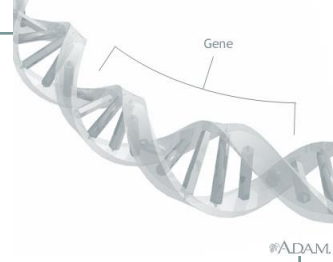
Dwi Setyati 2016

DNA: The Genetic Material



- Search for genetic material---is it composed of nucleic acid or protein/DNA or RNA?
    - Griffith's Transformation Experiment
    - Avery's Transformation Experiment
    - Hershey-Chase Bacteriophage Experiment
    - Tobacco Mosaic Virus (TMV) Experiment
- Nucleotides - composition and structure
- Double-helix model of DNA - Watson & Crick
- Organization of DNA/RNA in chromosomes
    - Prokaryotes
    - Eukaryotes

Timeline of events:

- 1890 Weismann - substance in the cell nuclei controls development.
- 1900 Chromosomes shown to contain hereditary information, later shown to be composed of protein & nucleic acids.
- 1928 Griffith's Transformation Experiment (incorrectly guessed protein!)
- 1944 Avery's Transformation Experiment (DNA not RNA)
- 1953 Hershey-Chase Bacteriophage Experiment (DNA not protein)
- 1953 Watson & Crick propose double-helix model of DNA
- 1956 First demonstration that RNA is viral genetic material.

DNA SBG MATERI GENETIK

DNA dibuktikan sebagai materi genetik oleh :

- 1 Frederick Griffith
- 2. Percobaan Mac Leod dan Mc Carty
- 3 Percobaan Hershey-Chase

Griffith's Experiments

- Griffith worked with 2 strains of S. pneumoniae
- 1st strain had a smooth capsule that protected the bacterium from body's defense systems (S)
- Virulent – (full of poison) able to cause disease
- 2nd strain lacked capsule and didn't cause disease (R)
- Mice injected with (S) strain died; mice injected with (R) strain lived

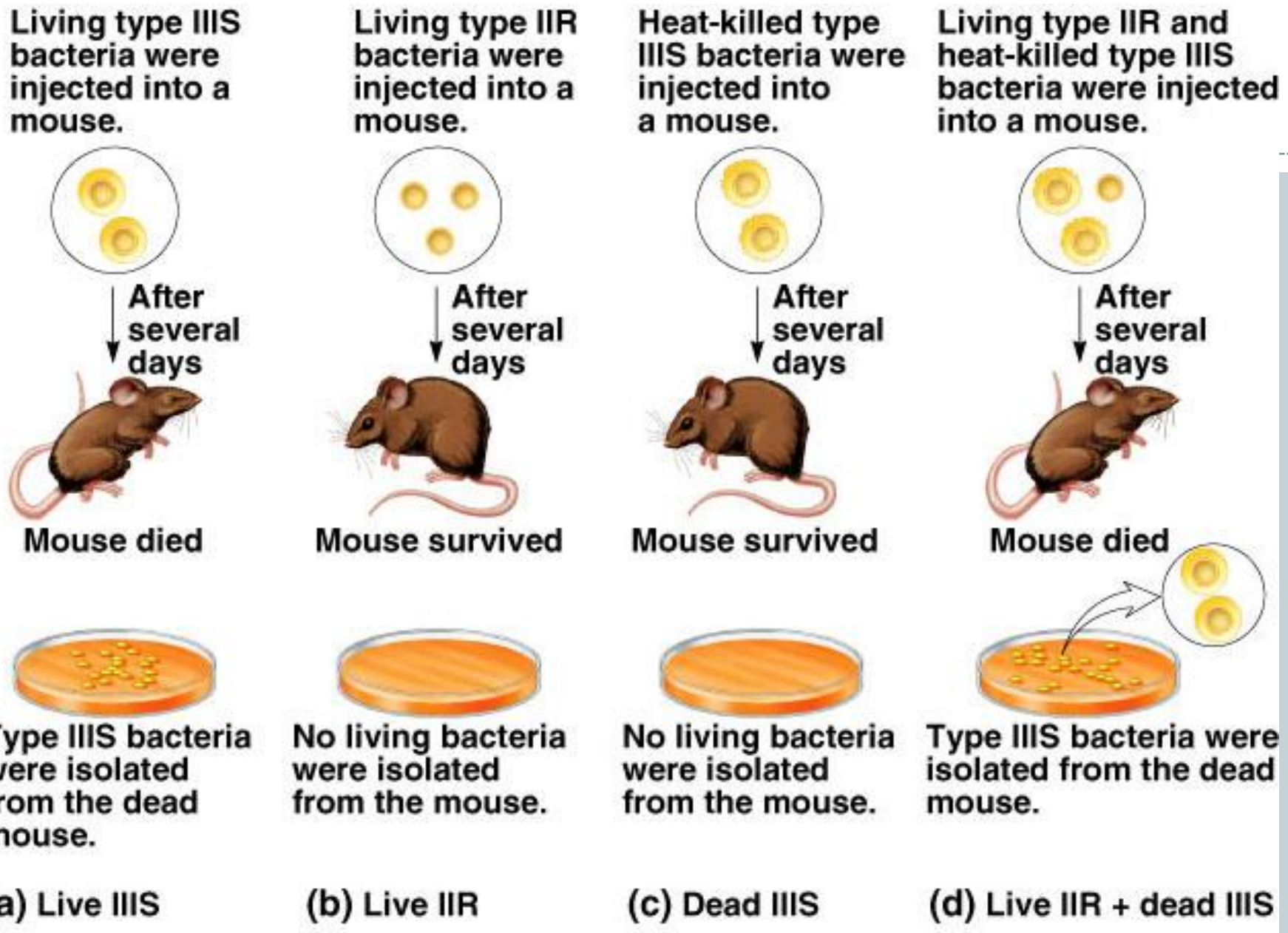
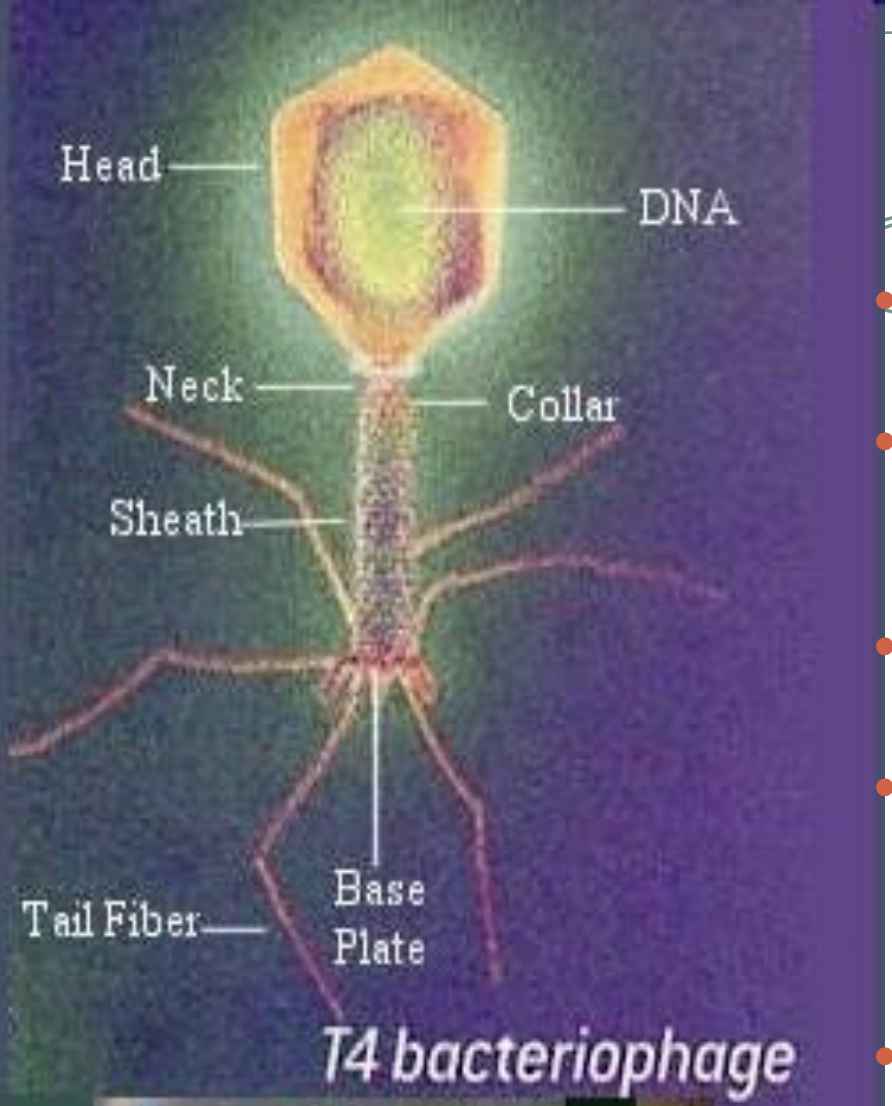
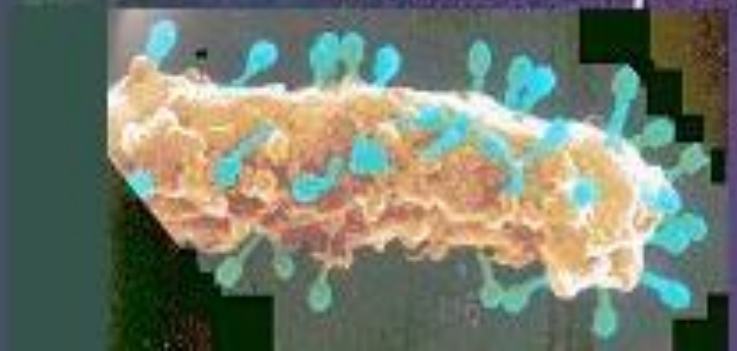

Living type IIIS bacteria were injected into a mouse.
After several days
Mouse died
Type IIIS bacteria were isolated from the dead mouse.
(a) Live IIIS
Living type IIR bacteria were injected into a mouse.
After several days
Mouse survived
No living bacteria were isolated from the mouse.
(b) Live IIR
Heat-killed type IIIS bacteria were injected into a mouse.
After several days
Mouse survived
No living bacteria were isolated from the mouse.
(c) Dead IIIS
Living type IIR and heat-killed type IIIS bacteria were injected into a mouse.
After several days
Mouse died
Type IIIS bacteria were isolated from the dead mouse.
(d) Live IIR + dead IIIS

Figure 9.2

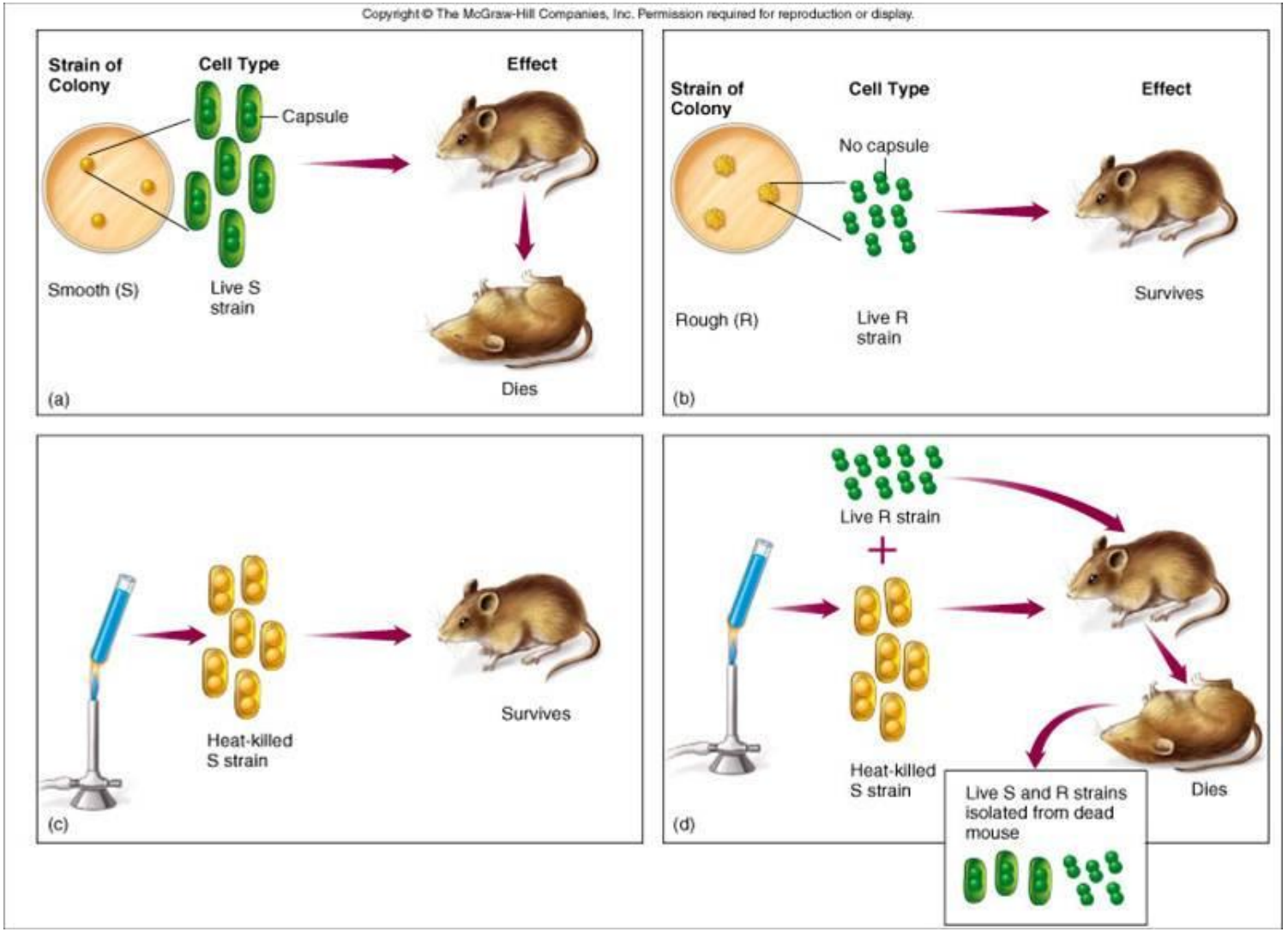
Griffith's Conclusions

- Something from the dead type IIIS transformed type IIR into type IIIS
- Called this process transformation
- The unknown substance was termed the transforming principle





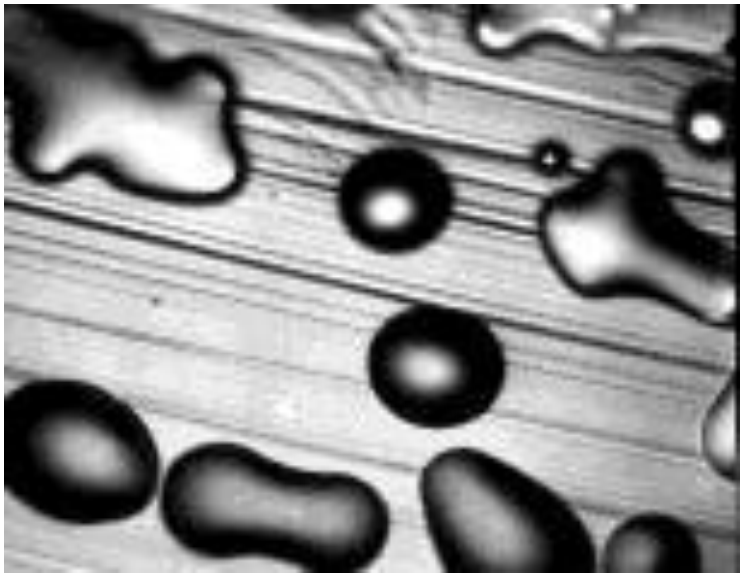
- Griffith injected mice with dead *S* bacteria – mice lived
- Griffith injected mice with heat-killed *S* bacteria-mice still lived
- Meaning the capsule was not involved with killing the mice
- He mixed harmless live *R* bacteria with the harmless heat-killed *S* bacteria-mice died
- Transformation- a change in genotype caused when cells take up foreign genetic material



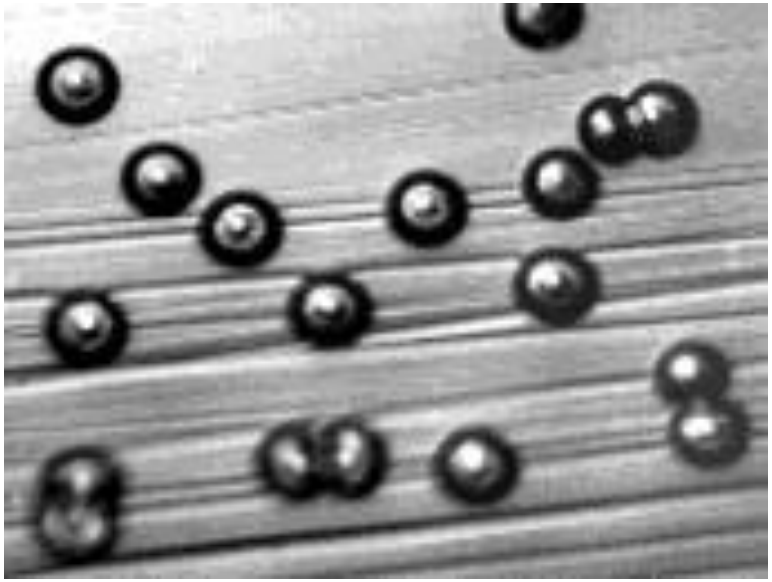
Griffith's experiment, 1928 Streptococcus pneumoniae (Figure 9.25)

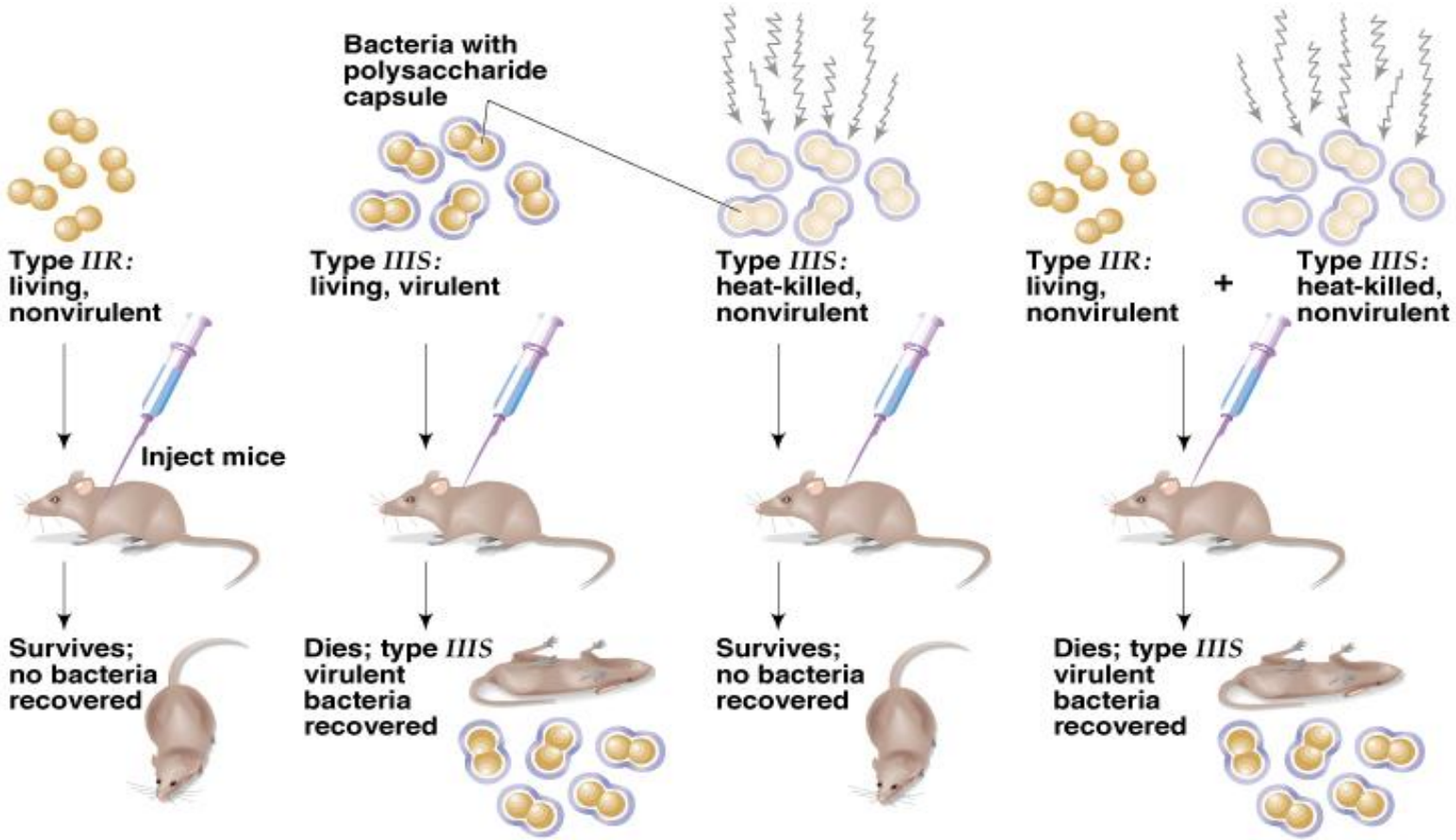
Avery el.al.'s Experiment

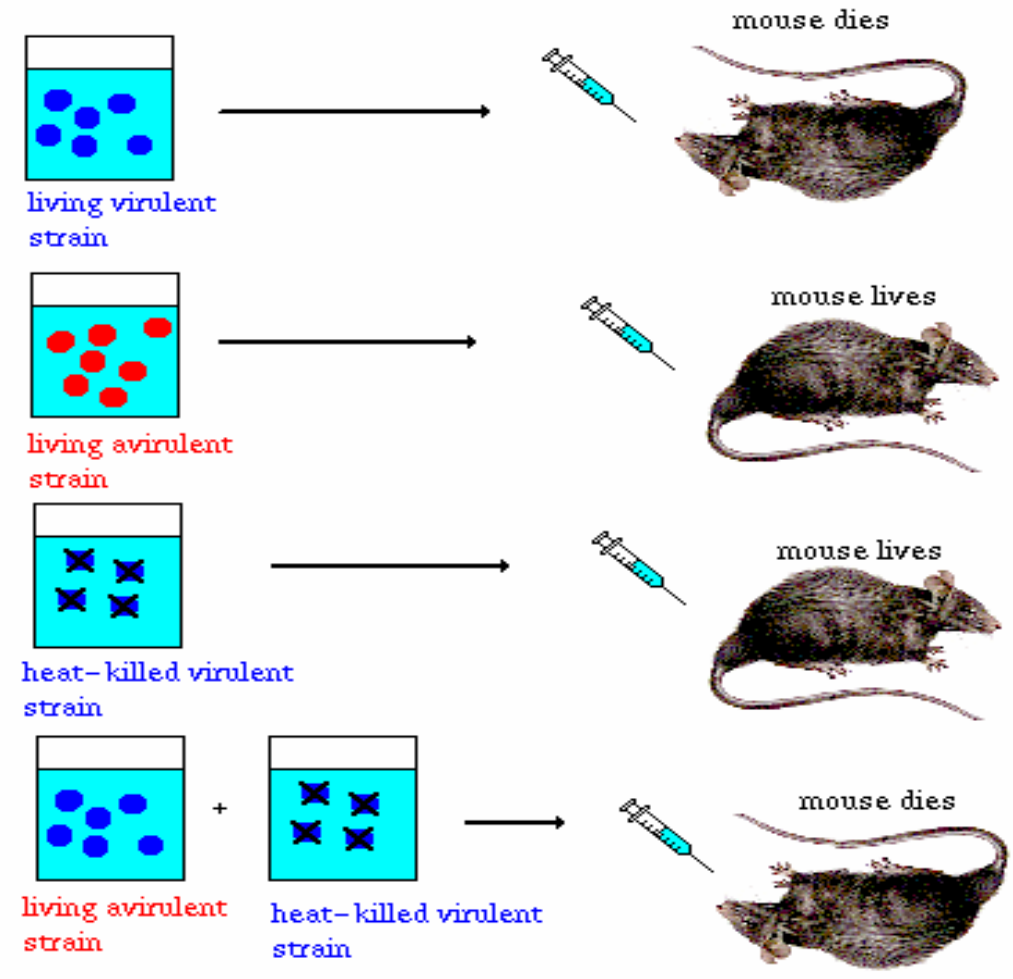
Smooth ***Streptococcus pneumoniae*** (pneumococci)



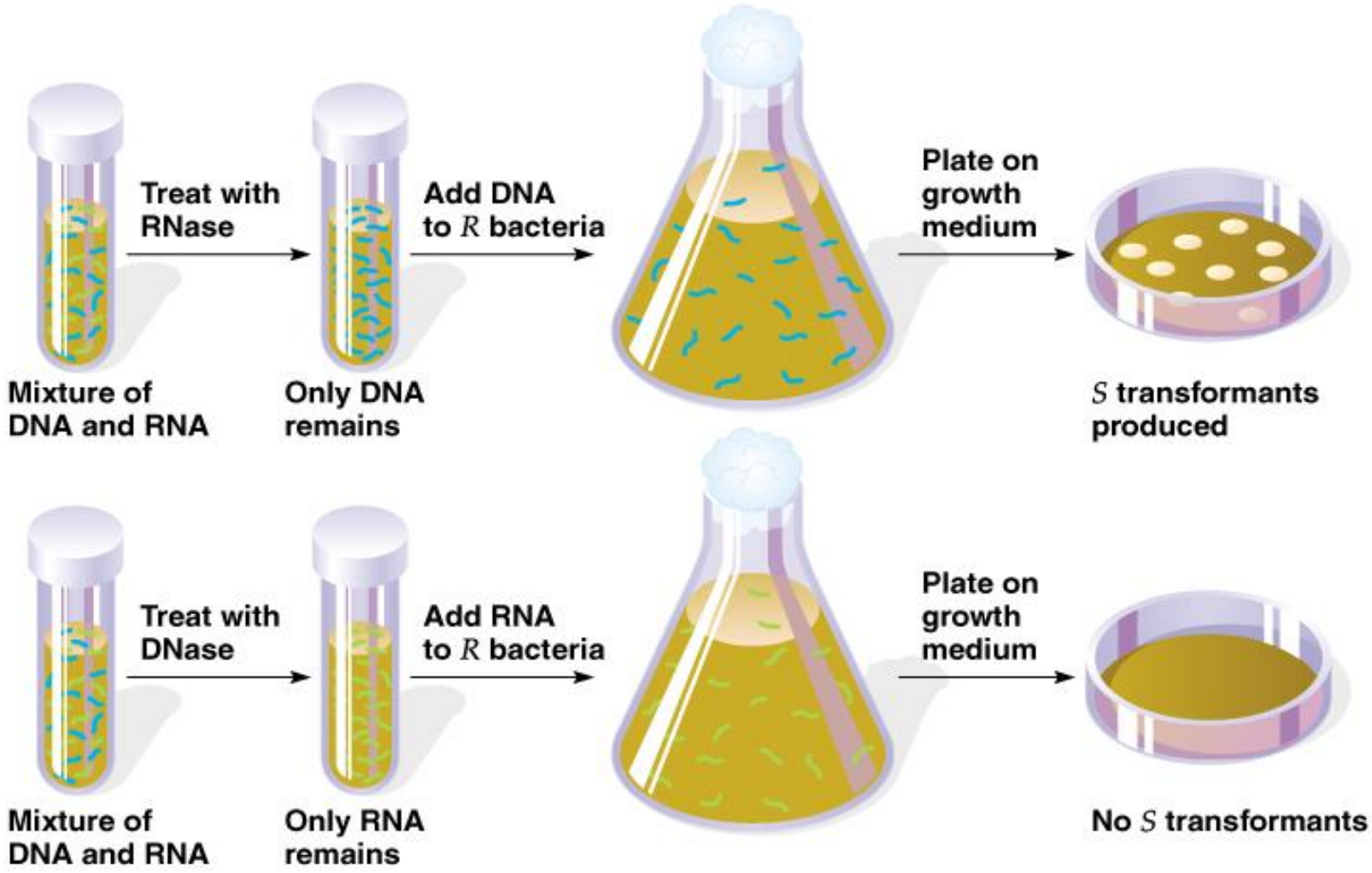
Rough ***Streptococcus pneumoniae*** (pneumococci)




Bacteria with polysaccharide capsule
Type IIR: living, nonvirulent
Type IIIS: living, virulent
Type IIIS: heat-killed, nonvirulent
Type IIR: living, nonvirulent
+
Type IIIS: heat-killed, nonvirulent
Inject mice
Survives; no bacteria recovered
Dies; type IIIS virulent bacteria recovered
Survives; no bacteria recovered
Dies; type IIIS virulent bacteria recovered



Sumber gambar :web.mit.edu/esgbio/ www/dogma/history2.html

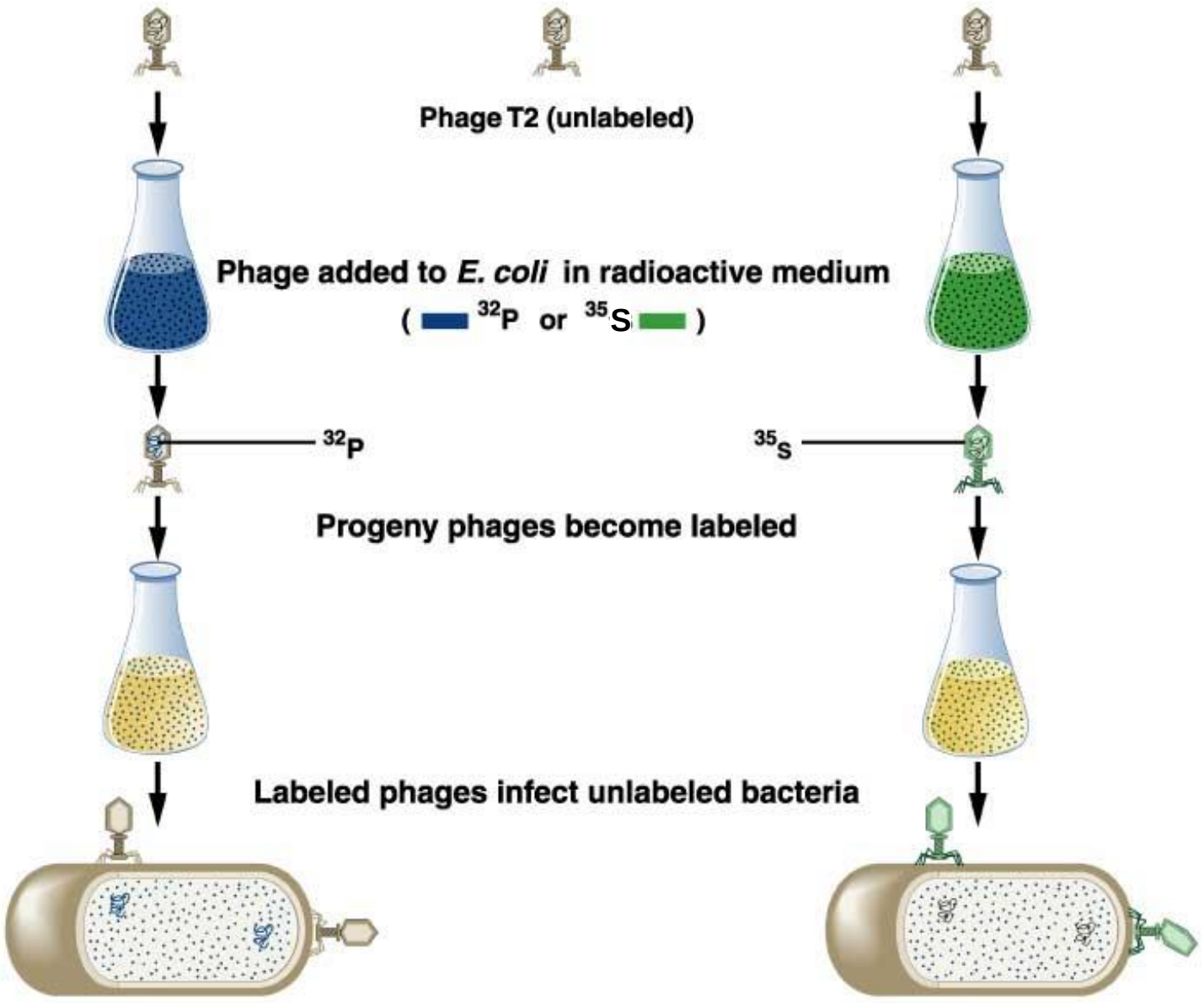


The Hershey and Chase experiment:

- Used radioisotopes to distinguish DNA from proteins
  - ^{32}P labels DNA specifically
  - ^{35}S labels protein specifically

- Infect non-radioactive *E. coli* with *r*adioactively-labeled phages
- Remove phage coats from cells
- Is ^{32}P or ^{35}S inside bacteria?

Hershey & Chase Experiment



Hershey & Chase Experiment

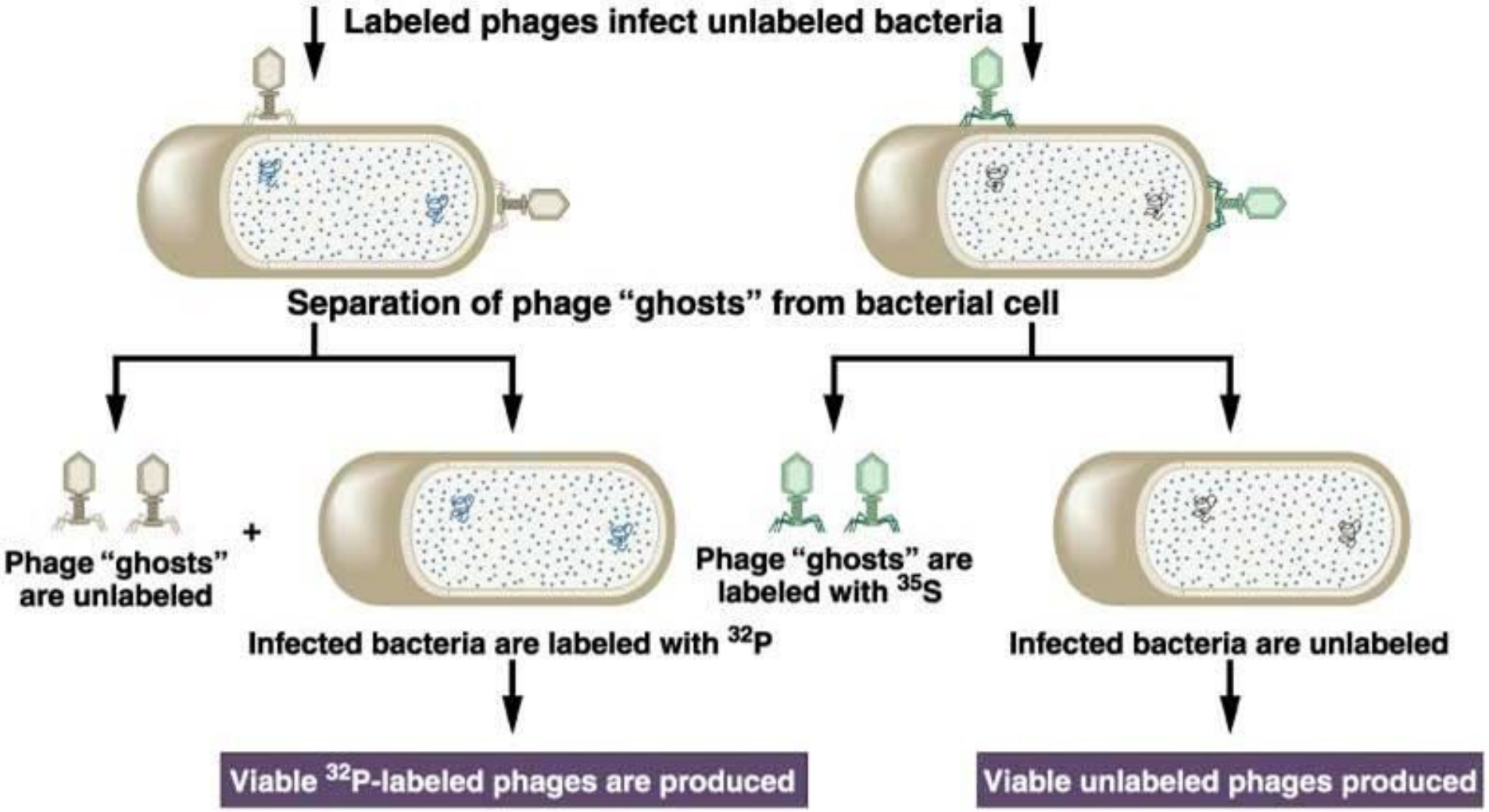


Fig. 2.6: Hershey-Chase Bacteriophage Experiment - 1953

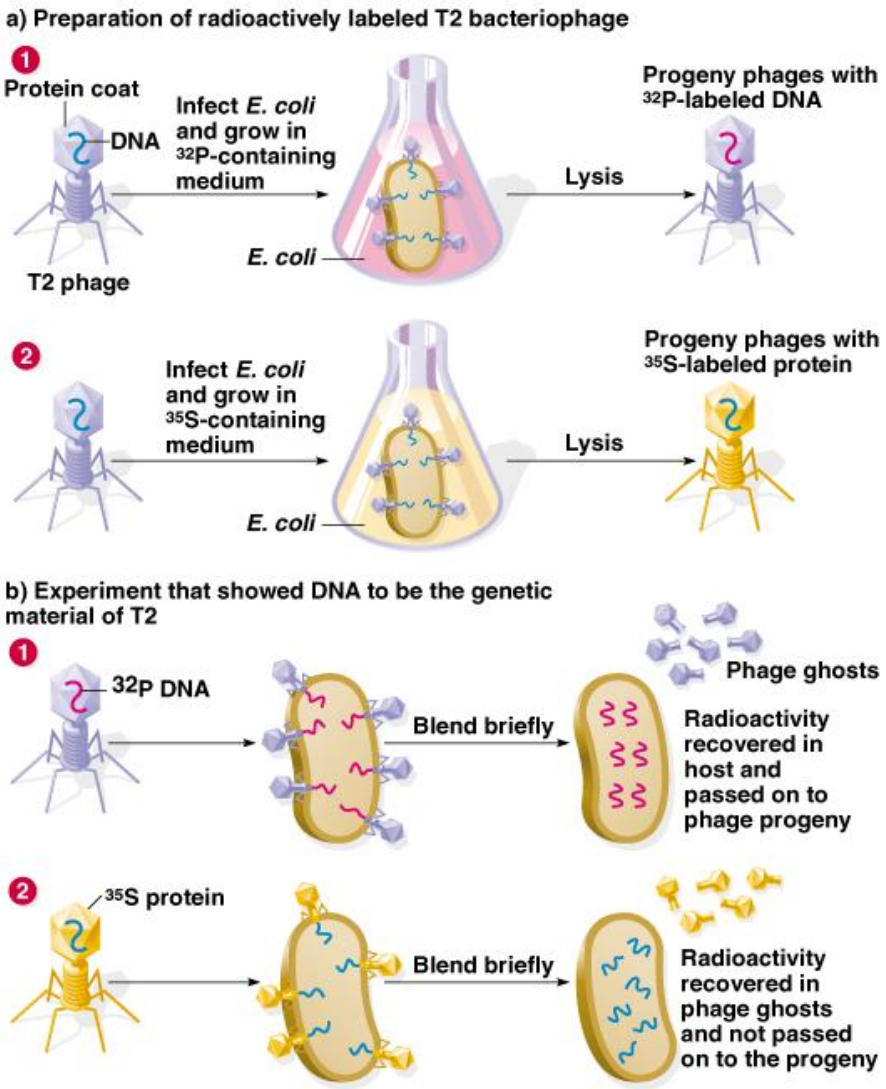
1. T2 bacteriophage is composed of DNA and proteins:

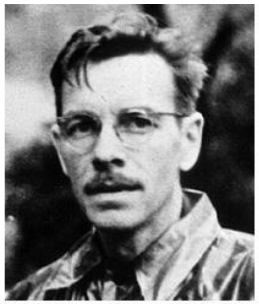
2. Set-up two replicates:

   - Label DNA with ^{32}P
   - Label Protein with ^{35}S

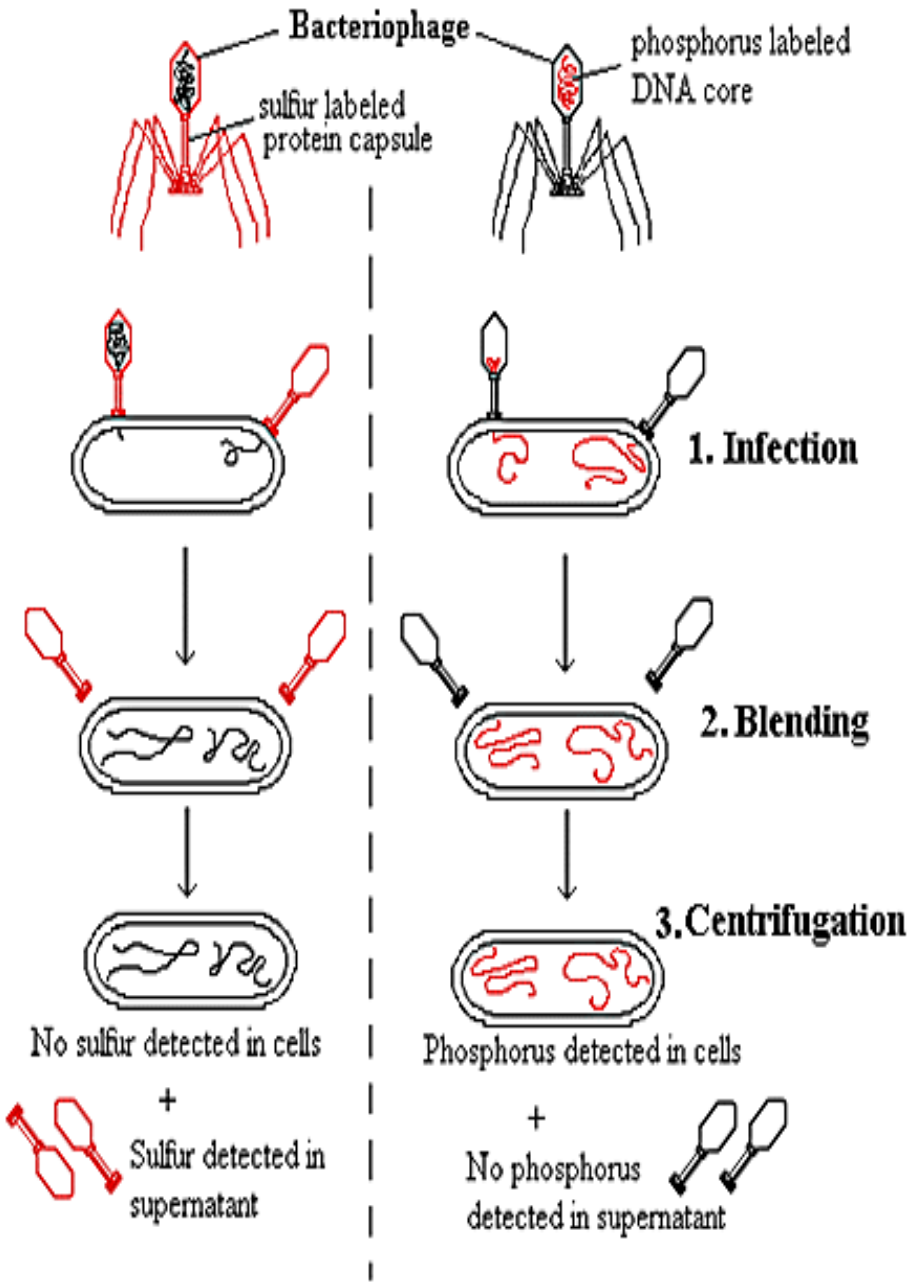
3. Infected *E. coli* bacteria with two types of labeled T2

4. ^{32}P is discovered within the bacteria and progeny phages, whereas ^{35}S is not found within the bacteria but released with phage ghosts.





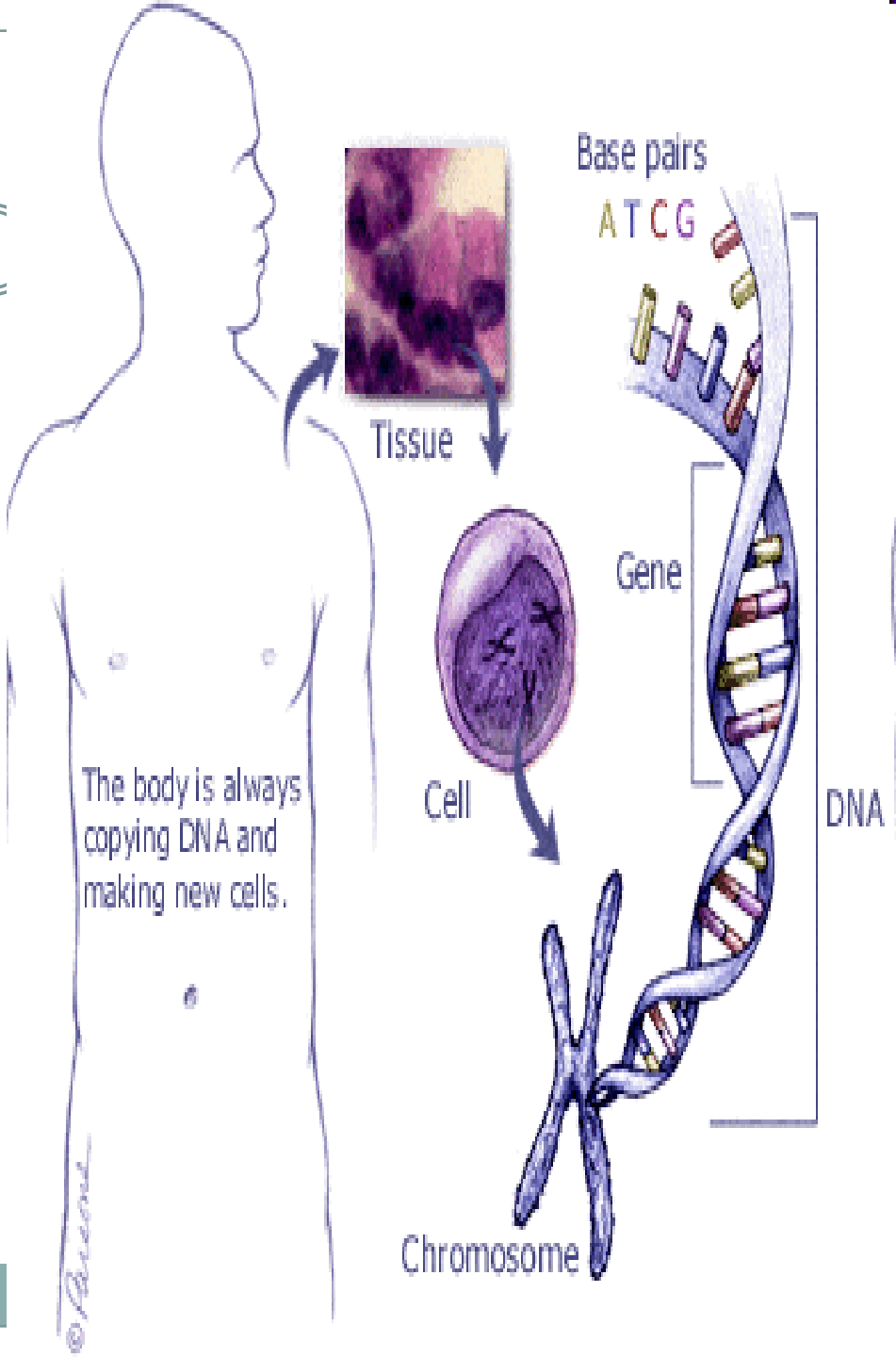
Alfred Hershey



The Hershey-Chase Experiment

- Step 1 – grew *E. coli* contained radioactive sulfur (35S) protein coat incorporated the sulfur
- Grew second batch *E. coli* with radioactive phosphorus (32P) would become part of the phages' DNA
- Step 2 Labeled phages used to infect two separate batches of *E. coli*

- Step 3 Used centrifuge tubes to separate the bacteria (heavy) from the viral parts (lighter)
- Concluded that the DNA of viruses is injected into the bacterial cells, while most of the viral proteins remain outside
- Experiments have shown that DNA is the molecule that stores genetic information in living cells

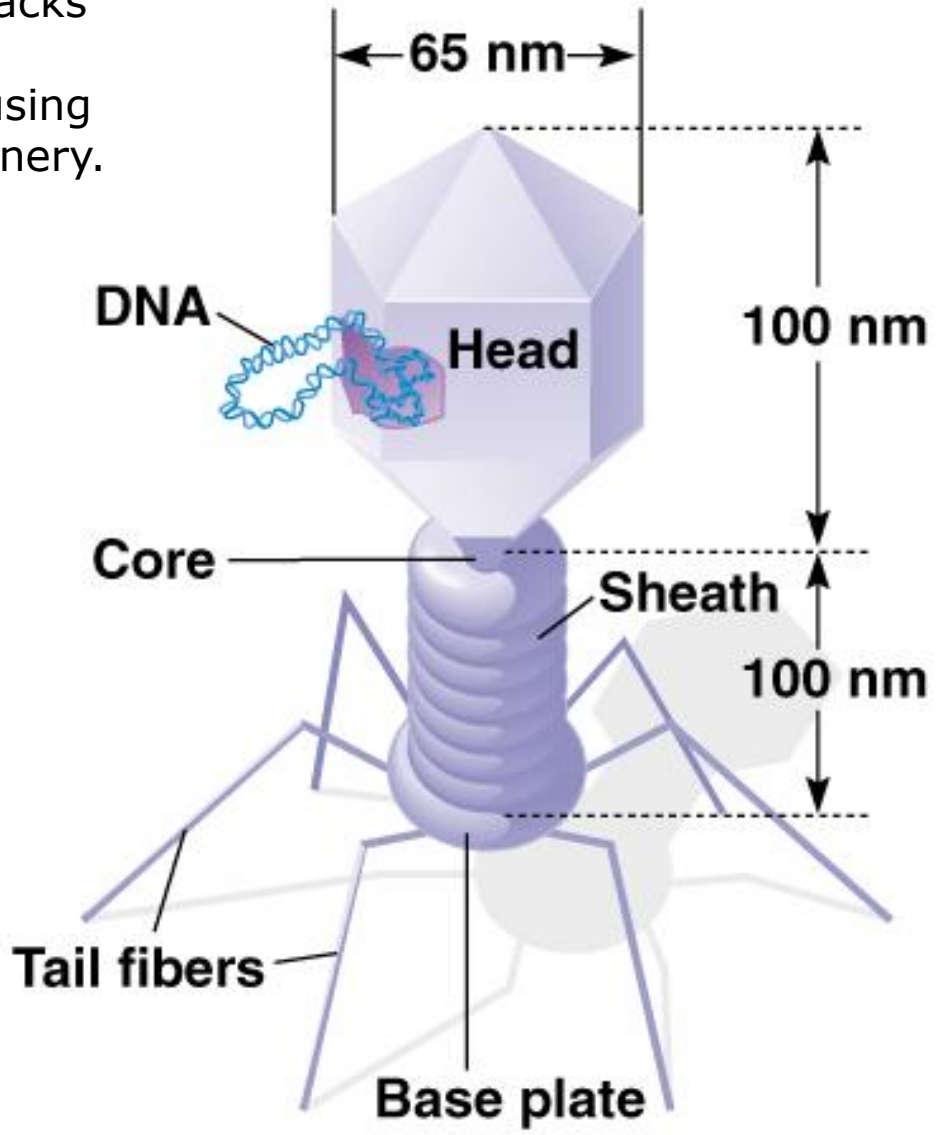


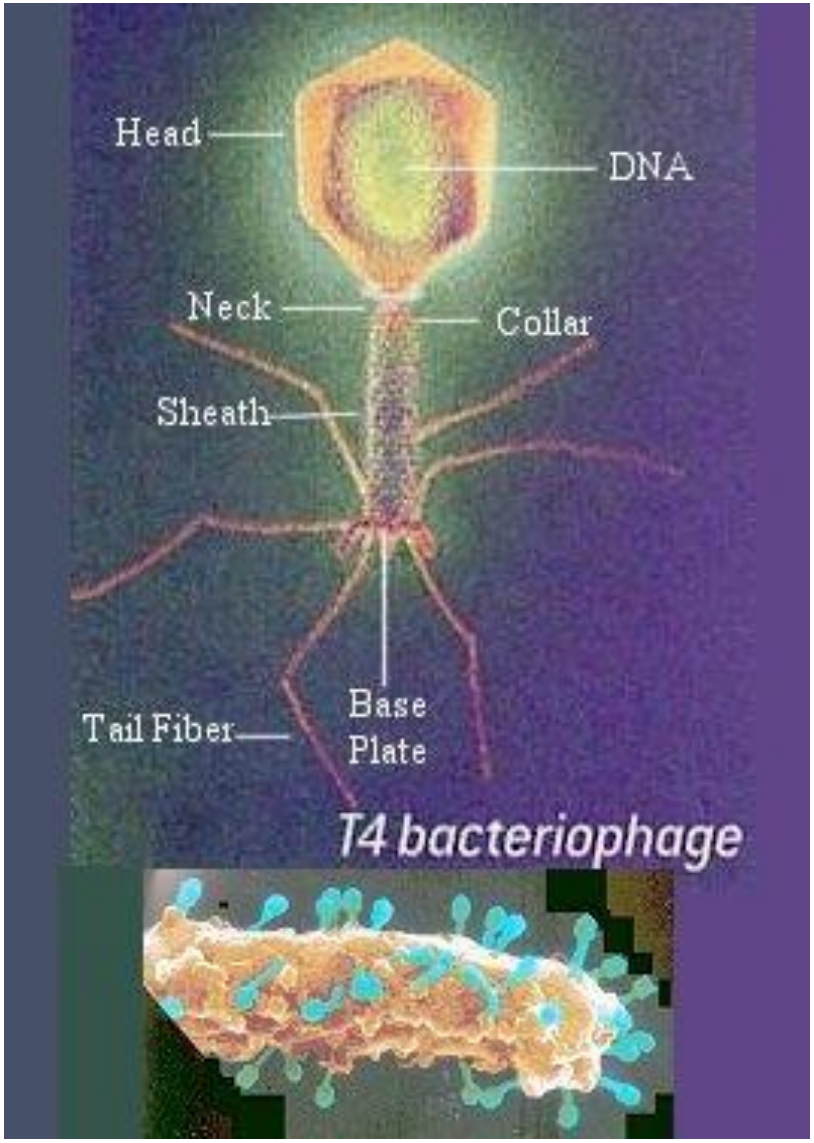
Hershey-Chase Bacteriophage Experiment - 1953

Bacteriophage = Virus that attacks bacteria and replicates by invading a living cell and using the cell's molecular machinery.

Fig. 2.4
Structure of T2 phage

Bacteriophages are composed of DNA & protein





http://universe-review.ca/I11-09-virus1.jpg

Fig. 2.7 (2nd edition)
Gierer & Schramm Tobacco Mosaic Virus (TMV) Experiment - 1956
Fraenkel-Conrat & Singer - 1957

Demonstrated that RNA is the genetic material of TMV.

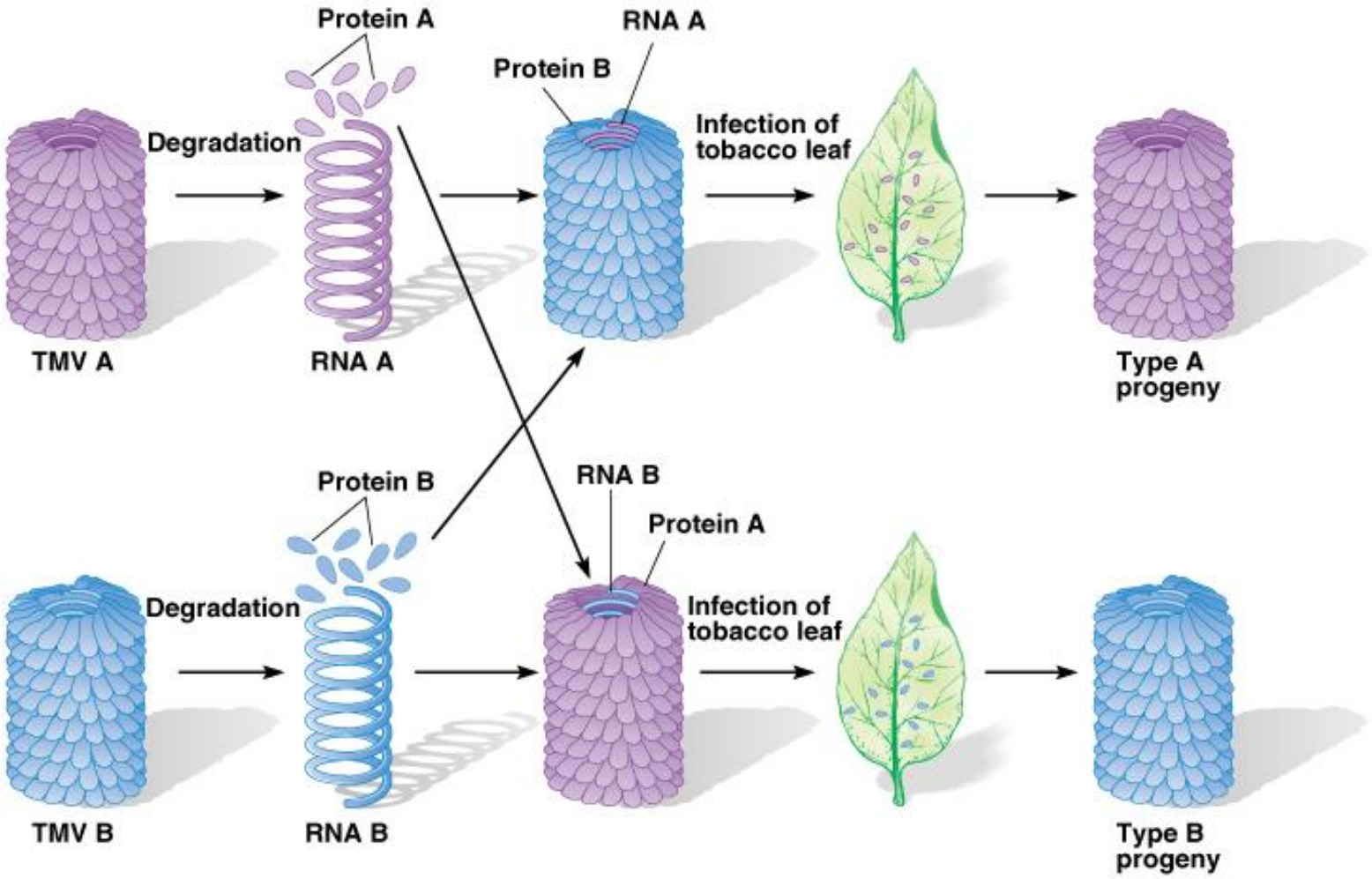


TABLE 9.1

Examples of DNA- and RNA-Containing Viruses

Virus	Host	Nucleic Acid
Tomato bushy stunt virus	Tomato	RNA
Tobacco mosaic virus	Tobacco	RNA
Influenza virus	Humans	RNA
HIV	Humans	RNA
f2	*E. coli*	RNA
Qβ	*E. coli*	RNA
Cauliflower mosaic virus	Cauliflower	DNA
Herpes virus	Humans	DNA
SV40	Primates	DNA
Epstein-Barr virus	Humans	DNA
T2	*E. coli*	DNA
M13	*E. coli*	DNA

Conclusions about these early experiments:

Griffith 1928 & Avery 1944:

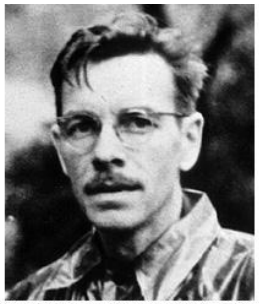
DNA (not RNA) is transforming agent.

Hershey-Chase 1953:

DNA (not protein) is the genetic material.

Gierer & Schramm 1956/Fraenkel-Conrat & Singer 1957:

RNA (not protein) is genetic material of some viruses, but no known prokaryotes or eukaryotes use RNA as their genetic material.



Alfred Hershey
Nobel Prize in Physiology or Medicine
1969

Confirmation for DNA as the genetic material

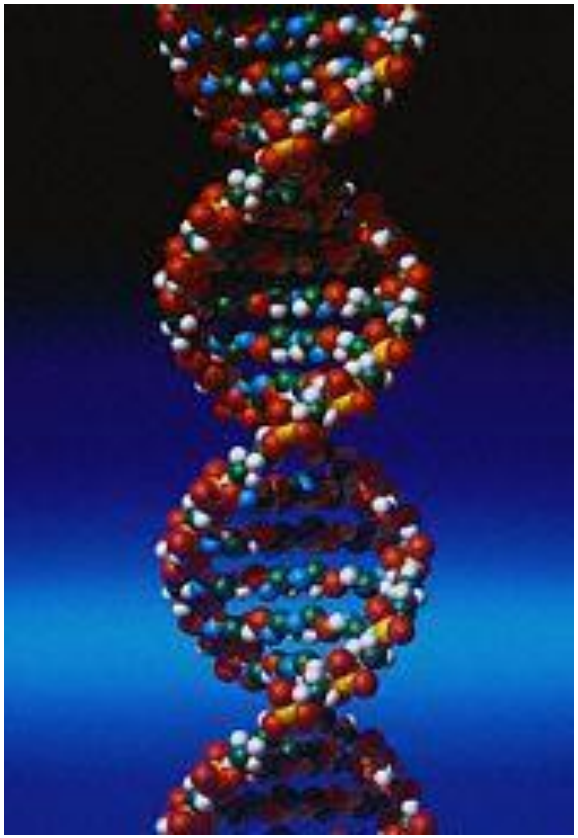
- In the 1940s geneticists doubted the use of DNA as the genetic material as it appeared to be monotonous repeats of 4 bases
- By 1953 Watson & Crick published the double-helical model of DNA structure and Chargaff demonstrated that the 4 bases were not present in equal proportions
- In 1952 Hershey and Chase demonstrated that bacteriophage infection comes from DNA, adding more evidence to support that DNA is the genetic material


STRUKTUR DNA

Why do we study DNA?

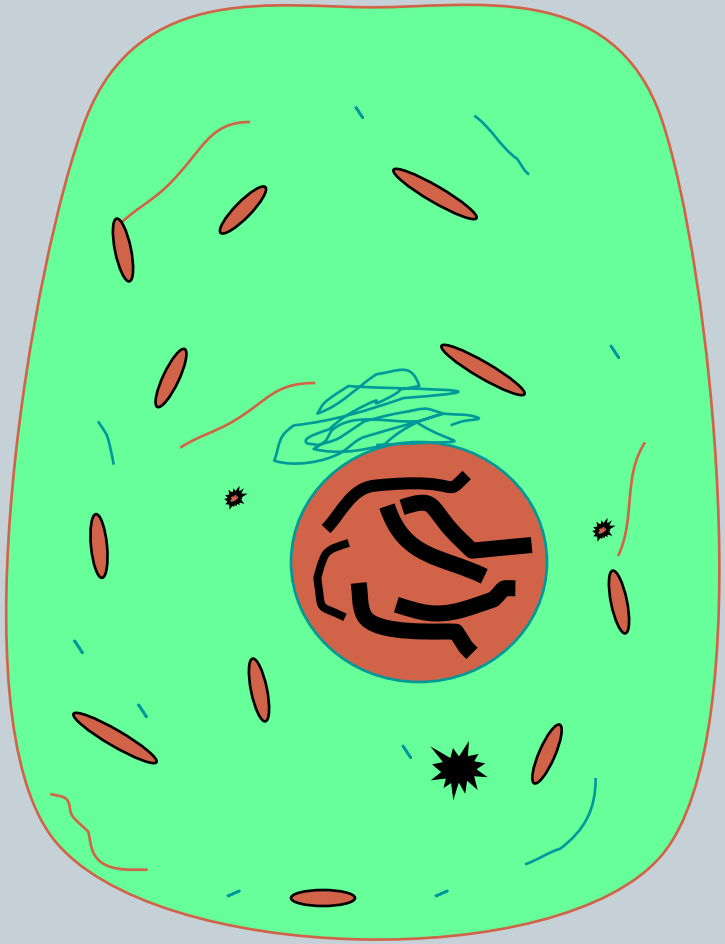
We study DNA for many reasons, e.g.,

- its central importance to all life on Earth,
- medical benefits such as cures for diseases,
- better food crops.



Chromosomes and DNA

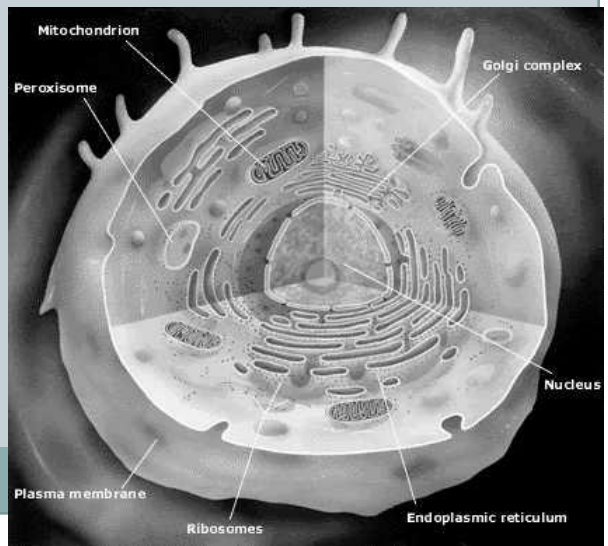
- Our genes are on our chromosomes.
- Chromosomes are made up of a chemical called DNA.



D.N.A. Function

- 1. D.N.A. can make a copy of itself, handy during?
- Mitosis & meiosis
- 2. D.N.A. contains the code for protein synthesis, the manufacture of proteins
- Problem, where does protein synthesis take place?
- Ribosomes, located? Outside the nucleus. D.N.A. can't leave the nucleus.

  So how does this get done?

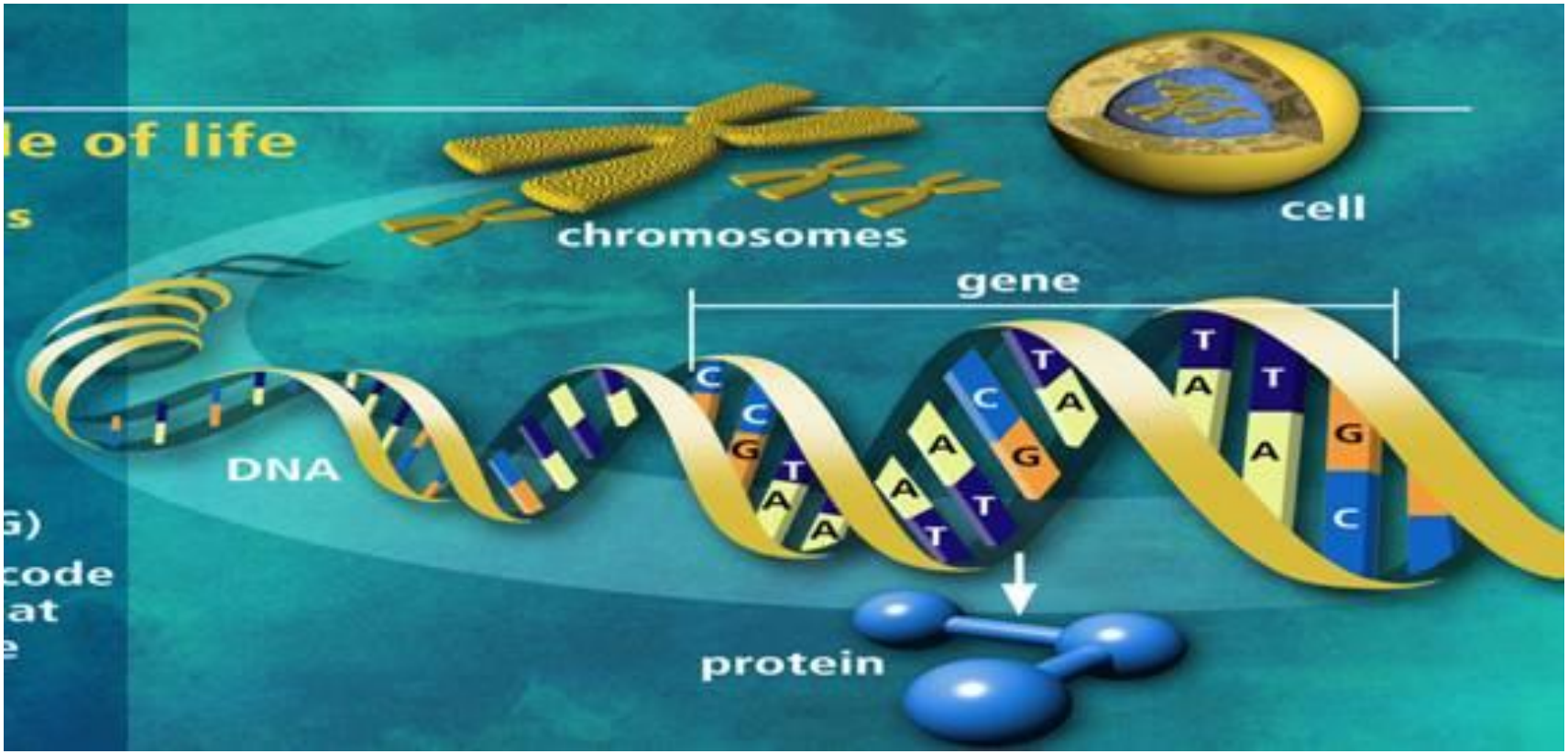


DNA

- DNA (Deoksi Nukleotida Acid)
  1. terdiri dari 2 Untai polinukleotida yang membentuk Double Heliks
  2. Dua untai polinokleotida dengan arah yang berlawanan polar (antiparalel) yaitu 5'-3' dengan 3'-5"
  3. Antar nukleotida membentuk ikatan fosfodiester
  4. Antar basa nitrogen membentuk ikatan hidrogen

  G dan C : 3 ikatan Hidrogen
  A dan T : 2 ikatan Hidrogen

- Fungsi DNA :
- Replikasi DNA sangat berperanan pada kelangsungan hidup organisme
- Pada proses transkripsi berperanan sebagai cetakan/templet untuk membentuk RNA
- Menyimpan dan mentransfer informasi genetik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chromosomes
cell
gene
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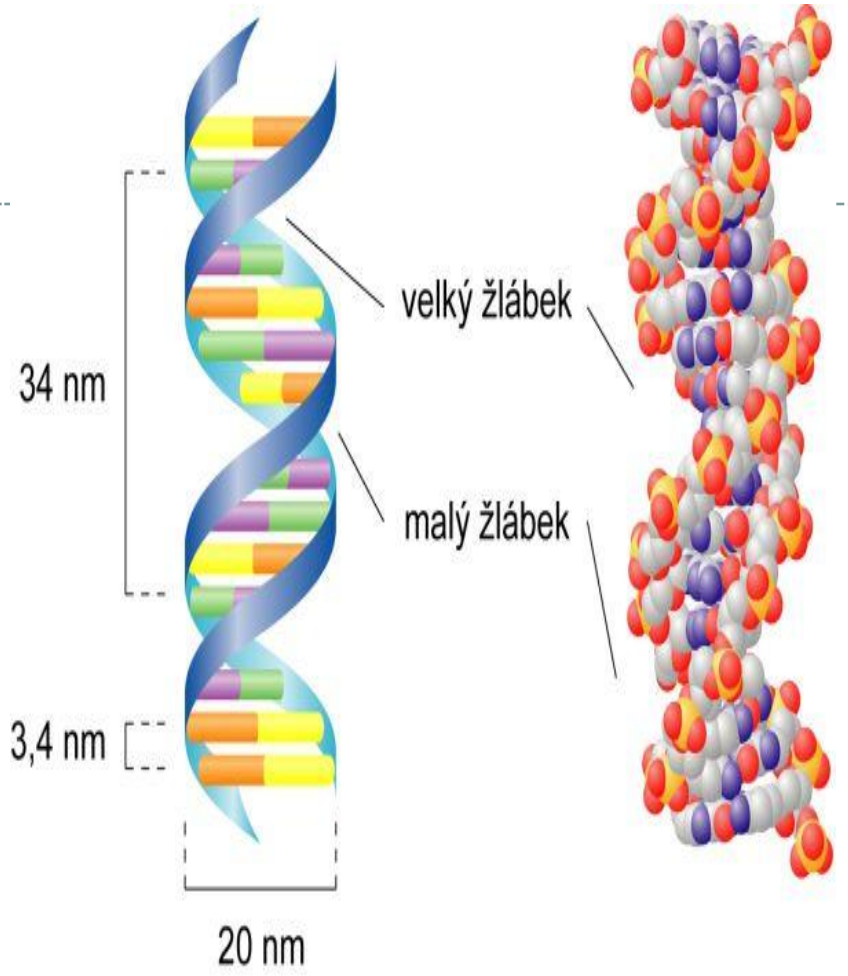
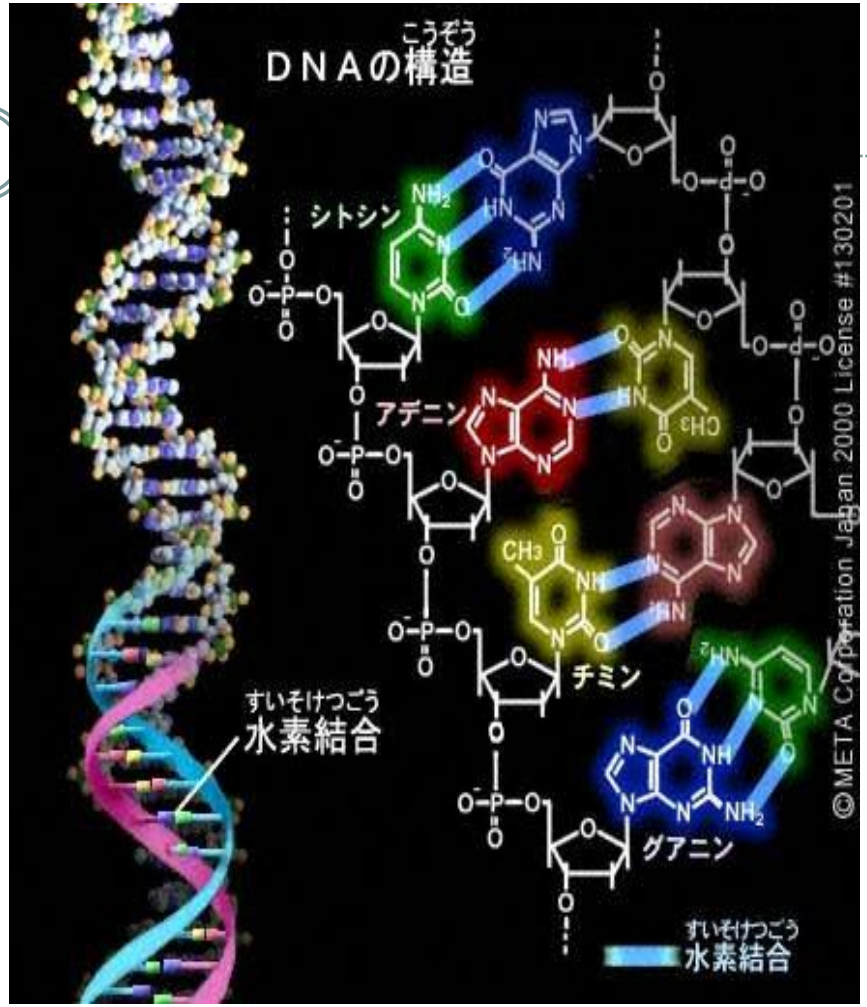
protein

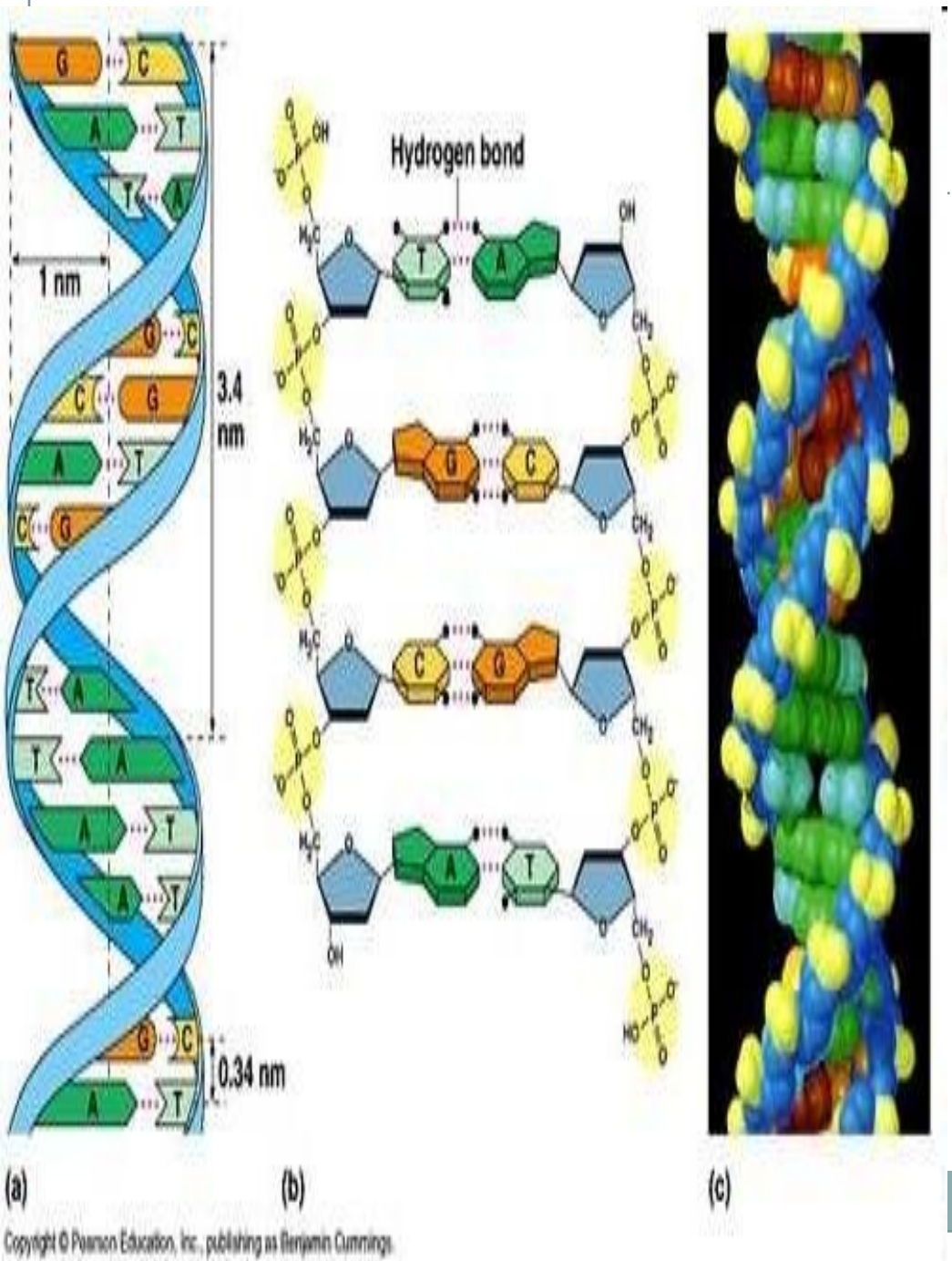




Double Helix DNA

:www.vscht.cz/.../ motor/index.obrazky.html

http://hinyoukika.cocolog-nifty.com/tlz/DNA.jpg

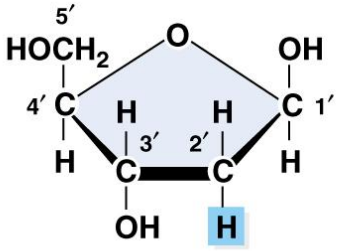


- Watson & Crick determined that a DNA molecule is a <u>double helix</u> – two strands twisted around each other
- Nucleotides – the subunits that make up DNA
- 3 parts: a phosphate group, a 5-carbon sugar, and a nitrogen-containing base

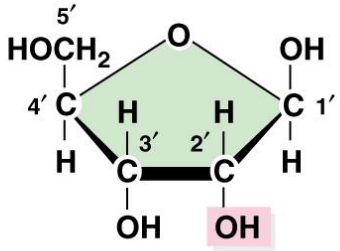
Nucleotide = monomers that make up DNA and RNA (Figs. 2.8)

Three components

1. Pentose (5-carbon) sugar
- **DNA = deoxyribose**
- **RNA = ribose**
- **(compare 2' carbons)**



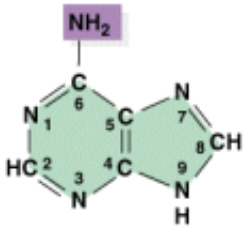
Deoxyribose



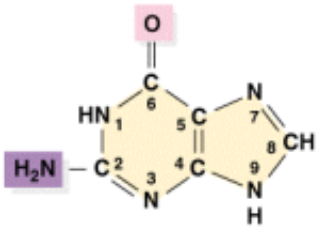
Ribose

2. Nitrogenous base

Purines (2 rings)
- **Adenine**
- **Guanine**

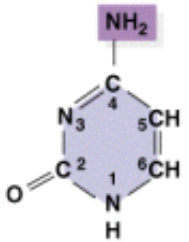


Adenine (A)

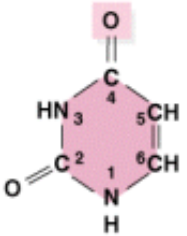


Guanine (G)

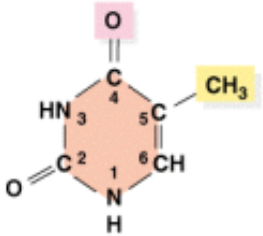
Pyrimidines (1 ring)
- **Cytosine**
- **Thymine (DNA)**
- **Uracil (RNA)**



Cytosine (C)

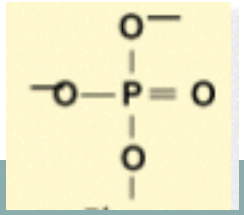


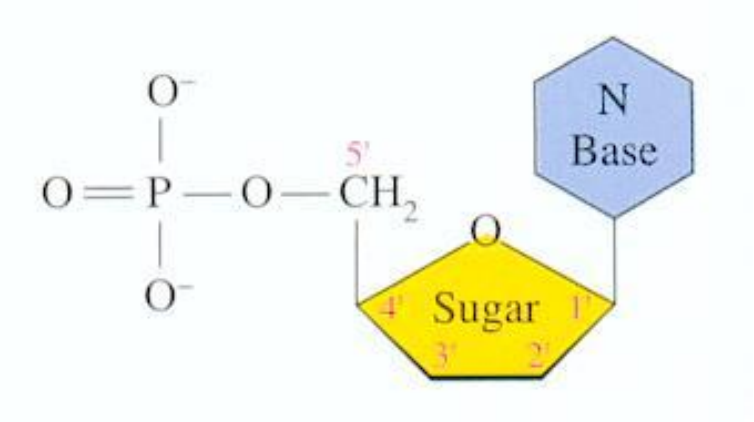
Uracil (U)
(found in RNA)



Thymine (T)
(found in DNA)

3. Phosphate group attached to 5' carbon





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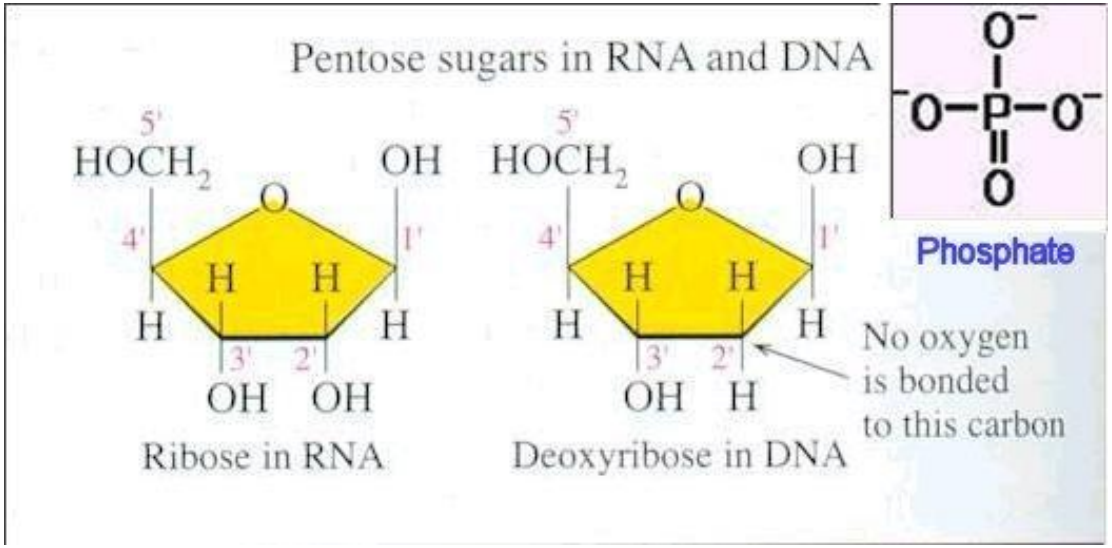

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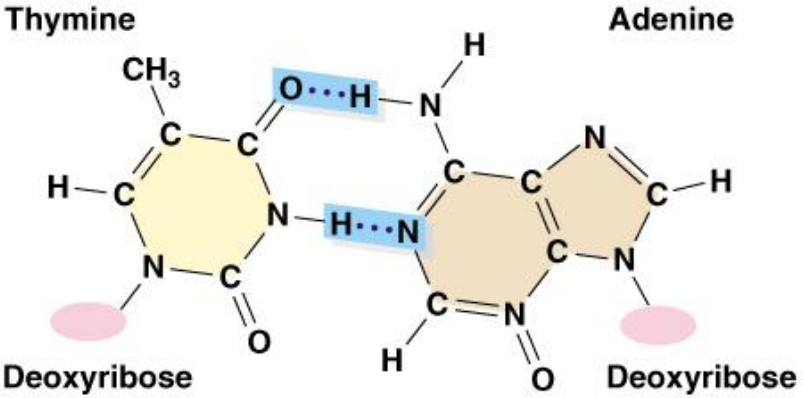
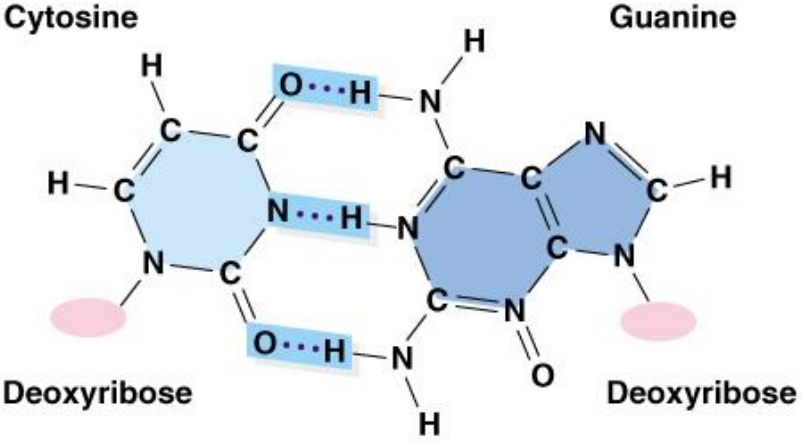
Fig. 2.13

a) Adenine-thymine base
(Double hydrogen bond)



b) Guanine-cytosine base
(Triple hydrogen bond)



DNA mempunyai komposisi basa yang khas

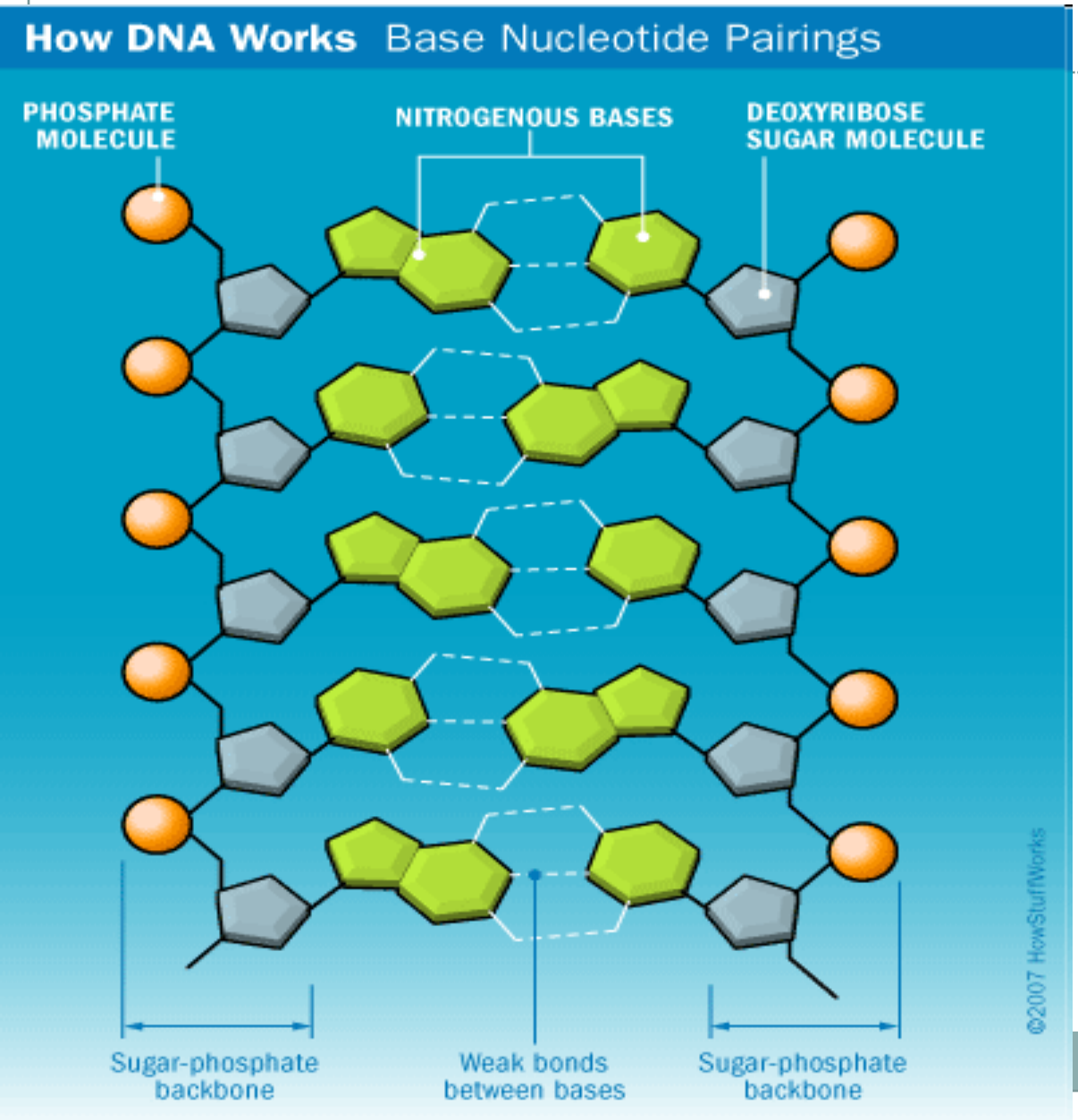
- Potongan DNA dr jaringan species yang sama, mempunyai komposisi yang sama
- Komponen basa DNA bervariasi dari spesies ke spesies yang lain
- Komponen DNA tidak berubah dengan bertambahnya umur dan perubahan lingkungan
- Jumlah Basa A=T dan Basa C=G

- **Structure of DNA**

- **Base composition studies of Erwin Chargaff**
  - indicated double-stranded DNA consists of ~50% purines (A,G) and ~50% pyrimidines (T, C)
  - amount of A = amount of T and amount of G = amount of C
  - (Chargraff's rules)
  - %GC content varies from organism to organism

Examples:	**%A**	**%T**	**%G**	**%C**	**%GC**
Homo sapiens	31.0	31.5	19.1	18.4	37.5
Zea mays	25.6	25.3	24.5	24.6	49.1
Drosophila	27.3	27.6	22.5	22.5	45.0
Aythya americana	25.8	25.8	24.2	24.2	48.4

Discovering DNA's Structure



- Chargaff's 1949 observations – the amount of adenine always equaled the amount of thymine; amount of guanine always equaled the amount of cytosine; but amount varied between different organisms

STRUKTUR DNA

Setiap utas DNA : **nukleotida --- rantai polinukleotida**
Nukleotida yang satu dengan nukleotida yang lain dihubungkan oleh ikatan **fosfodiester**

Fosfodiester : setiap gugusan fosfat menghubungkan atom C nomor 3 pada deoksiribosa sebuah nukleotida dengan atom C nomor 5 pada deoksiribosa nukleotida yang berikutnya.

Nucleotides are linked by phosphodiester bonds to form polynucleotides.

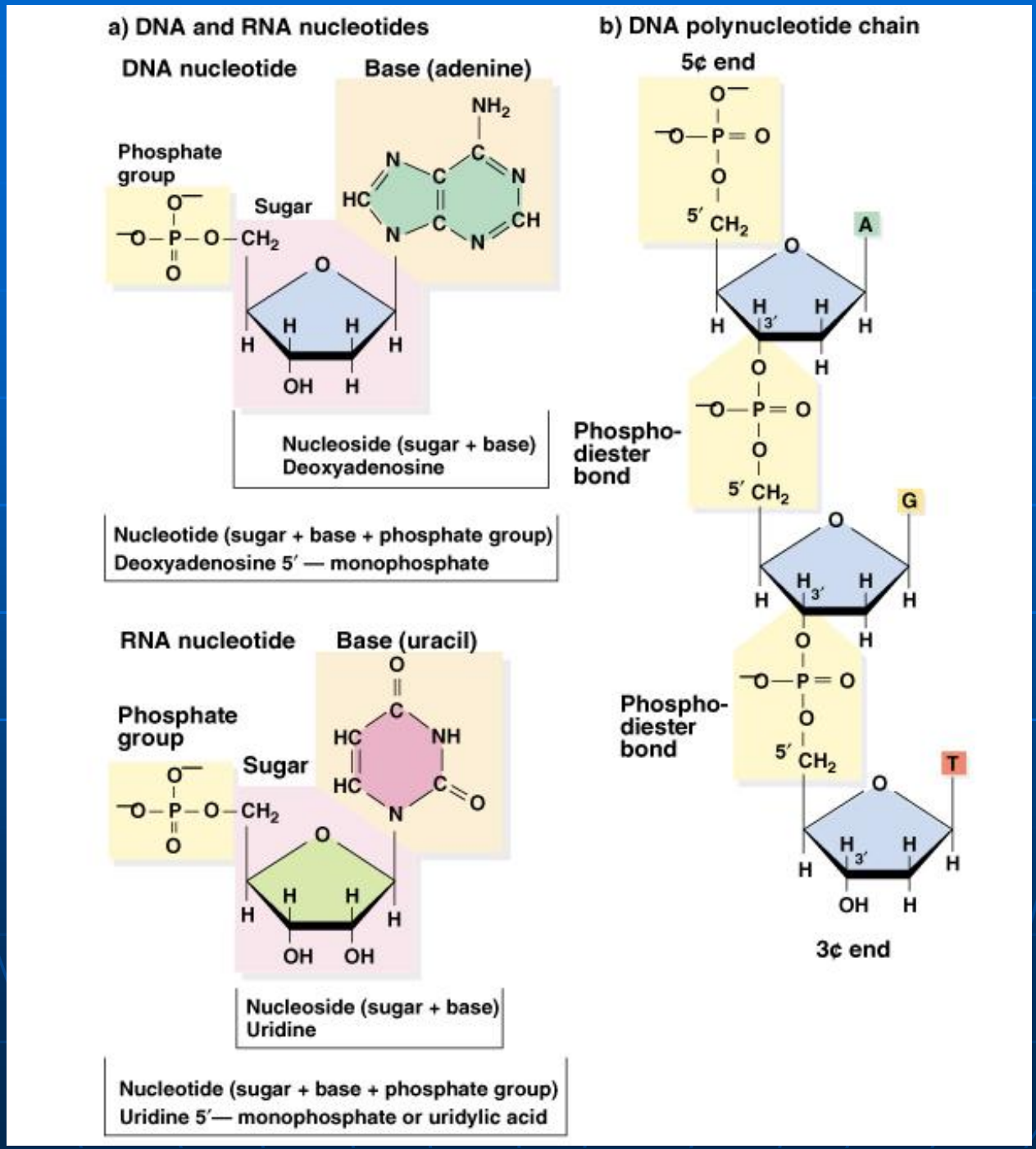
Phosphodiester bond

Covalent bond between the phosphate group (attached to 5' carbon) of one nucleotide and the 3' carbon of the sugar of another nucleotide.

This bond is very strong, and for this reason DNA is remarkably stable. DNA can be boiled and even autoclaved without degrading!

5' and 3'

The ends of the DNA or RNA chain are not the same. One end of the chain has a 5' carbon and the other end has a 3' carbon.

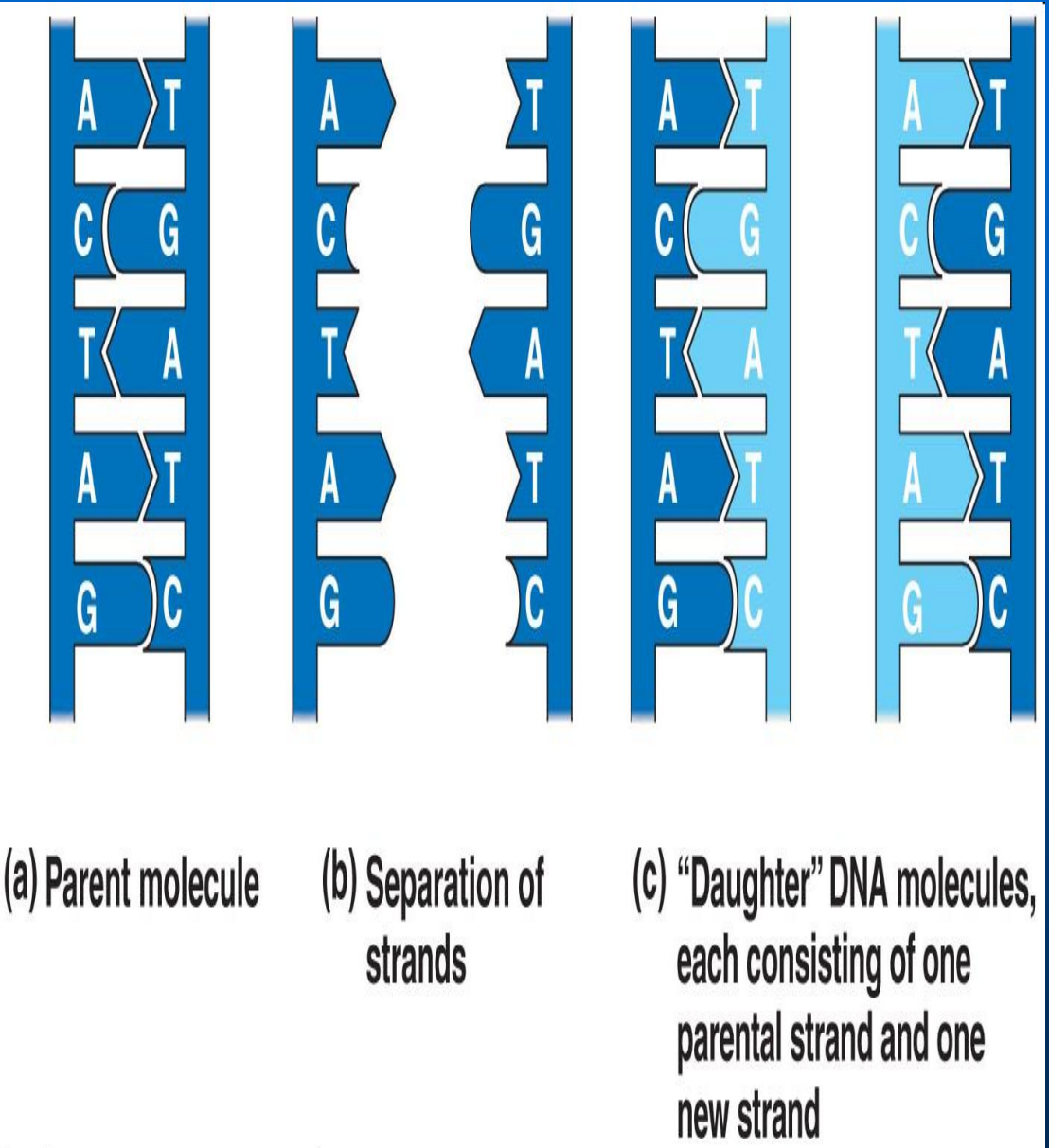

a) DNA and RNA nucleotides
DNA nucleotide
Base (adenine)
Phosphate group
Sugar
Nucleoside (sugar + base)
Deoxyadenosine
Nucleotide (sugar + base + phosphate group)
Deoxyadenosine 5′ — monophosphate
RNA nucleotide
Base (uracil)
Phosphate group
Sugar
Nucleoside (sugar + base)
Uridine
Nucleotide (sugar + base + phosphate group)
Uridine 5′— monophosphate or uridylic acid
b) DNA polynucleotide chain
5¢ end
Phospho-diester bond
Phospho-diester bond
3¢ end

STRUKTUR DNA

Basa dari satu rantai dengan basa rantai yang lain dihubungkan oleh ikatan hidrogen, A T : 2 H dan G C : 3 H Bua basa yang berhubungan dengan cara demikian disebut pasangan basa **komplementer**

Kedua utas heliks DNA bersifat **antiparalel** : setiap utas menuju arah yang berlawanan sehingga yang satu diakhiri dengan gugusan hidroksil 3 ` bebas dan lainnya dengan gugusan fosfat 5 `. Atau satu utas menjulur dari ujung 3 ` ke ujung 5 ` dan utasan lainnya dari ujung 5 ` ke ujung 3 `.

Pairing Between Bases

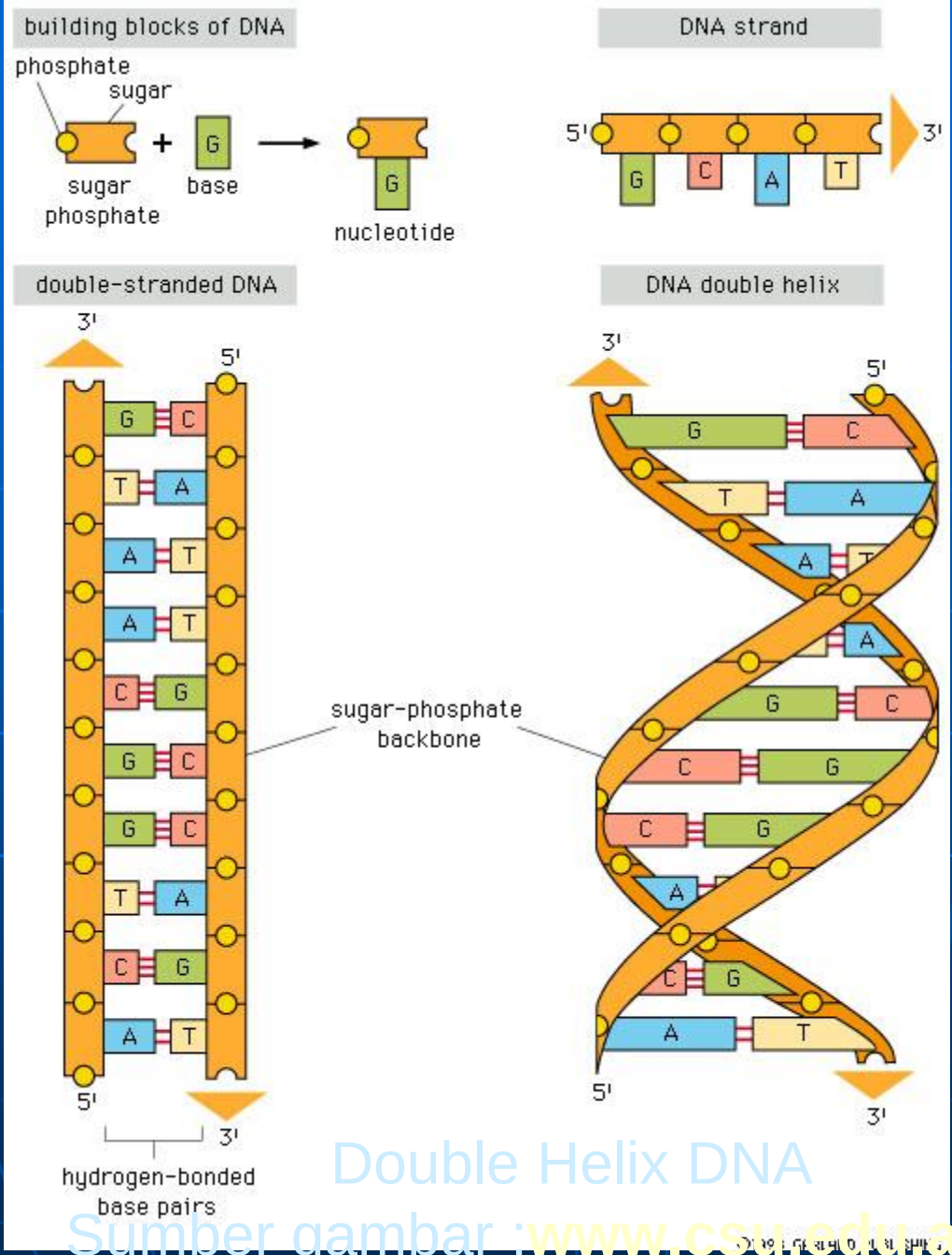


(a) Parent molecule

(b) Separation of strands

(c) "Daughter" DNA molecules, each consisting of one parental strand and one new strand

- Watson & Crick determined that a purine on one strand of DNA is always paired with a pyrimidine on the opposite strand
- Base-pairing rule – cytosine pairs with guanine and adenine with thymine
- Complementary base pairs – sequence of bases on strand determines the sequence of N bases on the other strand of DNA



Double Helix DNA

Sumber gambar :www.csu.edu.au/.../subjects/molbol/revision.htm

DNA Eukariot dan Prokariot

DNA EUKARIOT :

- Terdapat di nukleus, mitokondria dan kloroplast (pada tumbuhan dan alga)
- DNA pada kloroplas dan mitokondria hanya menyandikan protein untuk dirinya sendiri (kepentingan sendiri)

DNA PROKARIOT :

- Terdapat DNA kromosom dan plasmid
- Plasmid hanya menyandikan protein yang tidak terlalu penting untuk pertumbuhan sel, dapat digunakan untuk transfer DNA

- DNA manusia lk 2 m panjangnya, diameter nukleus lk 6 uM
- Jadi bagaimana DNA dikemas dalam nukleus yang sangat kecil?

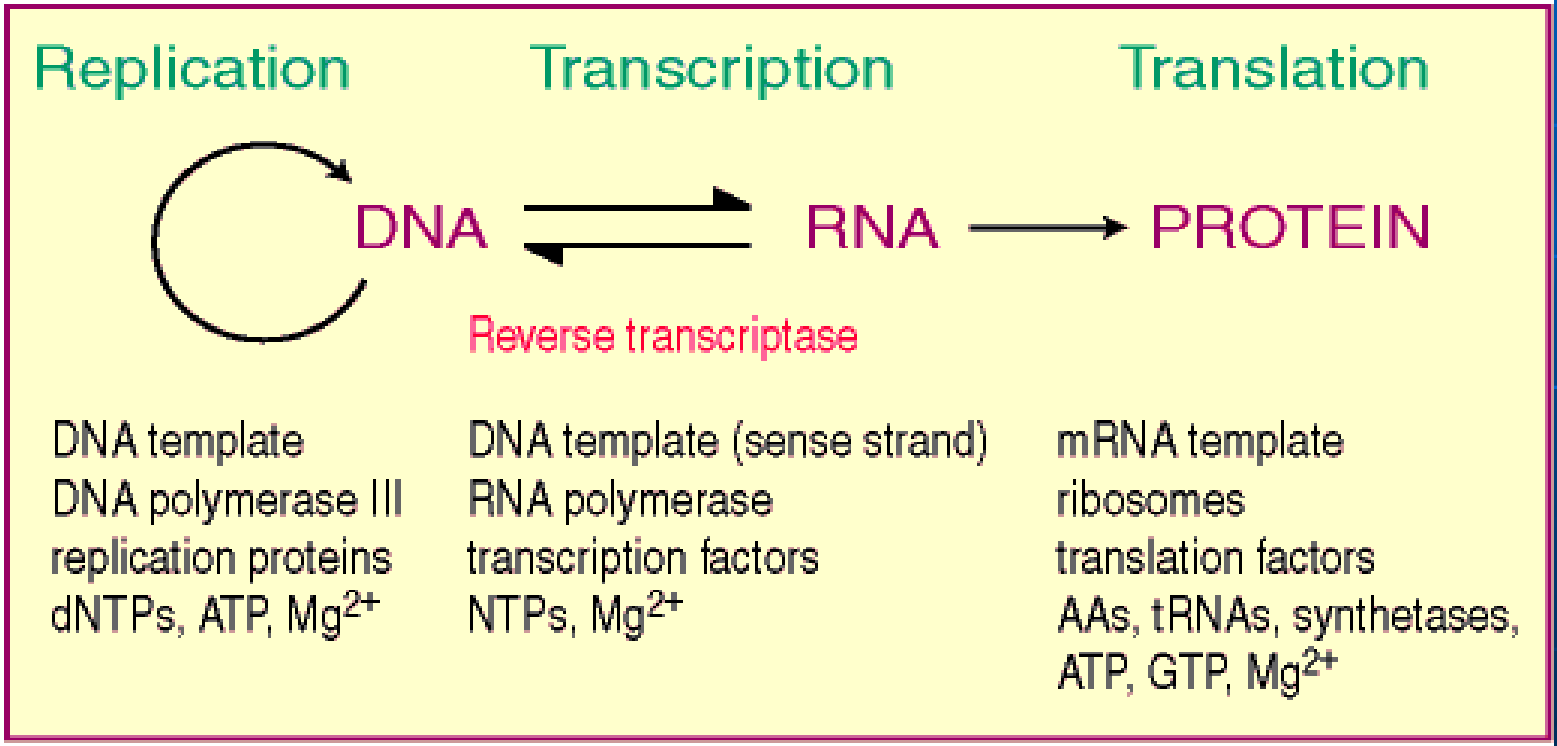
DNA by the Numbers

- Each cell has about 2 m of DNA.
- The average human has 75 trillion cells.
- The average human has enough DNA to go from the earth to the sun more than 400 times.
- DNA has a diameter of only 0.000000002 m.

The earth is 150 billion m or 93 million miles from the sun.

BACTERIAL GENETICS

I. CENTRAL DOGMA



http://web.indstate.edu/thcme/micro/bactGen/Bacgenet.htm

ORGANISASI DNA DALAM KROMOSOM

- Unit struktural dasar dari kromosom eukariot adalah nukleosom. Nukleosom tersusun atas DNA dan protein histon. Ada lima macam protein histon yaitu : H1, H2A, H2B,H3,H4

- DNA melingkar mengelilingi oktamer histon (H2A, H2B,H3,H4 masing-masing 2 molekul) dan sebagai pengunci adalah histon H1.

- Protein histon adalah protein sangat basa mengandung asam amino basa arginin dan lisin.

  Fungsi histon : memelihara integritas fungsi dan struktur kromatin

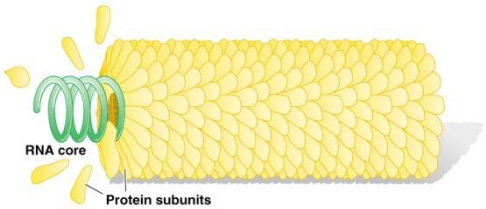
Organization of DNA/RNA in chromosomes

Genome = chromosome or set of chromosomes that contains all the DNA an organism (or organelle) possesses

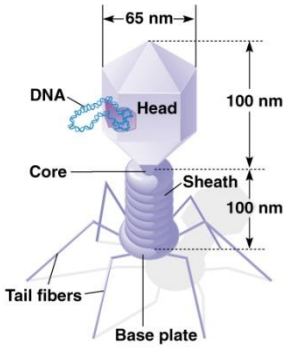
Viral chromosomes

1. **single or double-stranded DNA or RNA**
2. **circular or linear**
3. **surrounded by proteins**

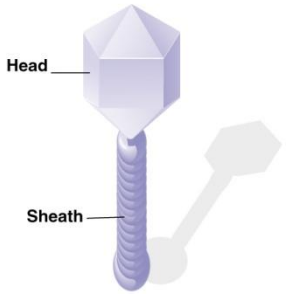
TMV



T2 bacteriophage



λ bacteriophage



Prokaryotic chromosomes

1. **most contain one double-stranded circular DNA chromosome**
2. **others consist of one or more chromosomes and are either circular or linear**
3. **typically arranged in arranged in a dense clump in a region called the nucleoid**

Problem:

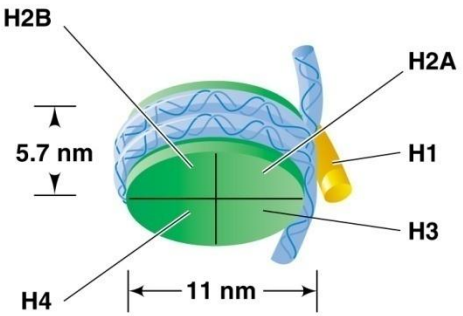
Measured linearly, the *Escherichia coli* genome (4.6 Mb) would be 1,000 times longer than the *E. coli* cell.

The human genome (3.4 Gb) would be 2.3 m long if stretched linearly.

Fig. 2.15
Chromosome released from lysed *E. coli* cell.

Packing of DNA into chromosomes:

1. **Level 1** — **Winding of DNA around histones to create a nucleosome structure.**

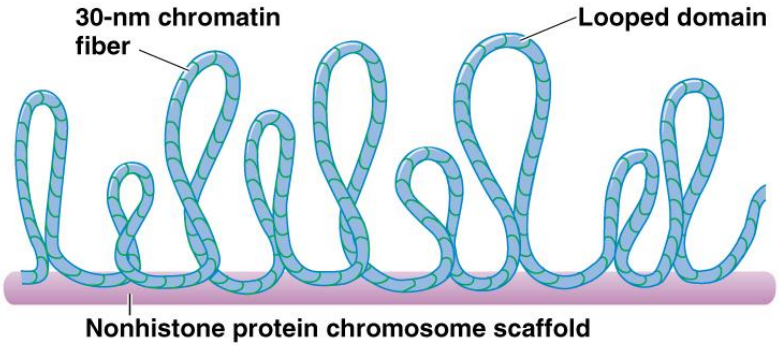


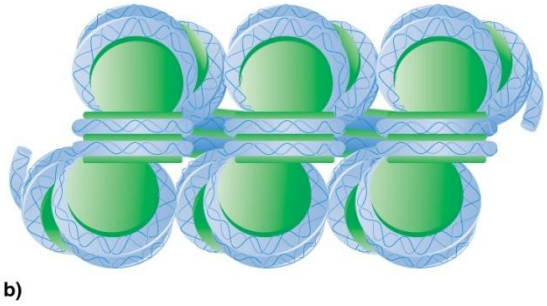
2. **Level 2** — **Nucleosomes connected by strands of linker DNA like beads on a string.**

3. **Level 3** — **Packaging of nucleosomes into 30-nm chromatin fiber.**

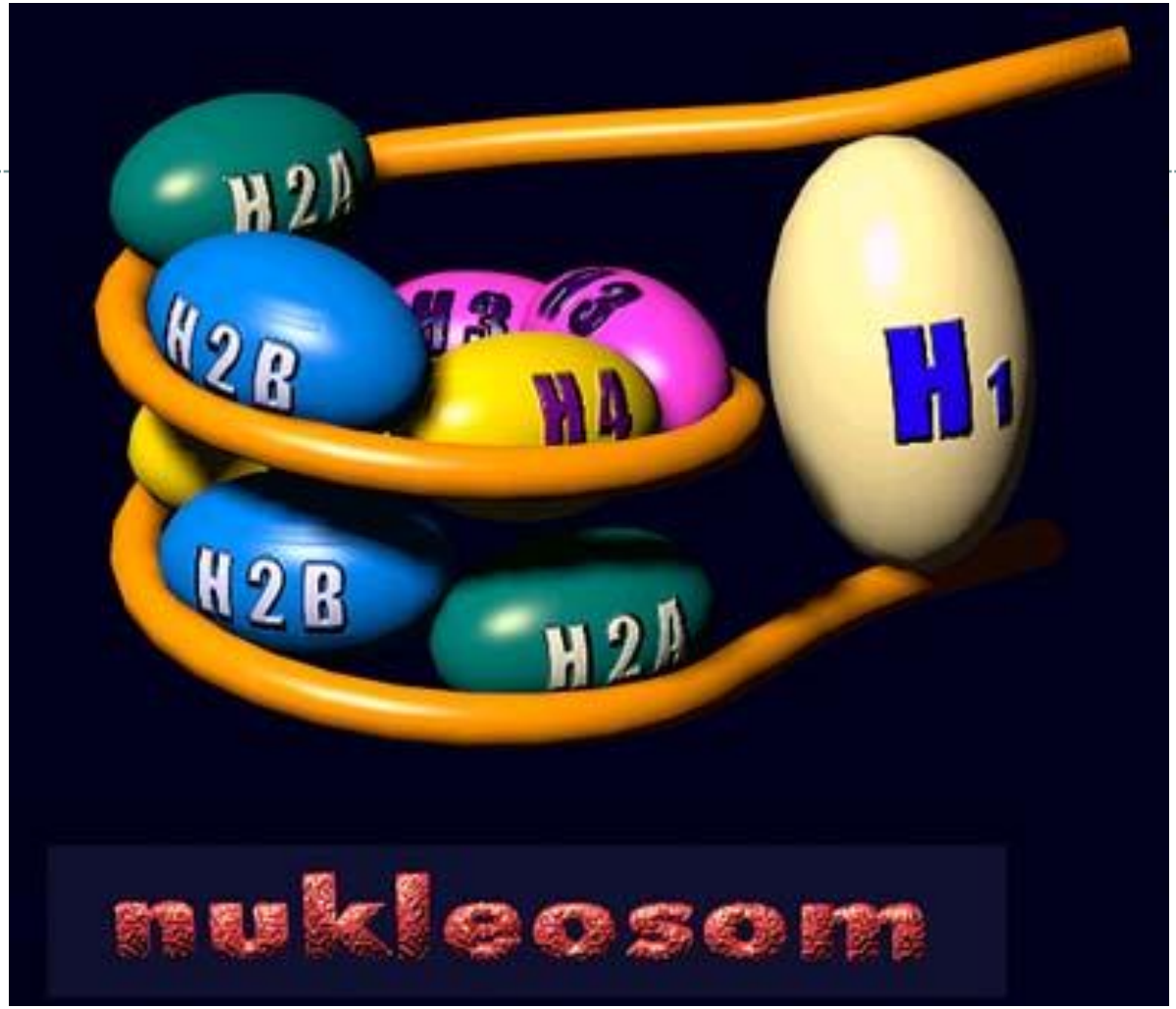
Beads-on-a-string form of chromatin

4. **Level 4** — **Formation of looped domains.**

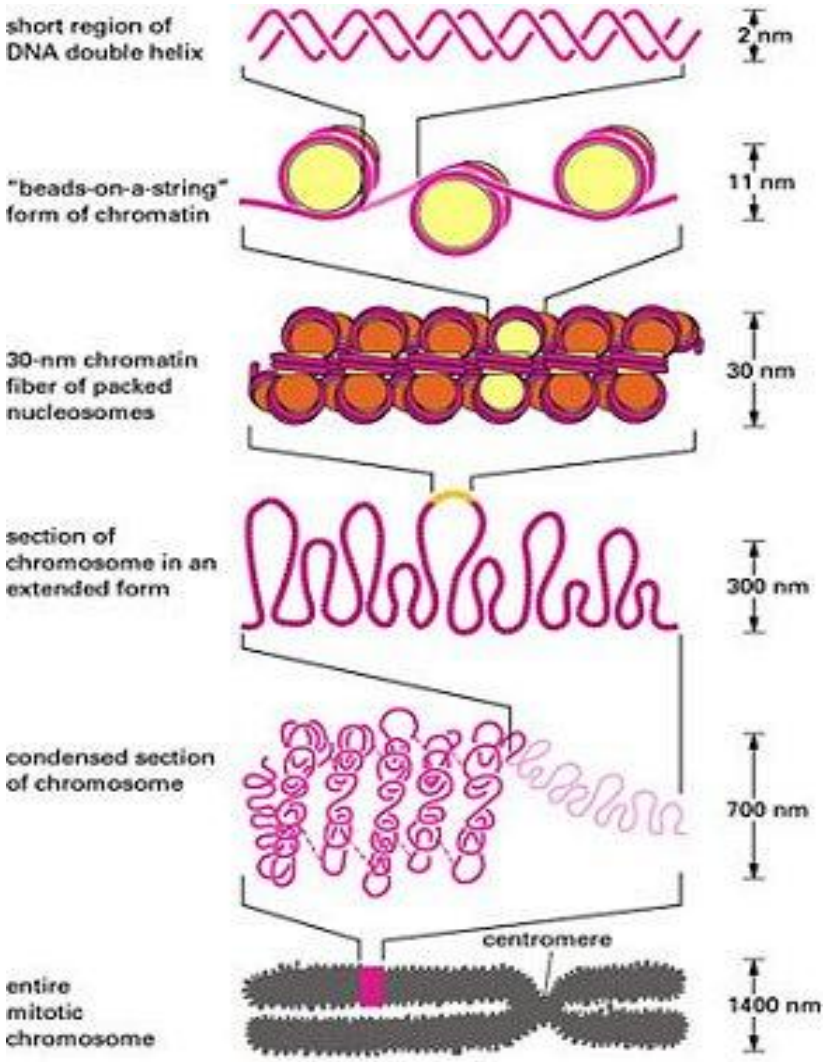


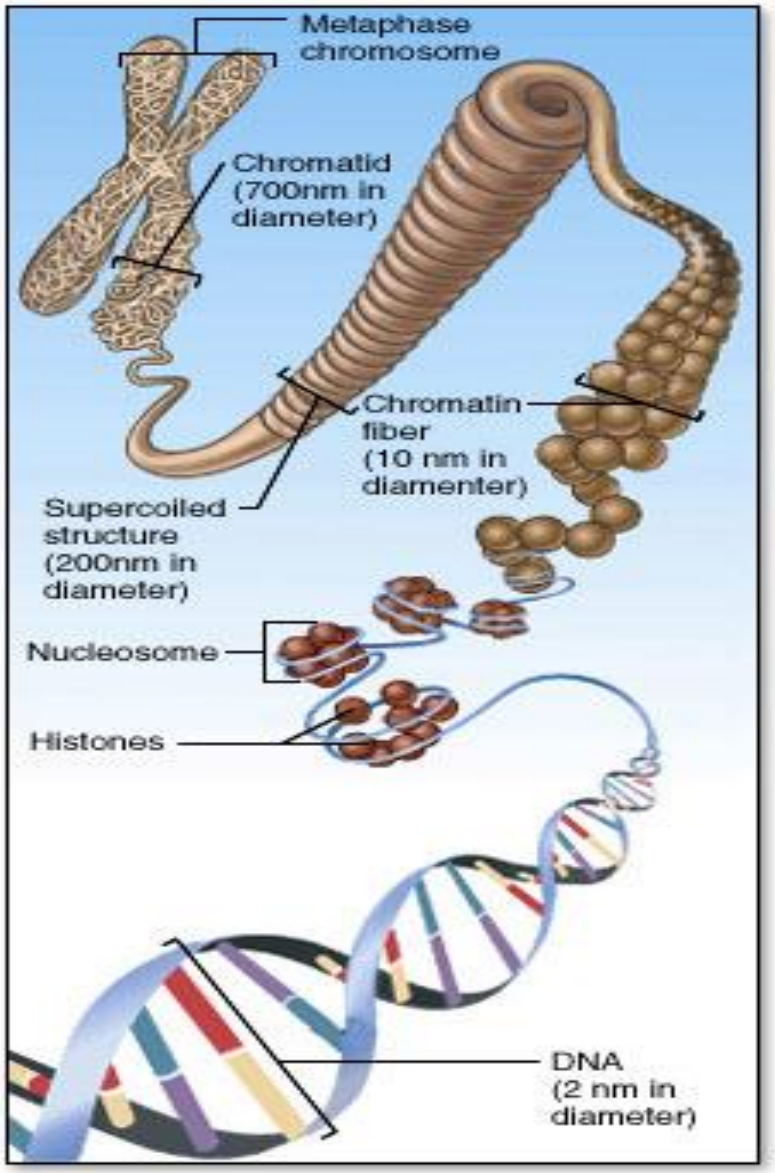


See Fig. 2.20



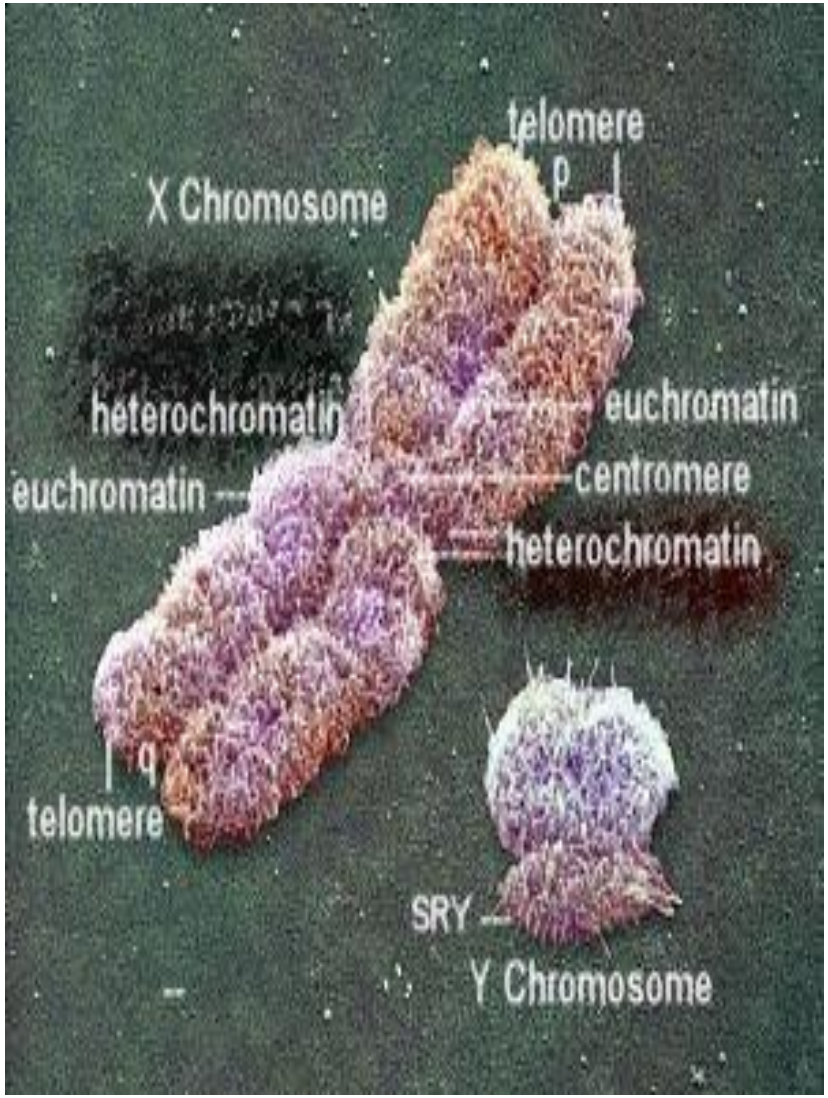
Sumber gambar :www.biologia.pl/ kurs/nukleosom.phtml

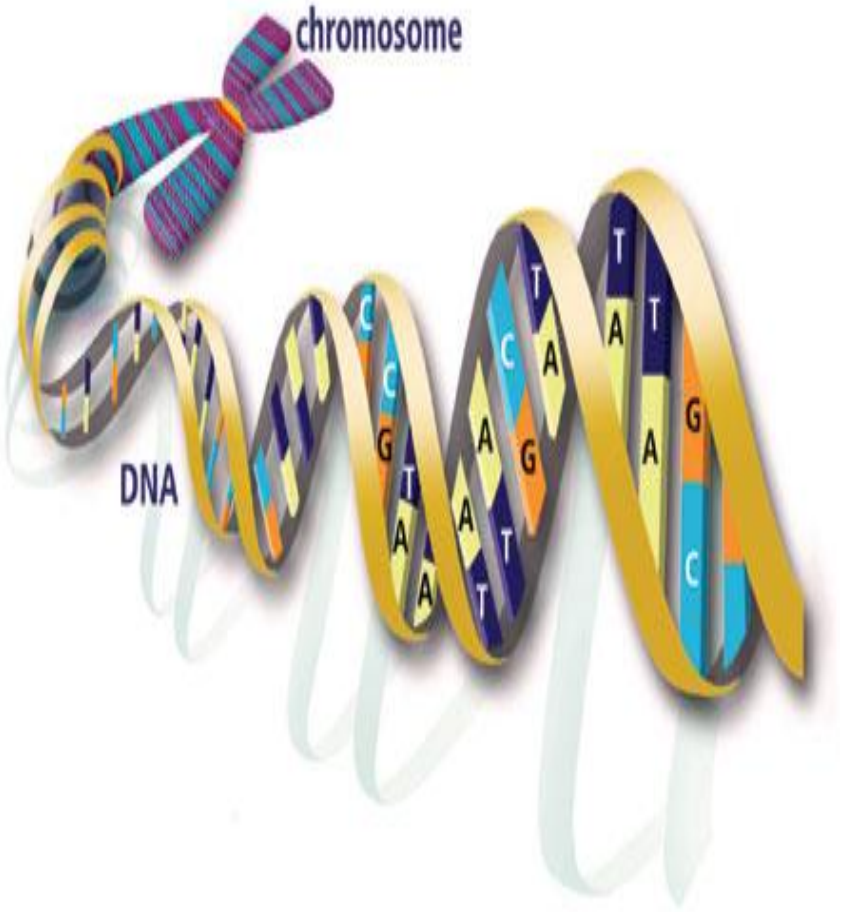




http://universe-review.ca/I11-25-chromosome.jpg

http://www.becomehealthynow.com/glossary/dna_bh.htm#





http://universe-review.ca/F11-monocell.htm#RNA

Sumber gambar :sina.eetezadi.de/.../dna-replikation-pcr/page/1

RNA

- Untai tunggal

- Diperlukan dalam sintesis protein
  - mRNA : membawa informasi genetik
  - t RNA : mengikat asam amino dan membaca pesan yang dibawa mRNA pada proses translasi
  - rRNA : penyusun ribosom

- Perbedaan RNA dan DNA :
  1. Pita RNA berupa pita tunggal
  2. Gula penyusun adalah ribosa
  3. Basa N pada kelompok pirimidin bukan Timin tetapi Urasil

THE REPLICATION OF DNA

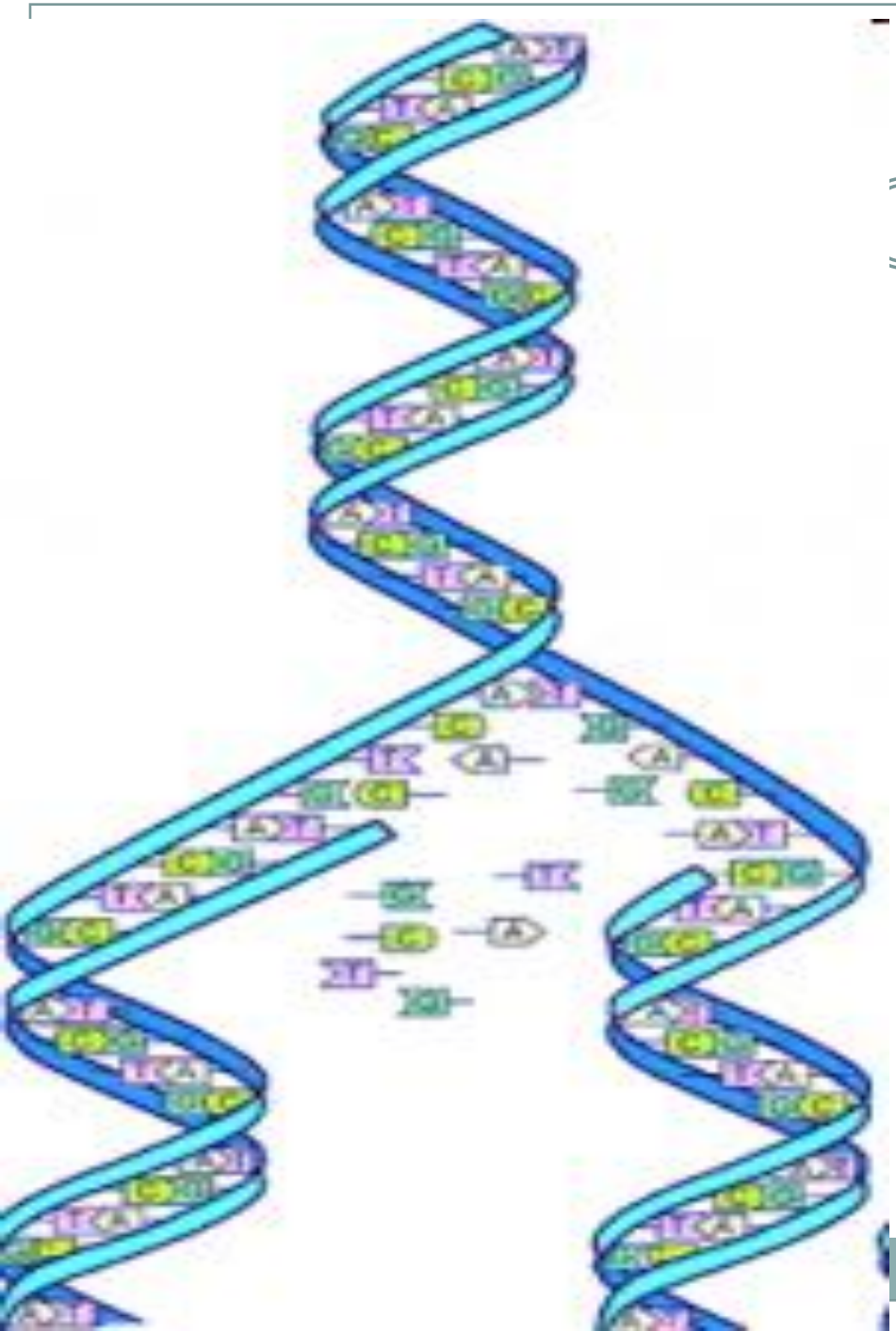
1. REPLIKASI DNA

- Replikasi adalah proses duplikasi/sintesis DNA yang terjadi sebelum sel membelah.
- Terjadi secara semikonservatif :

Satu double helix DNA akan membentuk dua double helix DNA baru yang masing-masing terdiri dari satu untai yang lama dan satu untai yang disintesis baru

INTRODUCTION

- DNA replication is the process by which the genetic material is copied
  - The original DNA strands are used as templates for the synthesis of new strands
- It occurs very quickly, very accurately and at the appropriate time in the life of the cell
  - This chapter examines how!

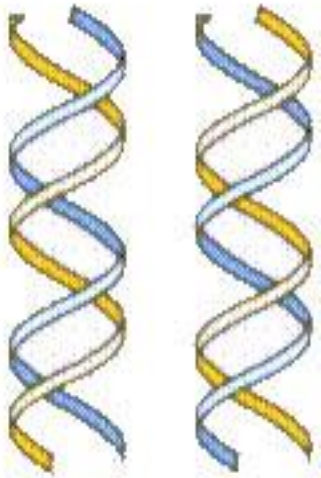


- Watson & Crick proposed that one DNA strand serves as a template, or pattern, on which the other strand is built
- DNA replication – the process of making a copy of DNA, which occurs during the (S) phase of the cell cycle

Semiconservative replication

Original DNA Helix

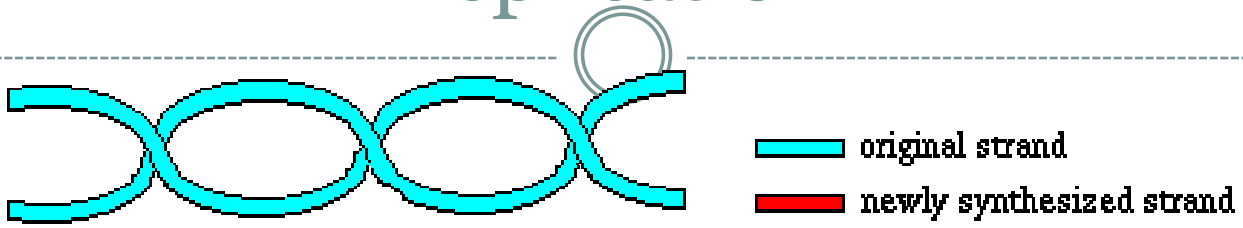
DNA helixes after one round of replication

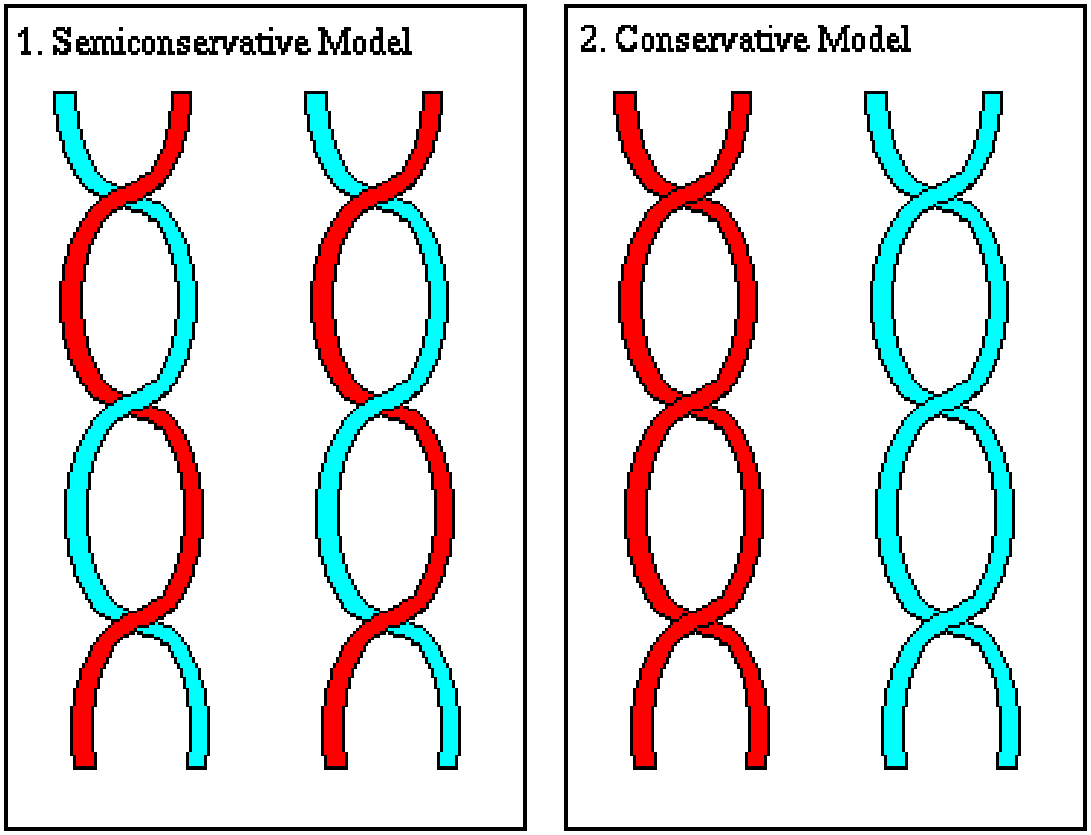


REPLIKASI DNA
semikonservatif

library.thinkquest.org/.../ RNA.htm

Conservative verses semiconservative replication





BAHAN REPLIKASI DNA

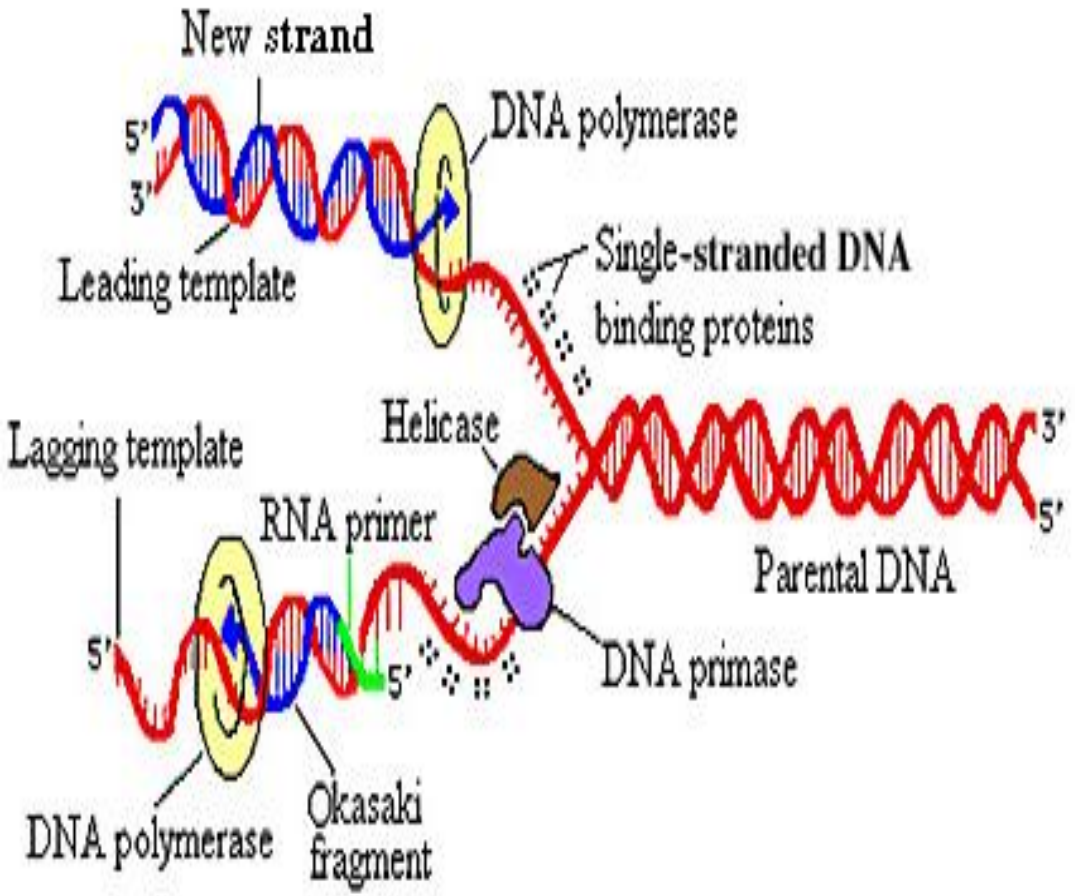
- Enzim yang berfungsi membuka gulungan DNA :

1. DNA girase (topoisomerase): membuka supercoil DNA
2. DNA helicase : membuka putaran segmen DNA di muka garpu replikasi
3. SSB (Single Strand Binding Protein) : mengikatkan diri pada rantai DNA yang terbuka sehingga tidak berdekatan lagi DNA cetakan/template
4. Primer/pemula yang dibentuk oleh enzim RNA primase
5. Deoksiribonukleotida (dNTP) : d ATP, d CTP, dGTP, d TTP
6. Enzim DNA polimerase yang mengkatalisis reaksi replikasi
7. Enzim DNA ligase untuk menyambung rantai/fragmen DNA

Sintesis DNA terjadi dengan arah 5’ – 3’

Reaksi penambahan nukleotida baru terjadi pada ujung 5’ hidroksi phospat menuju ujung 3’ OH (hidroksi bebas)

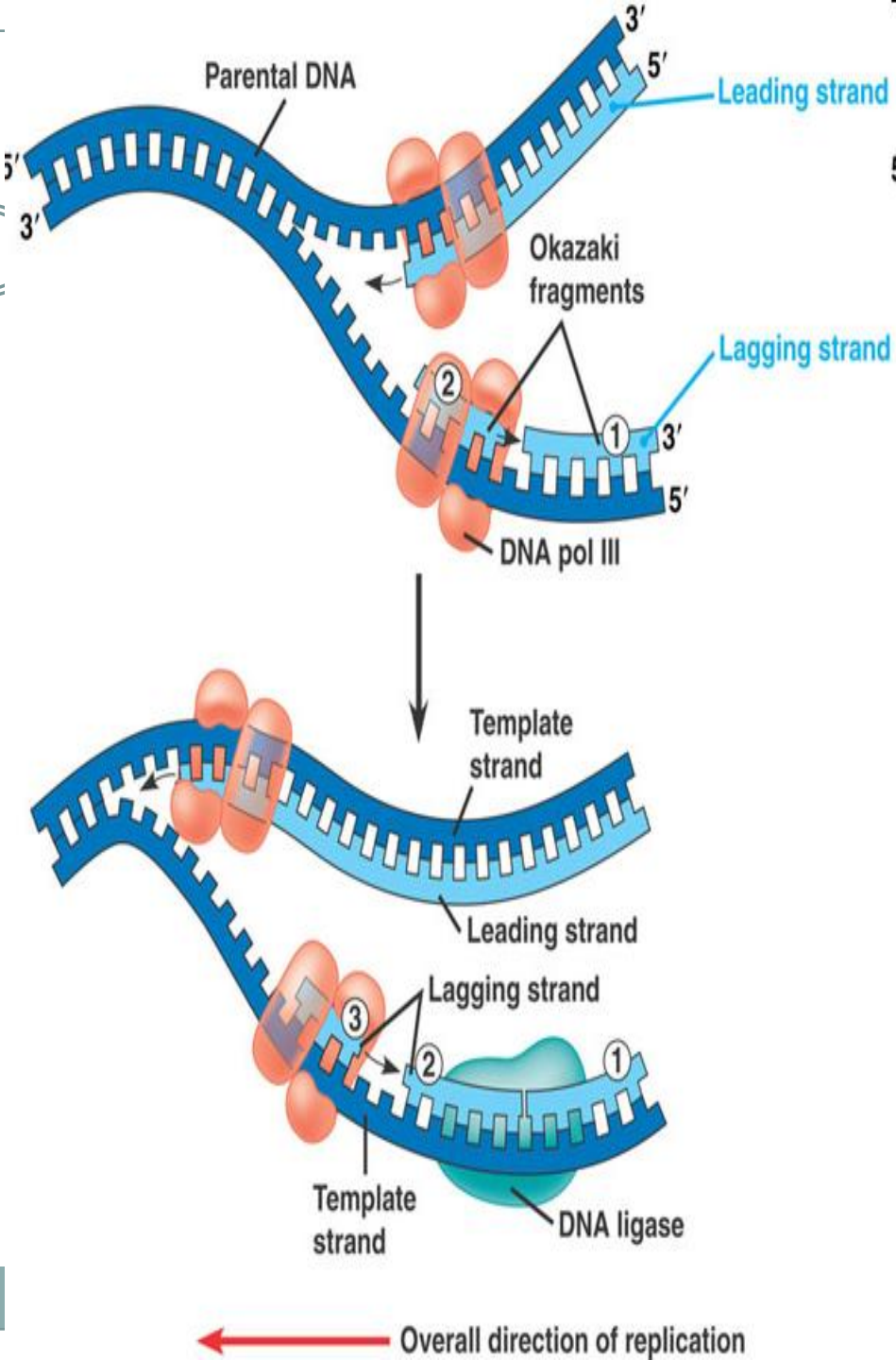
- Leading strand : rantai DNA disintesis terus menerus (kontinu) utuh dengan arah 5’ – 3’
- Lagging strand : rantai DNA disintesis terputus-putus (fragmen okazaki) dengan arah 5’ – 3’, fragmen tsb kemudian digabungkan dengan DNA ligase

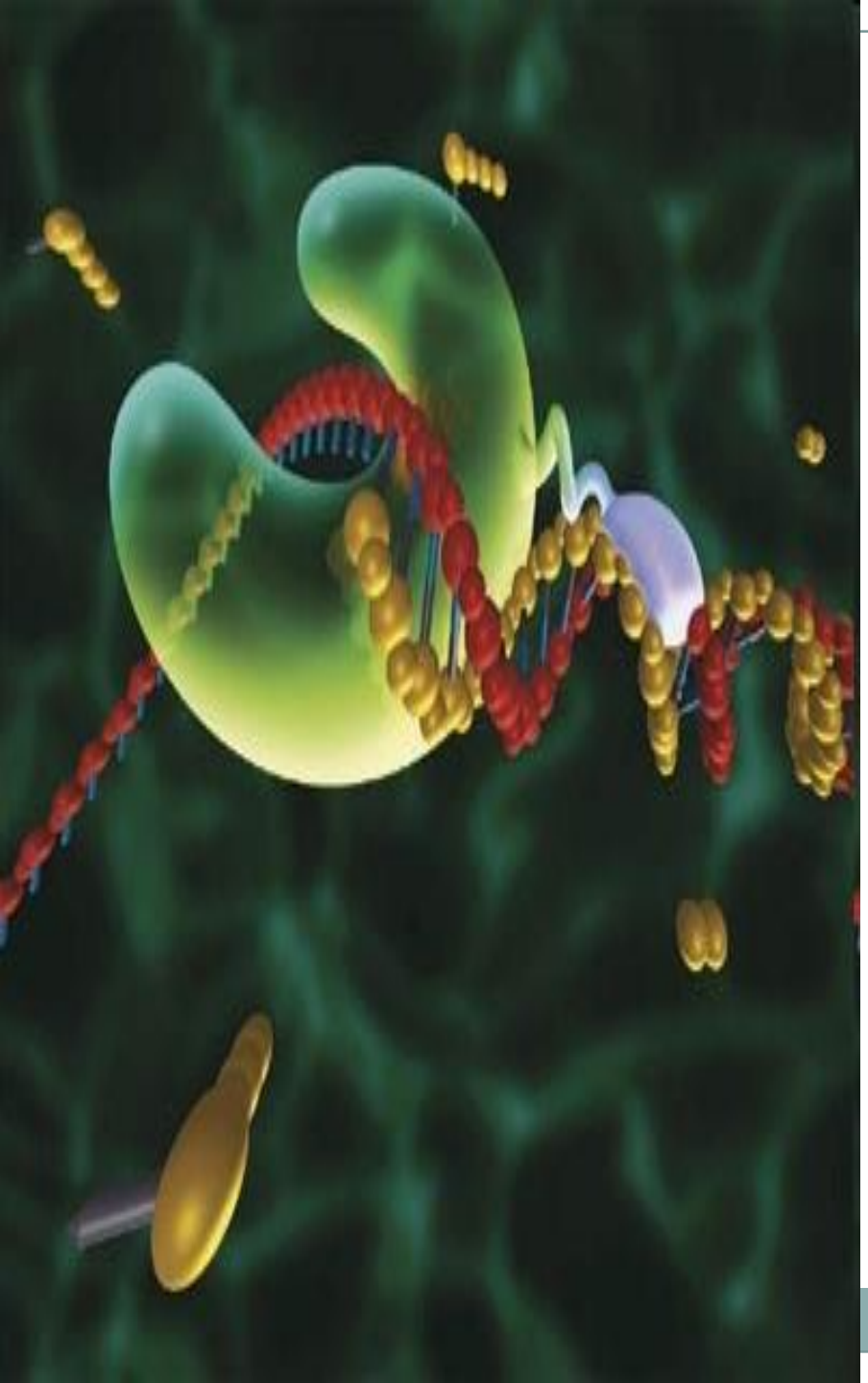


Collaboration of Proteins at the Replication Fork

- Step 1 – The double helix needs to unwind before replication can begin
- Accomplished by enzymes called DNA helicases which open the double helix by breaking the hydrogen bonds between the two strands

- Step 2. Additional proteins prevent the strands from assuming their double-helical shape
- Replication forks – areas where the double helix separates
- Enzymes known as DNA polymerases add nucleotides to the exposed nitrogen bases, according to the base-pairing rules – forming two double helixes

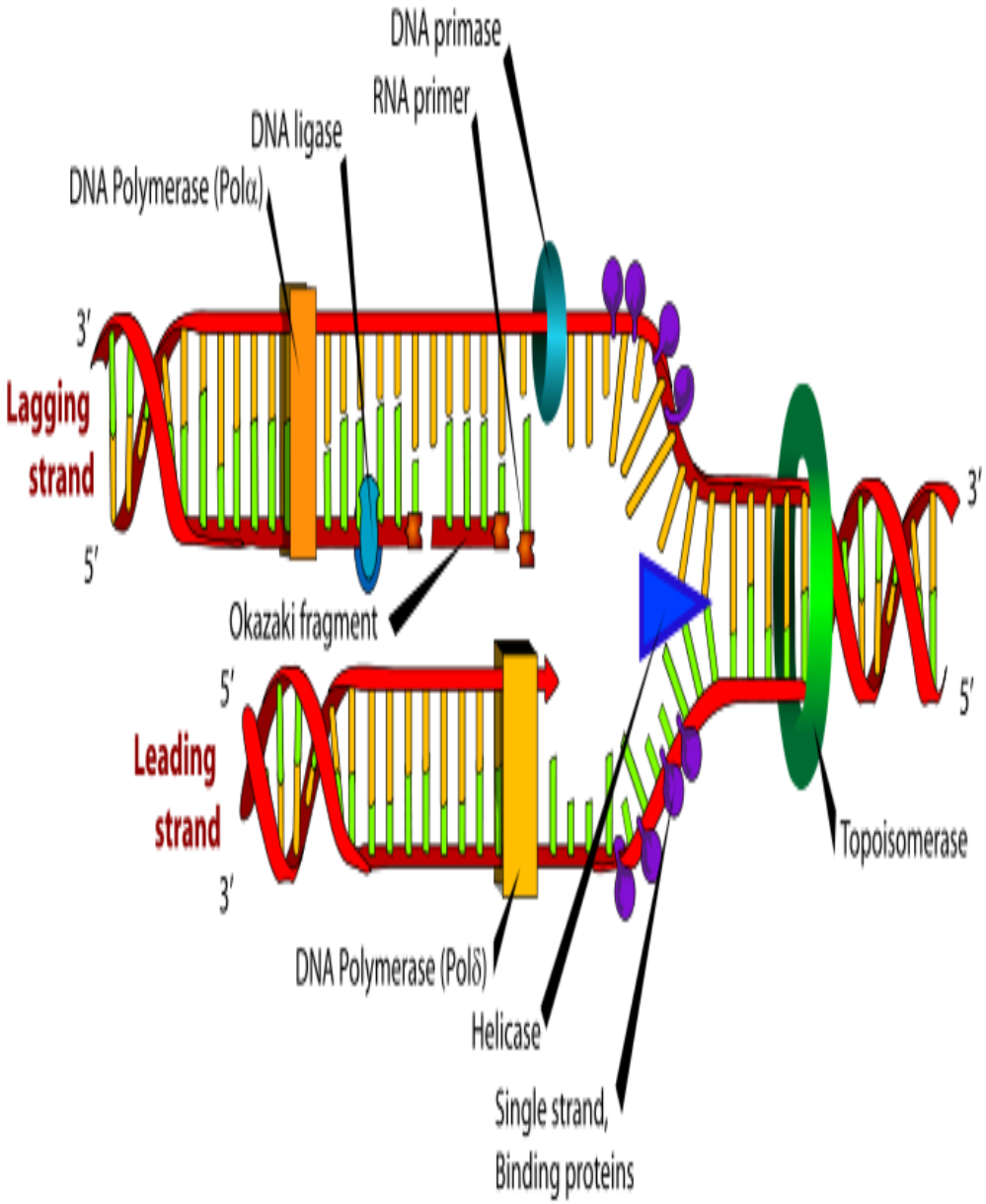




- Step 3 The process continues until all of the DNA has been copied & the polymerases are signaled to detach
- Nucleotide sequences are identical in the two DNA molecules
- Checking for errors – DNA polymerases are important in "proofreading" the nucleotides – can backtrack
- Errors in DNA replication about one error per 1 billion nucleotides

Rate of Replication

- Replication does not begin at one end & end at the other
- Prokaryotes usually have two replication forks
- Eukaryotic cells – length a problem – 33 days if done with a single point
- Each human chromosome is replicated in about 100 sections – replicated in about 8 hours



Garpu replikasi

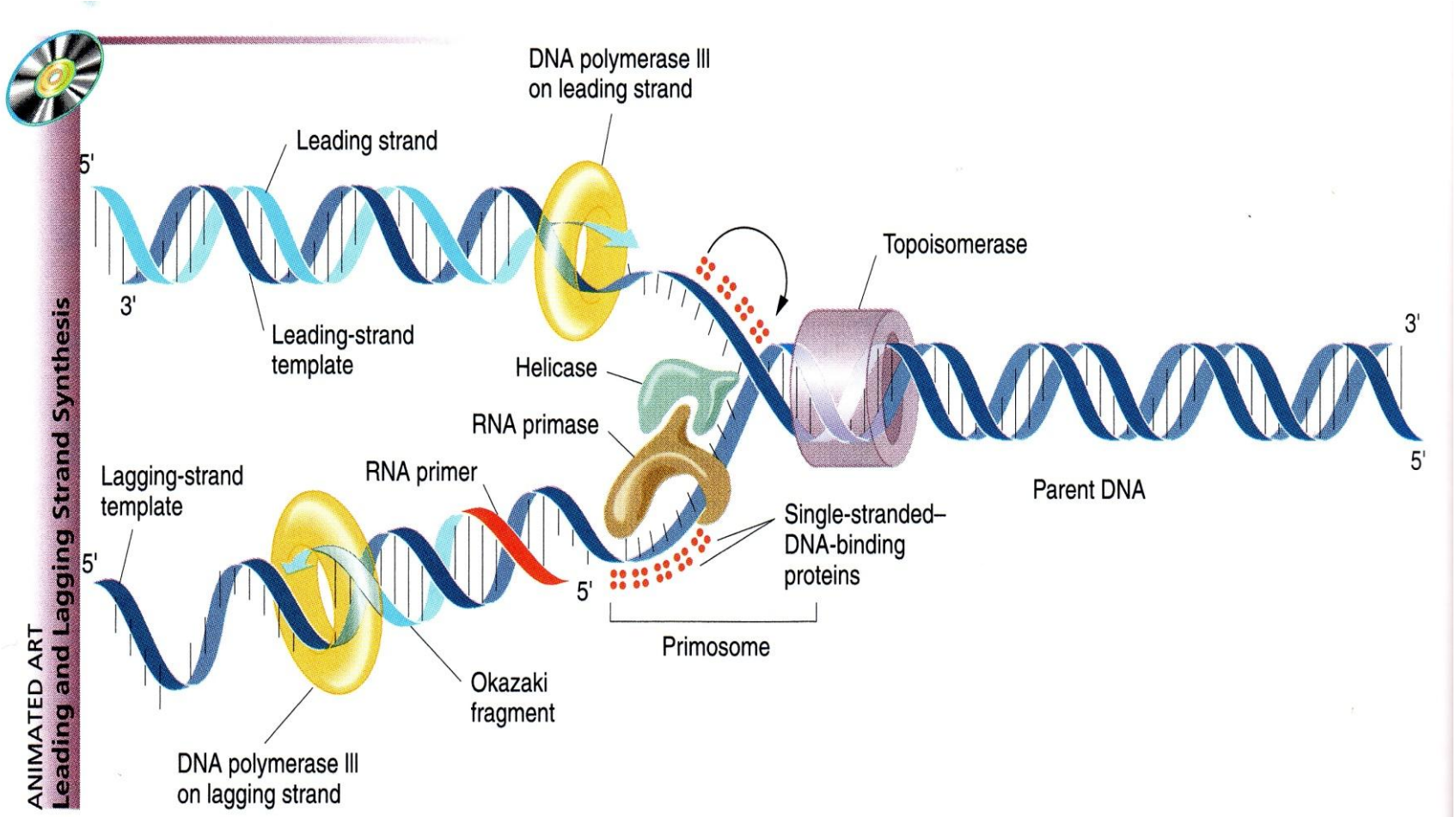
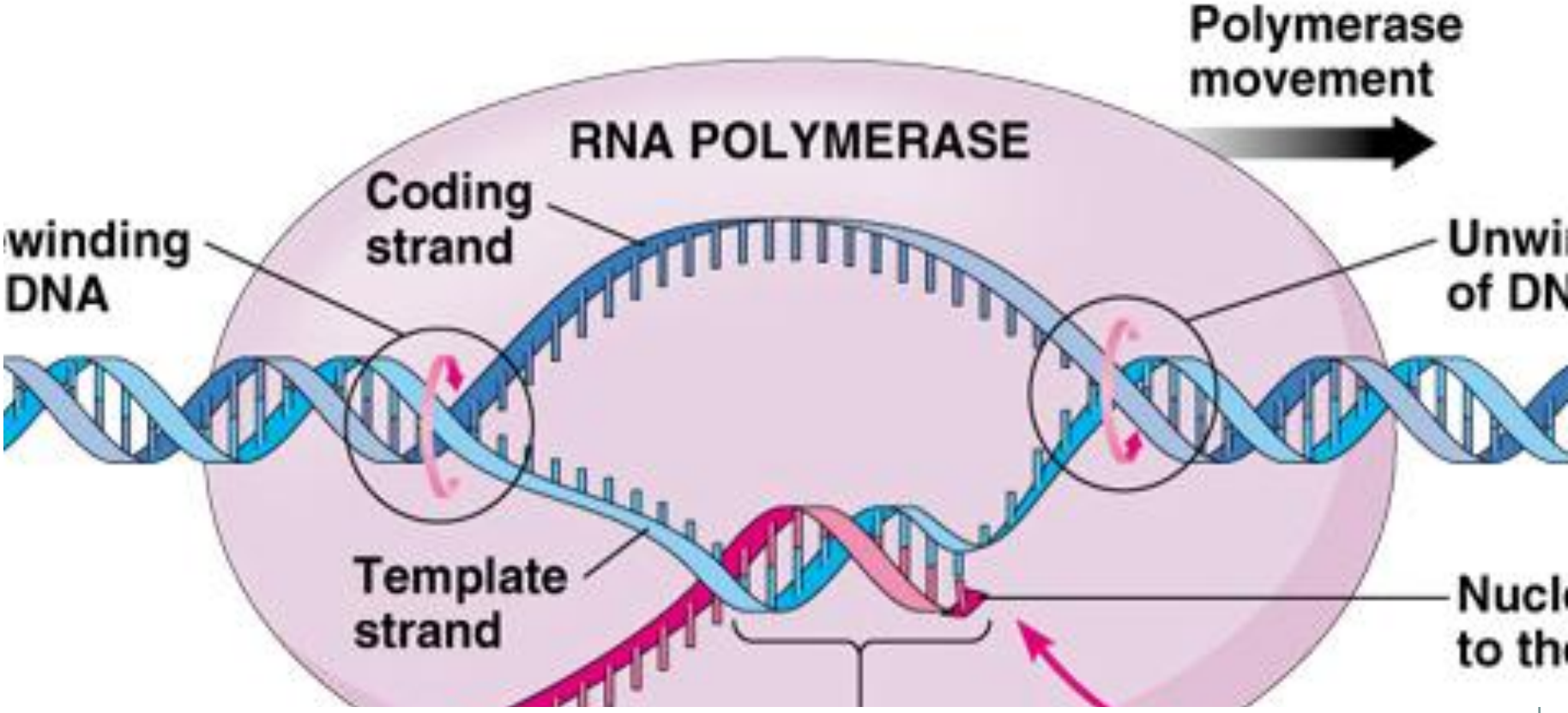


Figure 8-20 DNA replication fork.

How do we know DNA makes RNA




TERIMAKASIH.....