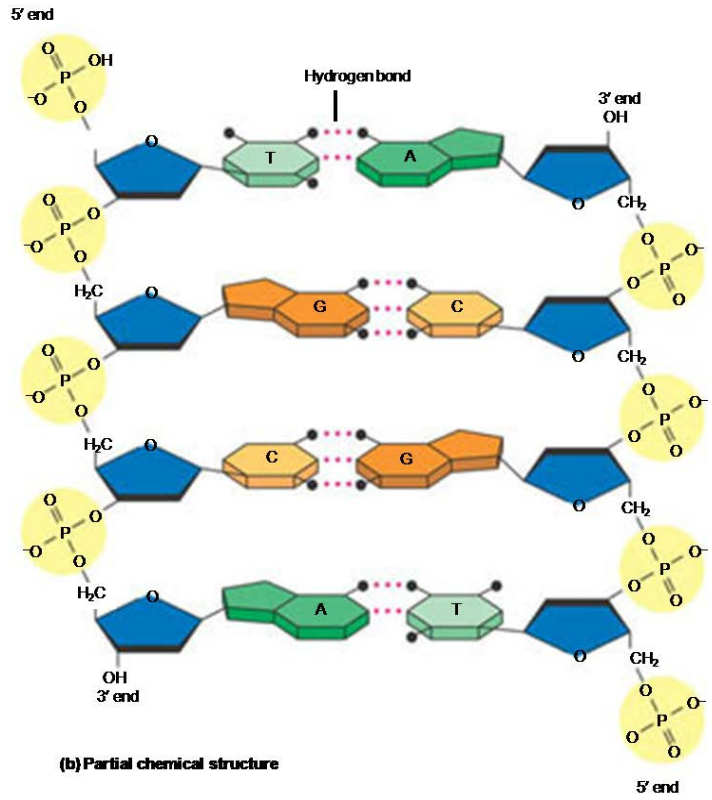


Figure 16.7b

-strong covalent bonds link the units of each strand , while weaker hydrogen bonds hold one strand to the other .

- notice that the strands are untiparallel , meaning that they are oriented in opposite directions .

- Watson and Crick reasoned that there must be an additional specificity of pairing , Dictated by the structure of the bases.

- Each base pair forms a different number of hydrogen bonds , **A**denine and **T**hymine form two hydrogen bonds, **C**ytosine and **G**uanine form three hydrogen bonds .

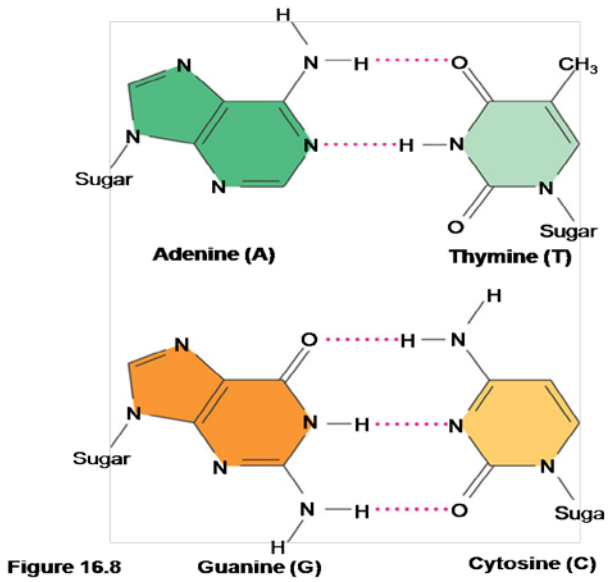


Figure 16.8

Figure 16.8 : Base Pairing in DNA

- In the DNA of any organism , the amount of (Adenine) equals the amount of (Thymine) and the amount of(Guanine) equals the amount of (Cytosine).

  - Watson-crick model explained the basis for Chargaff's rules .
  - the number of nitrogenous bases don't restrict the sequence of nucleotides along each DNA strand ; we can't depend on the number of nitrogenous bases to Know the exactly long of the sequence .

Concept 16.2: **Many proteins work together in DNA replication and repair**

-The relationship between structure and function is manifest(clear) in the double helix .

- The basic principle ***: Base Pairing To a Template Strand***

  1 **-Since the two strands of DNA are complementary , each strand acts as a template for building a new strand in replication**

  2 -In DNA replication The parent molecule unwinds and two new daughter strands are built based on base-pairing rules

Figure 16.9 : a model for DNA replication : the basic concept

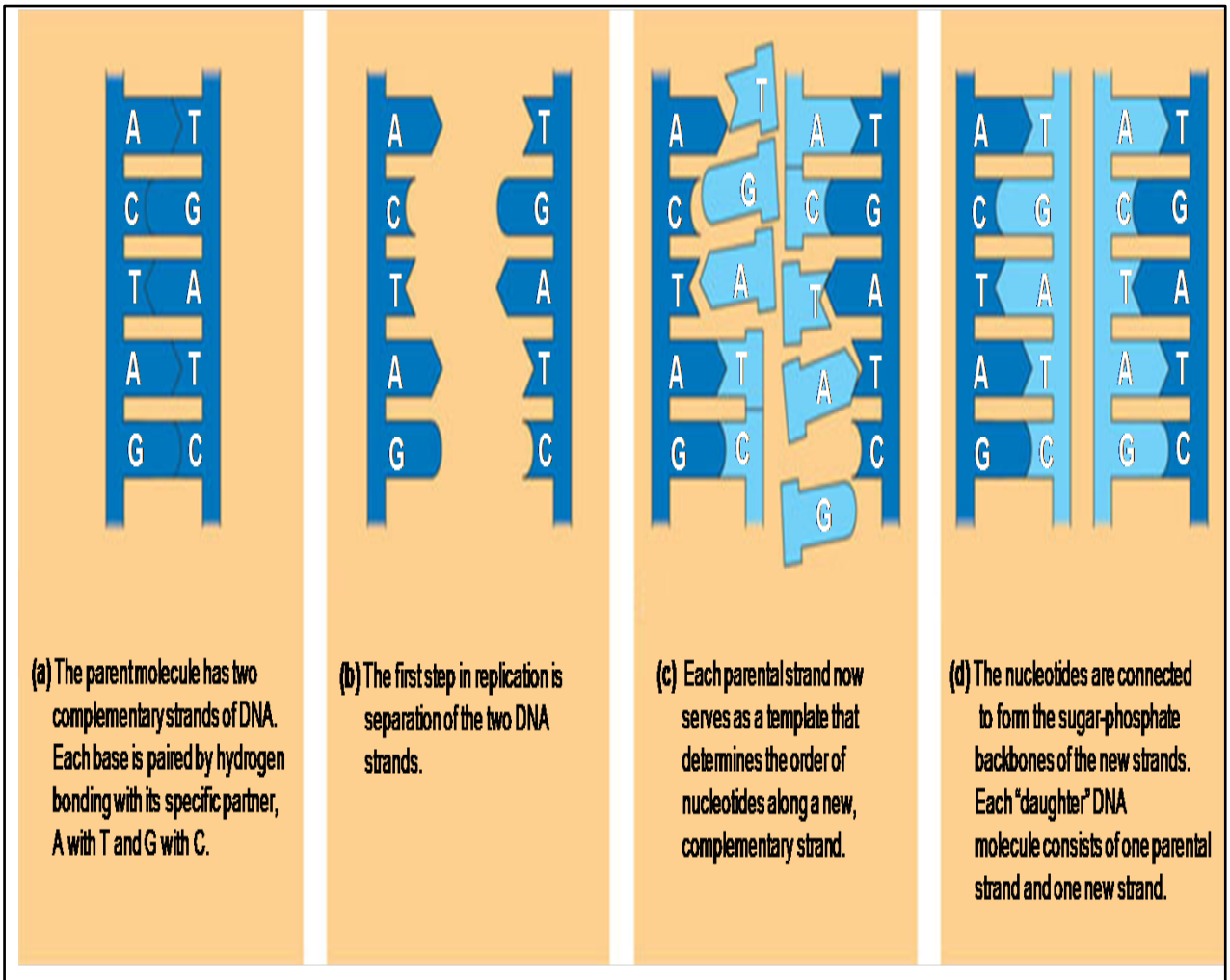


Figure 16.9 a–d

Note : - the figure shows a short section of double helix in untwisted form .

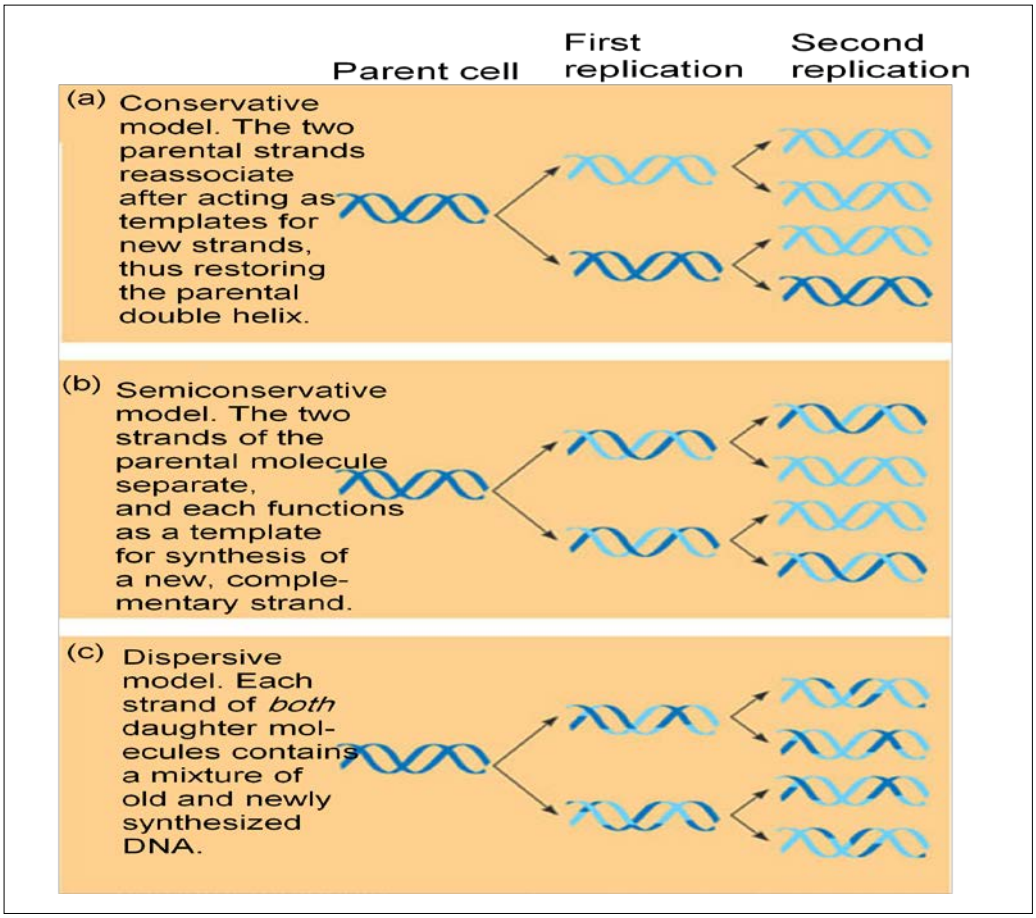
- **3- DNA replication is semi-conservative** "Each of the two new daughter molecules will have one old strand, derived from the parent molecule, and one newly made strand"

  Note : there was 3 possibilities for the replication >>

  (**conservative** Or **semi-conservative** or **dispersive**)

  Next figure represent the three prediction >>

Figure 16.10 : Three alternative models of DNA replication



- **An experiment performed by Meselson and Stahl Supported the semiconservative model of DNA replication >>**

- This inquiry only represent an evidence to be sure that **semi-conservative** model is the right model of DNA replication ☺

EXPERIMENT

Figure 16.11 :Maselo & Stahl experiment

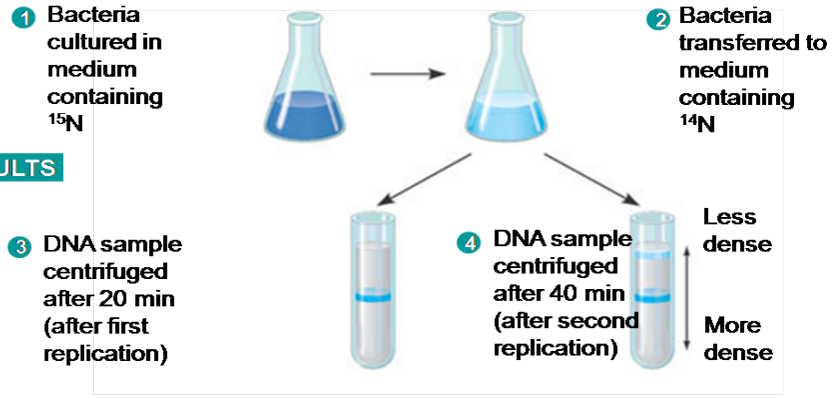
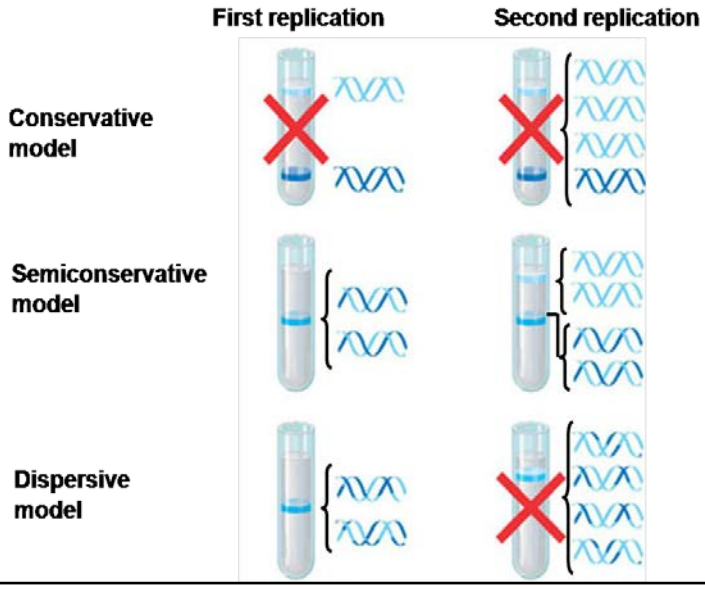


Figure 16.11

CONCLUSION Meselson and Stahl concluded that DNA replication follows the semiconservative model by comparing their result to the results predicted by each of the three models in Figure 16.10. The first replication in the ^{14}N medium produced a band of hybrid (^{15}N–^{14}N) DNA. This result eliminated the conservative model. A second replication produced both light and hybrid DNA, a result that eliminated the dispersive model and supported the semiconservative model.



Compartment between the experiment results and the 3 predictions .

❖ DNA Replication: *A Closer Look*

- The copying of DNA is remarkable in its speed and accuracy
- More than a dozen enzymes and other proteins participate in DNA replication

Note : scientists knew much more about how "replication mechanism" works in bacteria than in eukaryotes , so we will discuss the DNA replication in bacteria (in E.coil type), however the most of the process is fundamentally similar of prokaryotes and eukaryotes .

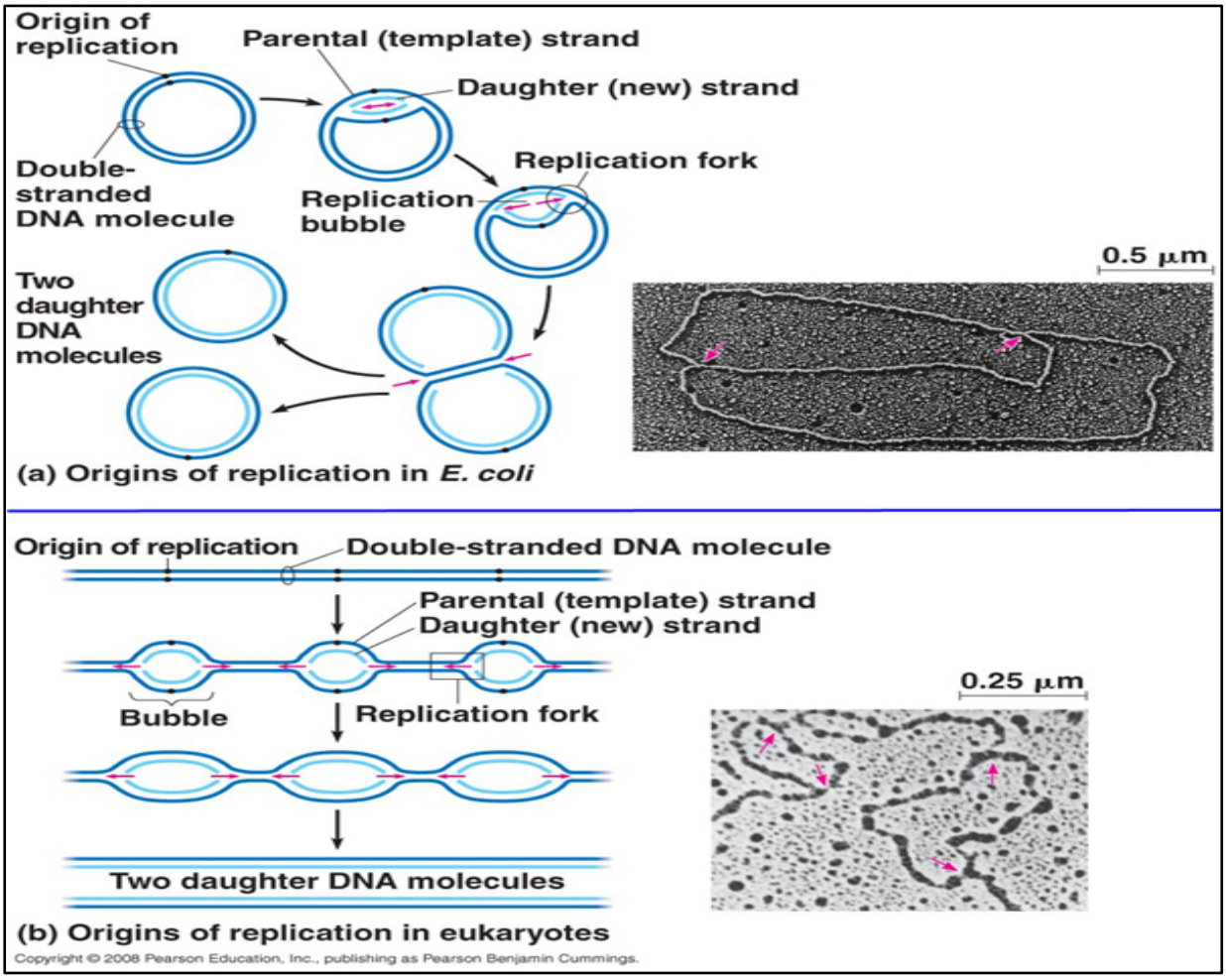
a) Getting Started

- Begins at special sites called origins of replication, where the two strands are separated

Origins of replication : short stretches of DNA having a specific sequence of nucleotides .

- E.coil chromosome , like many other bacterial chromosomes , is circular and has a single origin , while in eukaryotes chromosome may have hundreds or even a few thousand replication origins .

Figure 16.12 : Origins of replication in E.coil and eukaryotes



Explanation of Figure 16.12

Linear chromosome Replication (eukaryots)

1-Proteins that initiate DNA replication recognize the origins of replication and attach to the DNA , separating the two strands and opening up a replication bubbles .

2- The bubbles expand laterally, as DNA replication proceeds in both directions .

3- Eventually, the replication bubbles fuse, and synthesis of the daughter strands is complete.

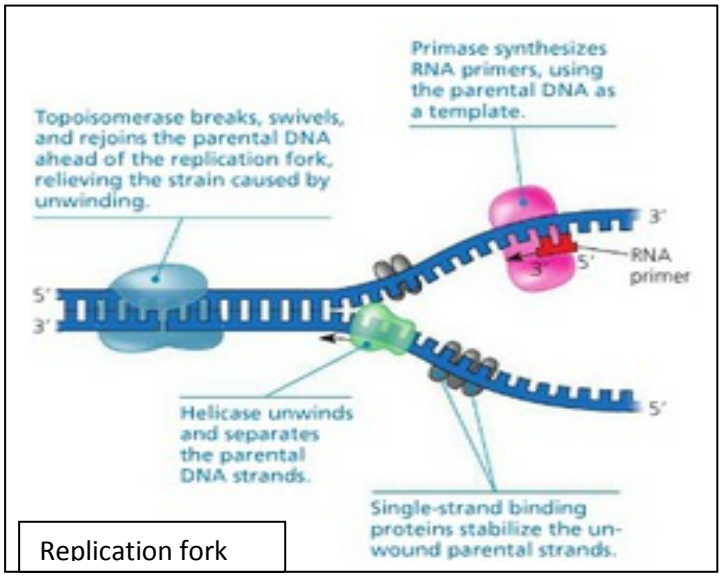
*multiple duplication bubbles form and eventually fuse, and thus speeding up the copying .

Circular chromosome Replication (ex: bacteria)

- In the circular chromosome of E.coil and other bacteria only one origin of replication .

- parental strands **separate** at the origin and **form** a replication bubble with 2 forks , and **proceeds** in both direction until the 2 forks meet on the other side .

Replication Fork : (Y) shaped region where the parental strands of DNA are being unwound (several Kinds of proteins participate in the unwinding) .
Helicase : enzyme which untwist the double helix at the replication forks , to make them available as template strands .
Single-Strand Binding Protein : bind to the unpaired DNA strands and stabilizing them .
Topoisomerase : enzyme breaks , swivels and rejoins the parental DNA ahead of the replication fork , relieving the strain caused by unwinding .



Replication fork

Note :
-the enzymes that synthesize DNA cant initiate the synthesis of a polynucleotide ; they can only add nucleotides to the end of an already existing chain that is (base-paired with the template strand .

-The initial nucleotide chain that is produced in DNA synthesis is actually a short stretch of RNA , not DNA << **this RNA chain is called Primer** , and synthesized by **Primase** >>

Primase : enzyme starts an RNA chain from a single RNA nucleotide , adding RNA nucleotides using the parental DNA strand as a template according to base- pairing rule .

☒ The completed primer , generally 5 to 10 nucleotides long .
-look to the figure , the DNA strand will start from the(3 end) of the RNA primer.

B) Synthesing of the new DNA strand

- When primer is formed , another type of enzymes catalyze the synthesis of the new DNA strand >>

DNA polymerases : ***catalyze*** the synthesis of new DNA strand by ***adding*** nucleotides to a growing strand .

example : DNA polymerase I , DNA Polymerase III , they play a major role in DNA replication of E.coil .

DNA Polymerase requires a ***Primer** and ***DNA template strand** to work ☺

Figure 16.13 : Incorporation of a nucleotide into a DNA strand

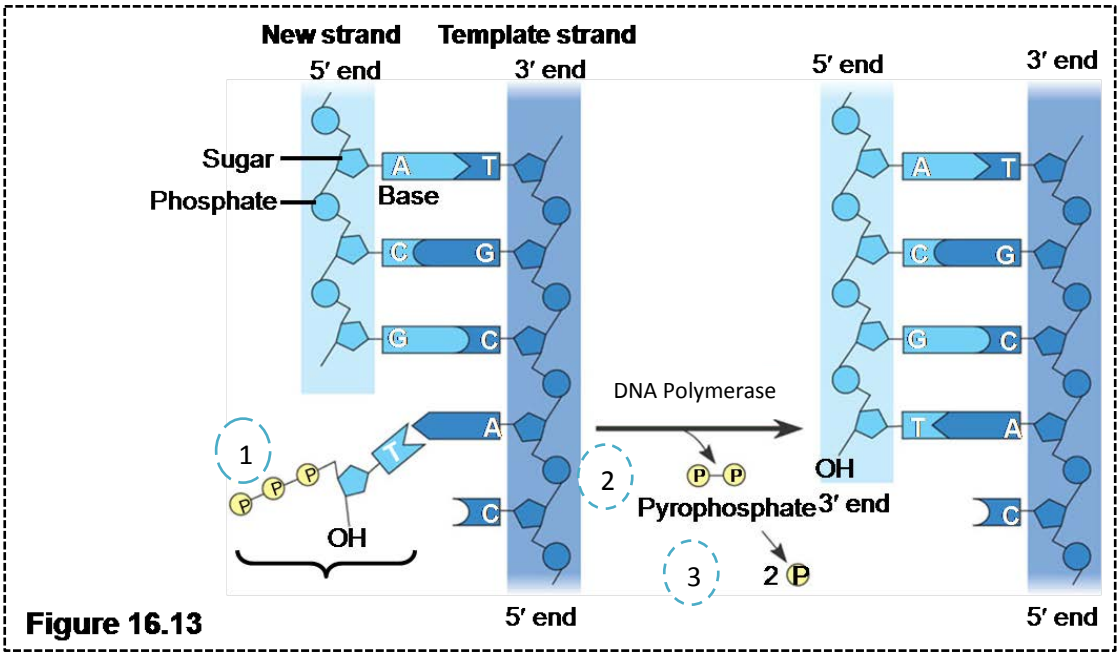


Figure 16.13

Main Idea : DNA polymerase catalyze the addition of a Nucleoside Triphosphate to the (3 end) of a growing DNA strand with the release of two phosphates .

1. Each nucleotide added to a growing DNA strand comes from **Nucleoside Triphosphate (** sugar + base + 3 phosphate groups **).**

2. The Nucleoside Triphosphate used for DNA synthesis is chemically reactive partly, because their triphosphate tails have an unstable cluster of negative charge . as each monomer joins the growing end of DNA strand , two phosphate groups are lost as a molecule of pyrophosphate .

3. Pyrophosphate hydrolyzed to two molecules of inorganic phosphate .

C) Elongation (anti-parallel elongation)

We Know that :

☒ The two ends of a DNA strand are different .
☒ The two strands of DNA in a double helix are antiparallel (they are oriented in opposite direction to each other).

- How does the antiparallel structure of the double helix affect replication?

Figure 16.14 : synthesis of the leading and lagging strand during DNA replication

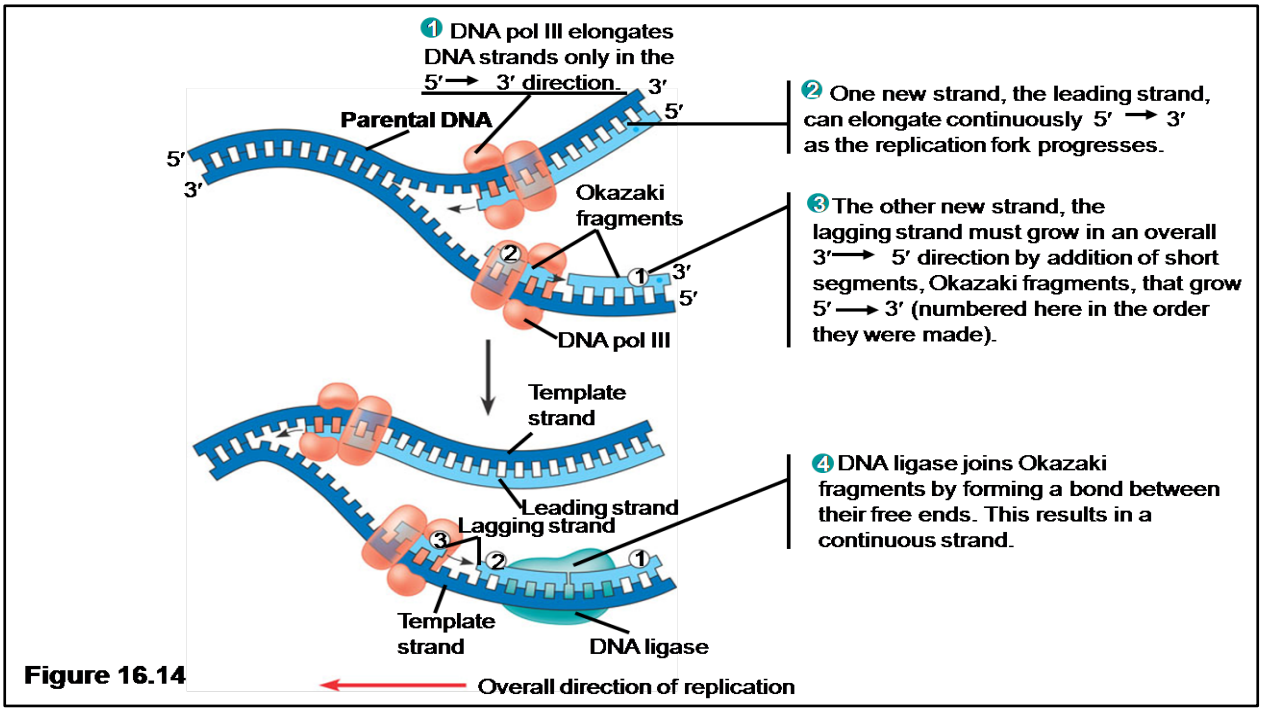


Figure 16.14

- DNA polymerases add nucleotides only to the free 3′ end of a growing strand . Along one template strand , so >
- **DNA polymerase III** can synthesize a complementary strand continuously, **moving toward the replication fork** . (**leading strand**)

- **DNA polymerase III** must work in the direction **away from the replication fork** to elongate the other new strand of DNA (**lagging strand)** ,which is discontinuously as a series of segments called Okazaki Fragments .
  look to figure (16.14)

- Only one primer is needed for synthesis of the leading strand ,but for synthesis of the lagging strand, each **Okazaki fragment** begin from a primer separately.
- The fragment s are about 1000-2000 nucleotides along E.coil , while in eukaryotes 100-200 nucleotides ☺

Figure 16.15 : Synthesis of the lagging strand

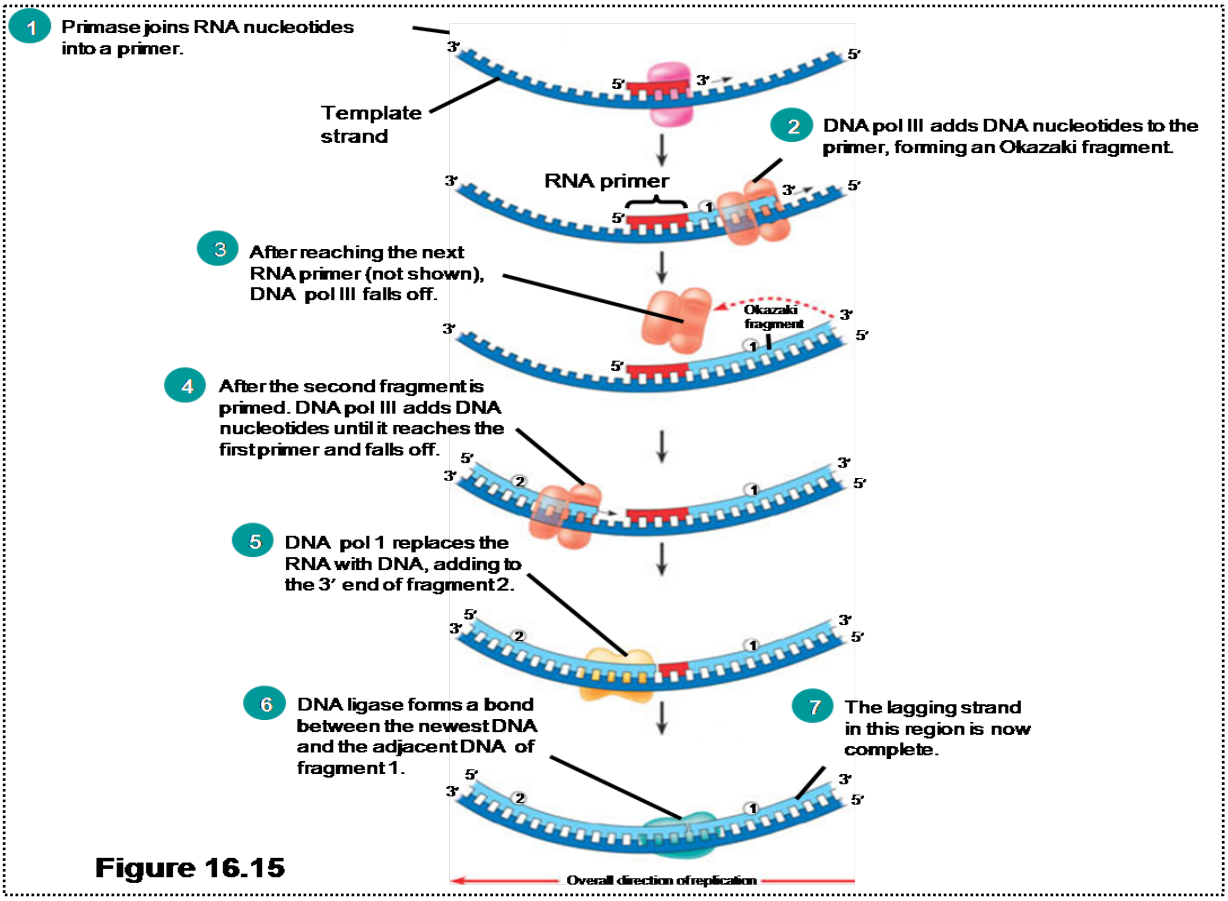


Figure 16.15

-This Figure illustrates the steps in the synthesis of the lagging strands . **Study steps carefully**

Notes:

1+2) Each Okazaki Fragment on the lagging strand must be **primed separately .**

5) **DNA Polymerase I** replaces the RNA with DNA , by adding nucleotides to the 3' end of the adjacent fragment . but it cannot join the fragments together .

6) DNA Ligase : join the sugar-phosphate backbones of all the Okzaki Fragments into a continuous DNA strand .

Table 16.1 Bacterial DNA replication proteins and their functions

Protein	Function for Leading and Lagging Strands	
Helicase	Unwinds parental double helix at replication forks	
Single-strand binding protein	Binds to and stabilizes single-stranded DNA until it can be used as a template	
Topoisomerase	Corrects "overwinding" ahead of replication forks by breaking, swiveling, and rejoining DNA strands	
	Function for Leading Strand	**Function for Lagging Strand**
Primase	Synthesizes a single RNA primer at the 5′ end of the leading strand	Synthesizes an RNA primer at the 5′ end of each Okazaki fragment
DNA pol III	Continuously synthesizes the leading strand, adding on to the primer	Elongates each Okazaki fragment, adding on to its primer
DNA pol I	Removes primer from the 5′ end of leading strand and replaces it with DNA, adding on to the adjacent 3′ end	Removes the primer from the 5′ end of each fragment and replaces it with DNA, adding on to the 3′ end of the adjacent fragment
DNA Ligase	Joins the 3′ end of the DNA that replaces the primer to the rest of the leading strand	Joins the Okazaki fragments

** All these proteins assist DNA replication as Enzymes ..

Figure 16.16 : A summary of bacterial DNA replication

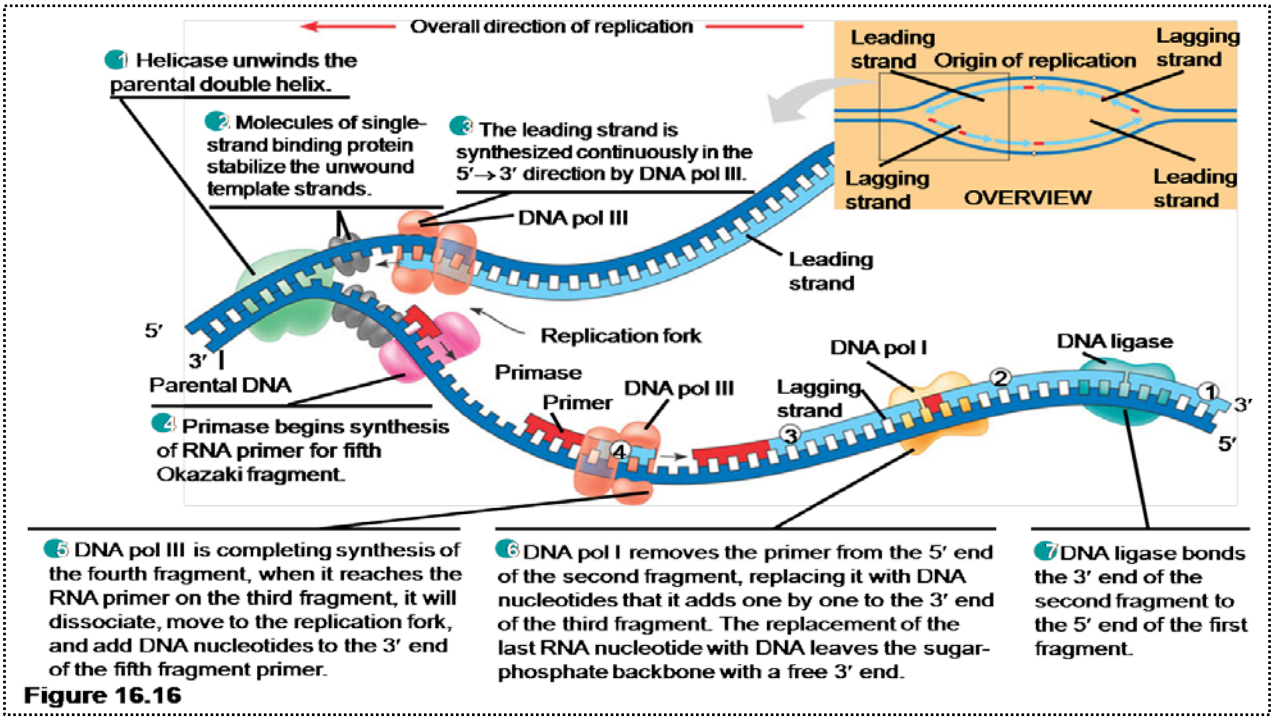


Figure 16.16

Note : **This diagram shows one replication fork , but replication usually occurs simultaneously at two forks , one at either end of a replication bubble as we studied before .**

➲ The DNA Replication Complex :

-various proteins that participate in DNA replication actually form a single large complex .

- DNA replication complex doesn't move along the DNA ; rather the DNA moves through the complex during replication process .

*in eukaryotic cells, multiple copies of the complex, perhaps grouped into "factories" maybe anchored to the nuclear matrix ..

- lagging strand is looped back through the complex to speed up the work of DNA polymerase , it doesn't have far to travel to reach the primer for the next fragment .
this looping of the lagging strand enables more Okazaki fragment to be synthesized in less time .

❖ Proofreading and Repairing DNA :

- DNA polymerases **proofread** each nucleotide against its template as soon as its added to the growing strand . upon finding an incorrectly paired nucleotide , the polymerase removes the nucleotide and then resume synthesis.

- In mismatch repair of DNA , **Repair enzymes** correct errors in base pairing (remove and replace incorrectly paired nucleotides that have resulted from replication errors .

  Note : incorrectly paired or altered nucleotides can also arise after the replication . and that requires frequent repair of various kinds of damage to existing DNA .

- DNA molecules are constantly subjected to potentially harmful chemical and physical agents (examples : radioactive emissions ,X-rays, Ultraviolet light ,certain molecules in cigarette smoke) can change nucleotides in way that effect encoded genetic information .next figure shows an example :

Figure 16.17 : Nucleotide excision repair of DNA damage .

a **repair system of DNA damage :**

- the Error is a covalent linking of Thymine bases that are adjacent on a DNA strand ,such Thymine dimer cause the DNA to buckle and interfere with DNA replication .
-**Nuclease** : is cutting enzyme
-**DNA Polymerase** : fills the gap with a new correct segment
-**Ligase** : join the new segment with the old DNA .

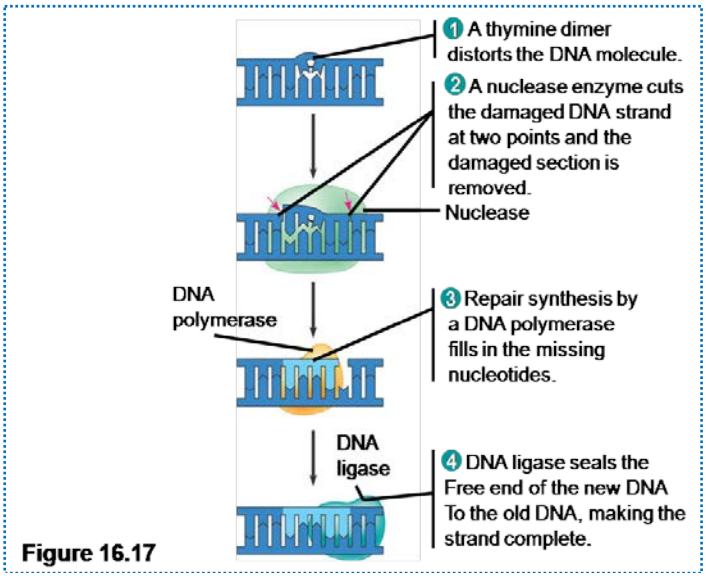


Figure 16.17

Concept 16.3 : **A chromosome consists of (a DNA molecule packed together with proteins)**

Bacterial Chromosome	**Eukaryotic Chromosome**
Consists of a one double-stranded **circular** DNA molecule associated with a small amount of protein **.**	Consists of one linear DNA molecule associated with a large amount of protein .
Consists of about **4.6 million nucleotide pairs ,** representing about **4.400 genes .**	In human somatic cell , chromosomal DNA is 1000 times more than in bacteria
The DNA molecule of E.coil measures about a millimeter in length	DNA molecule would be about 4cm long.
Chromosome is coiled and densely packed because of certain proteins associated with it . and it fills only part of the cell ,	Eukaryotic DNA is precisely combined with a large amount of protein (DNA + protein = chromatin)
DNA fills only part of the cell called **Nucleoid** (is not bounded by membrane) .	Chromosomes fit into the nucleus through a multi level system of DNA packing . look at figure below .

figure 16.21 : Chromatin Packing in a Eukaryotic

1. DNA the double helix

Shown here is a ribbon model of DNA>>
- with each ribbon representing one of sugar-phosphate back bone .

-phosphate groups along the backbone contribute a negative charge along the outside of each strand .

2. Histones

-Histones are proteins responsible for the first level of DNA packing in chromatin . they organize DNA within cells .

-Each histone contains about 100 amino acids , and its total mass in chromatin is approximately equal to mass of DNA .

-5% of its amino acids are positively charged and bind tightly to the negatively charged DNA .

-4 types of histon are most common in chromatin (H2A,H2B,H3,H4) and they are critical to the next level of DNA . a fifth type histone called (H1).
-Histons are very similar among eukaryotes .

3. Nucleosomes "beads on a string "

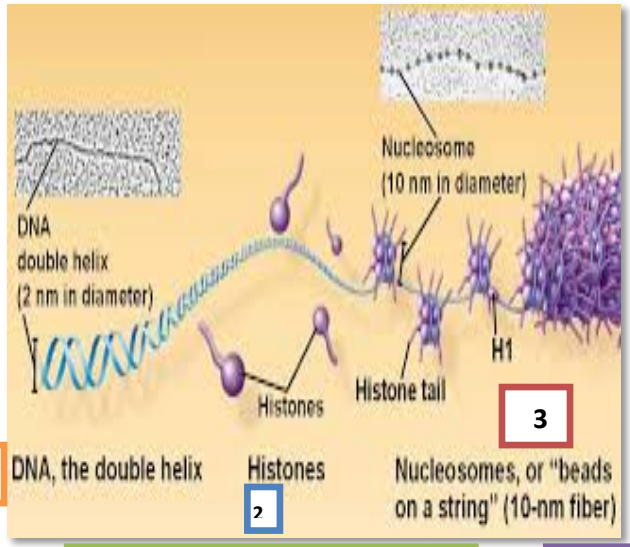
-chromatin in this level is 10 nm in diameter.

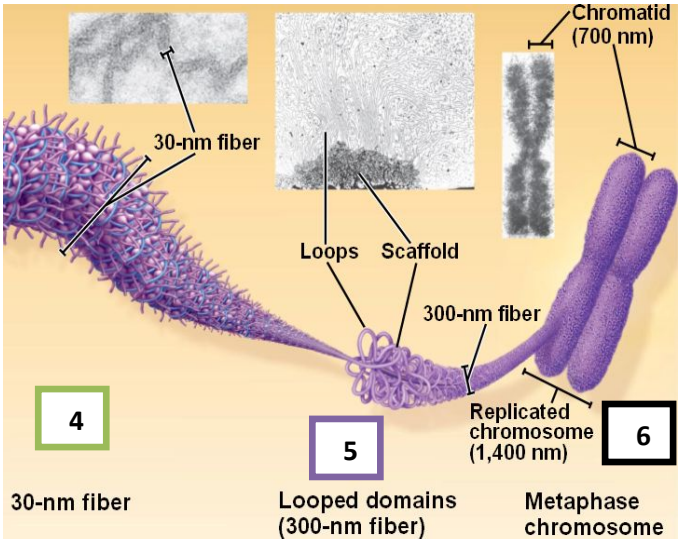
- each **bead** is a nucleosome(the basic unit of DNA packing) , and the **string** between beads is called linker DNA .

-nucleosome consists of DNA wound twice around a protein core (Two molecules of the 4 main histone types).

-histone tail = the amino end (N-terminus) , extends outward from the nucleosome.

-in the cell cycle , the histones leave DNA only briefly during DNA replication.





4. (30-nm fiber)

- this level is due to the interaction between the **histon tail** of one nucleosome and the **linker DNA** and **nucleosome in other side**.

-this interaction cause the extended 10-nm fiber to coil or fold forming chromatin fiber 30-nm in thickness.

-(H1) involved at this level

5- Looped Domains (300 - nm fiber)

-**the 30-nm fiber** forms looped Domains attached to chromosome **scaffold** made of proteins .

- Scaffold is rich in one type of topoisomerase . H1 molecules also appear to be present .

6- Metaphase

-mitotic Chromosome, the looped domains themselves coil and fold .

-the width in this level is 700-nm.

-particular genes always end up located at the same places in metaphase chromosomes.

Chromosomes is a dynamic structure (condensed , loosened , modified and remodeled ; as necessary)

Chromatin undergoes striking changes in its degree of packing during the course of the cell cycle >>

in Inter phase as example :

- **chromatin appears as a diffused mass within the nucleus (occupies a specific restricted area) and highly extended (less condensed and it shows the same levels of higher order packing).**

-although an inter phase chromosome lacks an obvious scaffold ,it's looped domains appear to be attached to the nuclear lamina on the inside of the nuclear envelope, and perhaps also to fibers of the nuclear matrix , these attachments help organize regions of chromatin where the genes are active .

- chromatin fibers of different chromosomes don't become entangled .

- there are 2 types of chromatin in this phase >
1. Heterochromatin :highly condensed state . visible as irregular clumps with light-microscope .
2. euchromatin : True chromatin . has the characteristics that we discussed above . The genes present in euchromatin only can be expressed (because of looser packing of euchromatin).

(when the cell prepares to mitosis) :
its chromatin coils and folds up (condense) , short and thick metaphase chromosome .

But , what is the pathway regulating these transformation ?
-scientists reached that the histones can undergo chemical modification that result in changes in chromatin organization .

Note :
there is an Inquiry prove that ☺ the researchers concluded that a specific phosphorylation of histone H2A is necessary for normal chromosome behavior during meiosis .

The END Of Chapter 16