River Valley High School
2025 JC1 H2 Biology

Lecture Topic 7: The Structure of Nucleic Acids and Gene Expression – DNA Replication

Name: _____ ( ) Class: 25J____ Date: _____

References

Title	Author
Biology (9th edition)	Campbell and Reece
Biological Science 1 and 2 (3rd Edition)	Taylor, Green and Stout
Molecular Biology of the Cell (5th edition)	Alberts, Johnson, Lewis, Raff, Roberts and Walter
Principles of Genetics (3rd edition)	Snustad and Simmons
Molecular Cell Biology (6th edition)	Lodish, Berk, Kaiser, Krieger, Scott, Bretscher, Ploegh and Matsudaira

H2 Biology Syllabus 9477 (2025)
Candidates should be able to use the knowledge gained in the following section(s) in new situations or to solve related problems.

Related Core Topics	Content
Transcription and Translation	Central Dogma – DNA to RNA, RNA to protein

Learning Outcomes

2A: The Structure of Nucleic Acids and Gene Expression

a. describe the structure and roles of DNA and RNA (tRNA, rRNA and mRNA) (knowledge of the structure and roles of mitochondrial DNA and chloroplast DNA is not required)

b. describe the process of DNA replication and how the end replication problem arises

Lecture Outline

I. Role of DNA as the Hereditary Material

II. Structure of DNA
- A. Building Blocks of Nucleic Acids
- B. Physical Structure of DNA

III. Replication of DNA
- A. DNA Strands as Templates for Replication
- B. Mechanism of DNA replication
- C. DNA Repair and Maintenance

Websites

URL	Description
http://learn.genetics.utah.edu/content/basics/	Website on the basics of genetics with videos and interactive activties
https://highered.mheducation.com/sites/9834092339/student_view0/chapter14/dna_replication.html	Animations on DNA replication (Chapter 14)* Chapter quiz to help reinforce concepts *Note: you would need to use a laptop/desktop and download a chrome extension (e.g. “Flash Player for Chrome”) to view the tutorial.
http://www.dnaftb.org/	Animations** and video clips for DNA-related content (under Molecules of Genetics). **Note that the animations will take some time to load.
https://www.dnalc.org/resources/nobel/crick_watson_wilkins.html	Animations which illustrate how the DNA structure was decoded

I. Role of DNA as the Hereditary Material

Introduction

- Life depends on the ability of cells to store, retrieve and translate the genetic instructions required to make and maintain a living organism.
- This hereditary information is passed on from a cell to its daughter cells at somatic cell division, and from one generation of an organism to the next through the organism's reproductive cells.
- These instructions are stored within every living cell as its genes, which are the information-containing elements that determine the characteristics of a species as a whole and of the individuals within it.

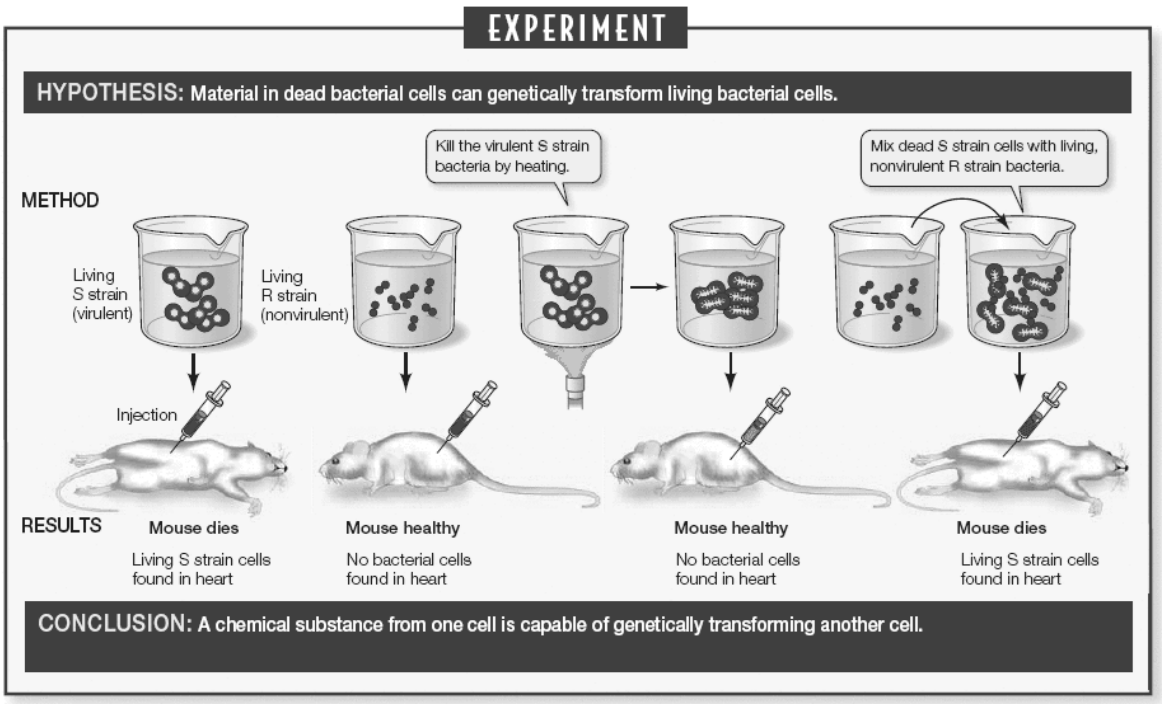
Characteristic properties

The hereditary material must be:

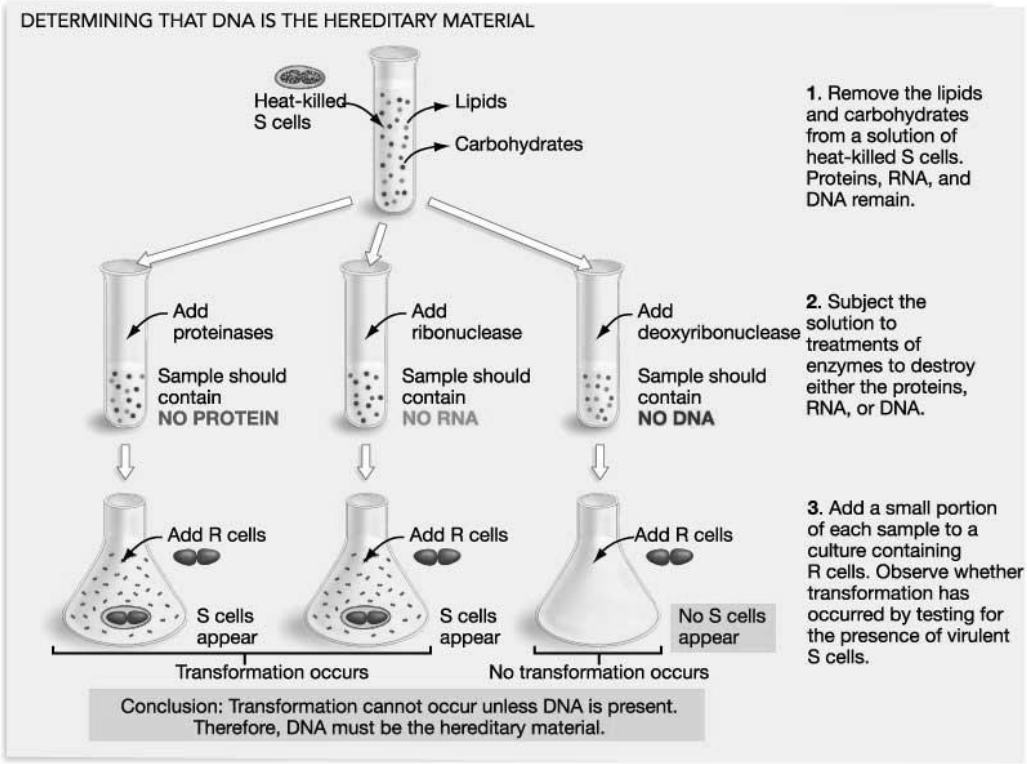
- chemically stable to be able to encode information without fail. It must not change easily due to age, nutrition, or environment.
- able to replicate accurately.
    - The molecule responsible for genetic information should act as a template for an accurate copy of itself during replication so that progeny cells share the same genetic information as the parental cell. Once this genetic material doubles in amount, it must be partitioned equally into two daughter cells.
    - During the formation of gametes, the genetic material is also replicated but is portioned such that each cell receives only one-half of the original amount of genetic material. Fertilisation of the egg by the sperm restores the original amount of DNA.
- capable of variation.
    - Without variation, all organisms would look alike and would not be capable of change and adaptation – conditions necessary for the evolutionary process.

Evidence that DNA is the hereditary material

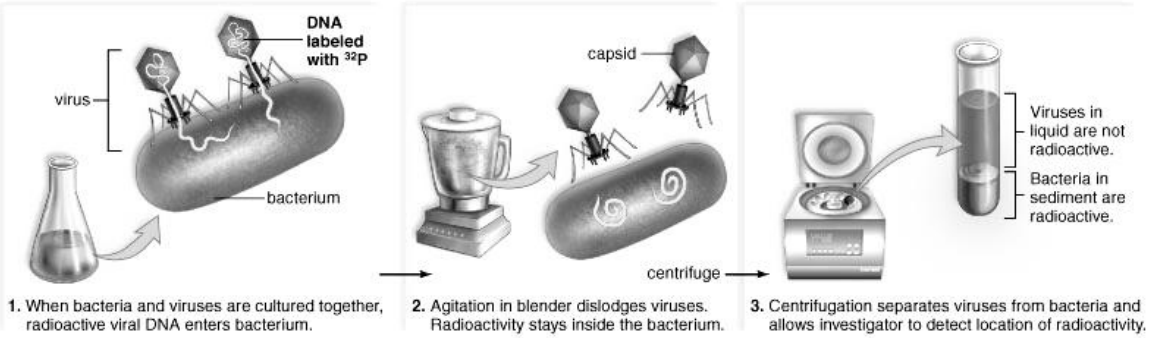
1. Chromosome analysis
2. Constancy of DNA within a cell
3. Correlation between mutagens and their effects on DNA
4. Experiments on bacterial transformation (Griffith, 1928)



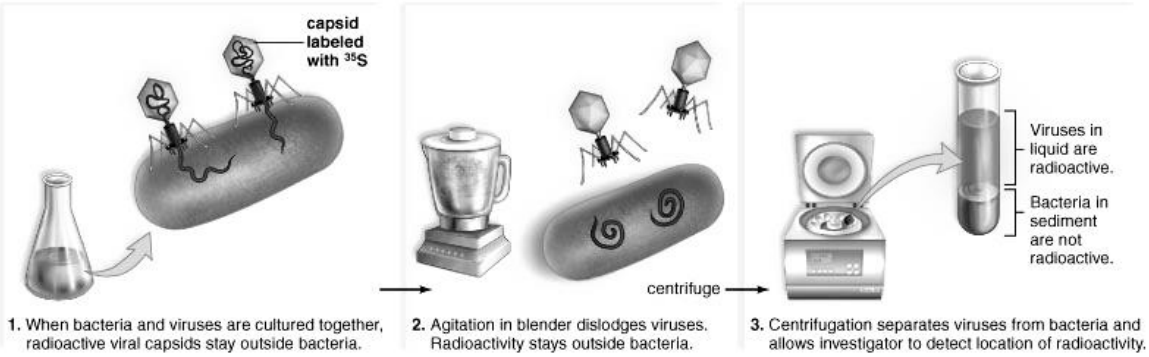
5. Experiments to identify the transforming principle
(Avery, MacLeod and McCarty, 1944) http://www.dnaftb.org/17/animation.html



6. Transduction experiments (Hershey and Chase, 1952)
https://tinyurl.com/HersheyChaseExpt
*Note: you would need to use a laptop/desktop and download a chrome extension (e.g. “Flash Player for Chrome”) to view the tutorial.



a. Viral DNA is labeled (yellow).



b. Viral capsid is labeled (yellow).

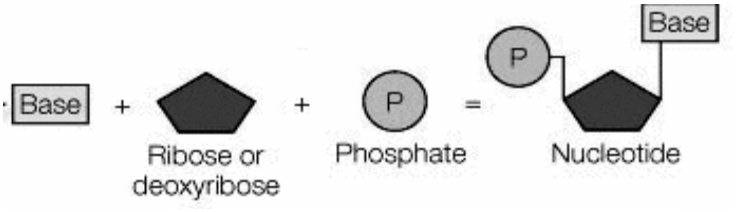
II. Structure of DNA

A. Building Blocks of Nucleic Acids

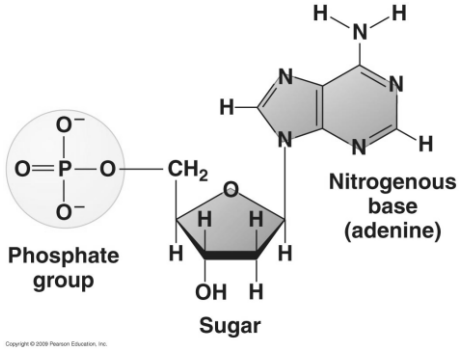
- Two types of nucleic acids:
  - **Deoxyribonucleic acid** (DNA)
  - **Ribonucleic acid** (RNA)
- Nucleic acids are macromolecules composed of repeating subunits (monomers) called **nucleotide**

Structure of a nucleotide

- Each nucleotide is composed of:
  - a pentose sugar (five-carbon sugar);
  - a nitrogenous base**;** and
  - a phosphate group



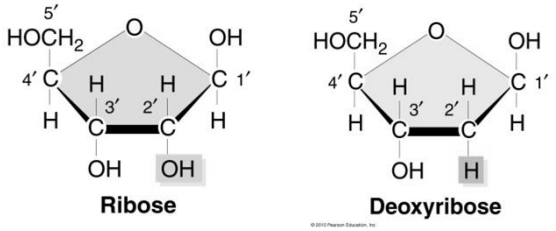
Diagrammatic representation of a nucleotide



Structure of a nucleotide

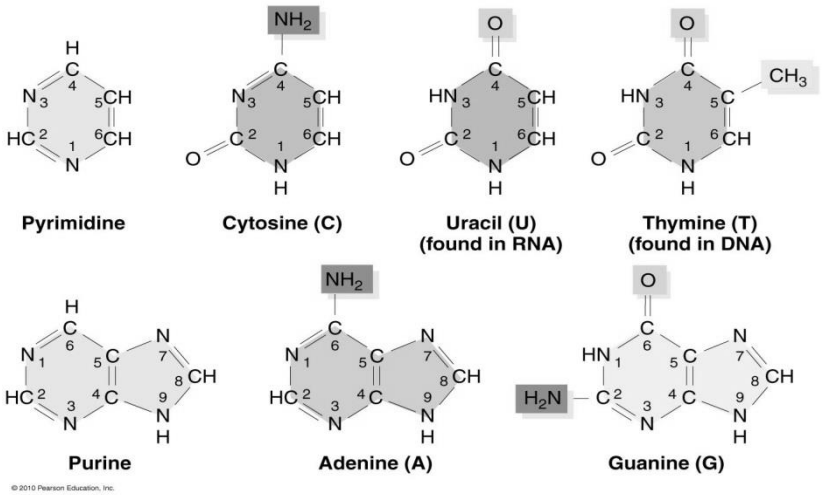
Pentose

- A five-carbon sugar that occurs as a ring form.
- Two types of pentose are found in nucleic acids: ribose (in RNA) and deoxyribose (in DNA).
- Main difference between ribose and deoxyribose:
  - hydroxyl group (-OH) at carbon atom 2 in ribose is replaced by a hydrogen atom (H).



Nitrogenous base

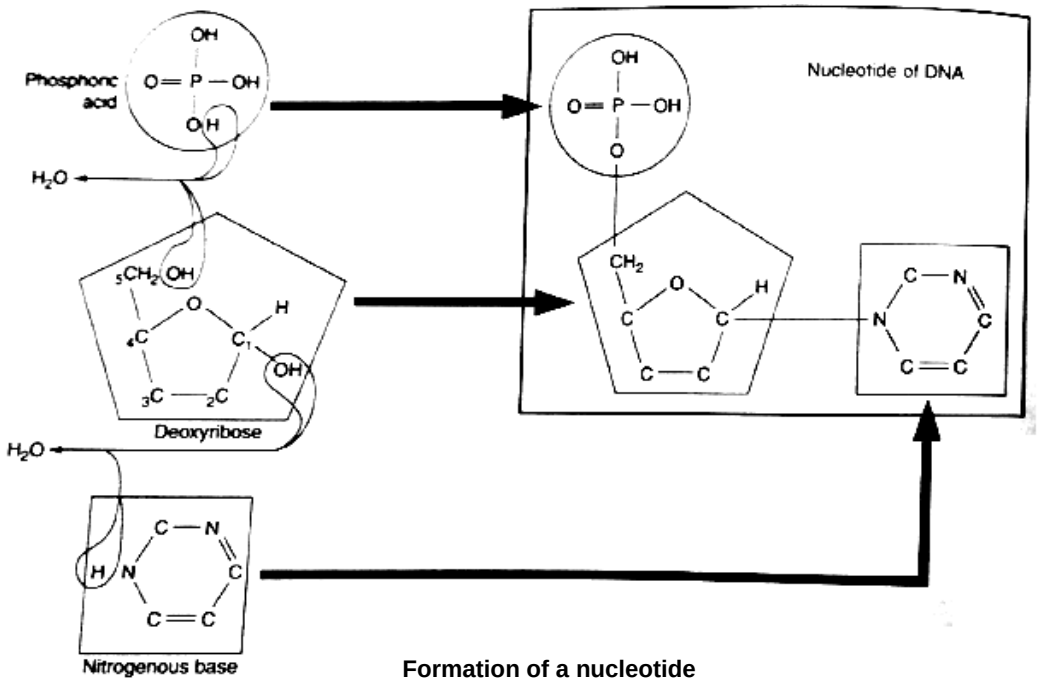
- Heterocyclic ring of carbon and nitrogen atoms.
- Each nucleic acid contains four different types of bases, which can be categorised into:
  - Purines (have a six-membered ring fused to a five-membered ring)
  - Pyrimidines (have one six-membered ring)



	Purine	Pyrimidine
DNA	Adenine (A) and Guanine (G)	Cytosine (C) and Thymine (T)
RNA	Adenine (A) and Guanine (G)	Cytosine (C) and Uracil (U)

Formation of Nucleotide

- The three components, i.e. pentose, phosphoric acid and a nitrogenous base, join to form a **nucleotide** via **condensation reaction**.
  - 1' carbon of the pentose is linked via a **glycosidic bond** to the nitrogenous base.
  - 5' carbon of pentose is linked via a **phosphoester bond** to the phosphate group.



Formation of a nucleotide

- Different nucleotides are formed according to the sugars and bases they contain. *(Refer to table below: Nucleotide and Nucleic Acid Nomenclature)*

TABLE 8–1 Nucleotide and Nucleic Acid Nomenclature

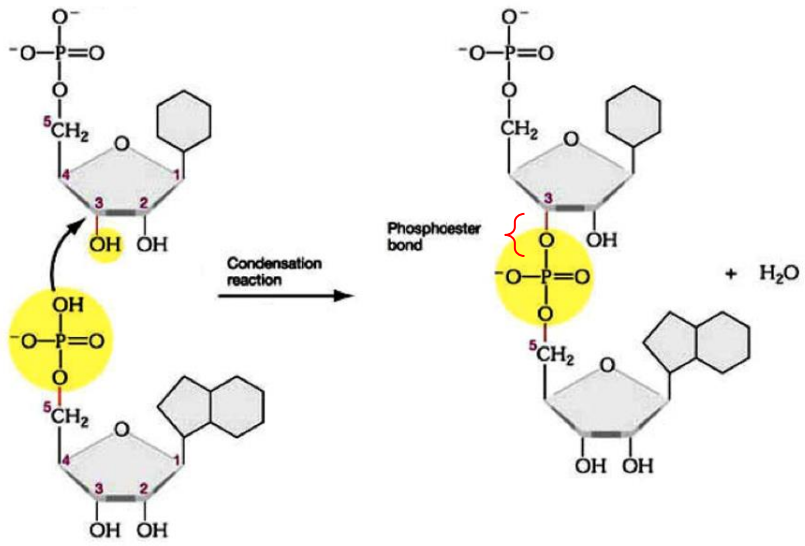
Base	Nucleoside	Nucleotide	Nucleic acid
Purines			
Adenine	Adenosine Deoxyadenosine	Adenylate Deoxyadenylate	RNA DNA
Guanine	Guanosine Deoxyguanosine	Guanylate Deoxyguanylate	RNA DNA
Pyrimidines			
Cytosine	Cytidine Deoxycytidine	Cytidylate Deoxycytidylate	RNA DNA
Thymine	Thymidine or deoxythymidine	Thymidylate or deoxythymidylate	DNA
Uracil	Uridine	Uridylate	RNA

Note: "Nucleoside" and "nucleotide" are generic terms that include both ribo- and deoxyribo- forms. Also, ribonucleosides and ribonucleotides are here designated simply as nucleosides and nucleotides (e.g., riboadenosine as adenosine), and deoxyribonucleosides and deoxyribonucleotides as deoxynucleosides and deoxynucleotides (e.g., deoxyriboadenosine as deoxyadenosine). Both forms of naming are acceptable, but the shortened names are more commonly used. Thymine is an exception; "ribothymidine" is used to describe its unusual occurrence in RNA.

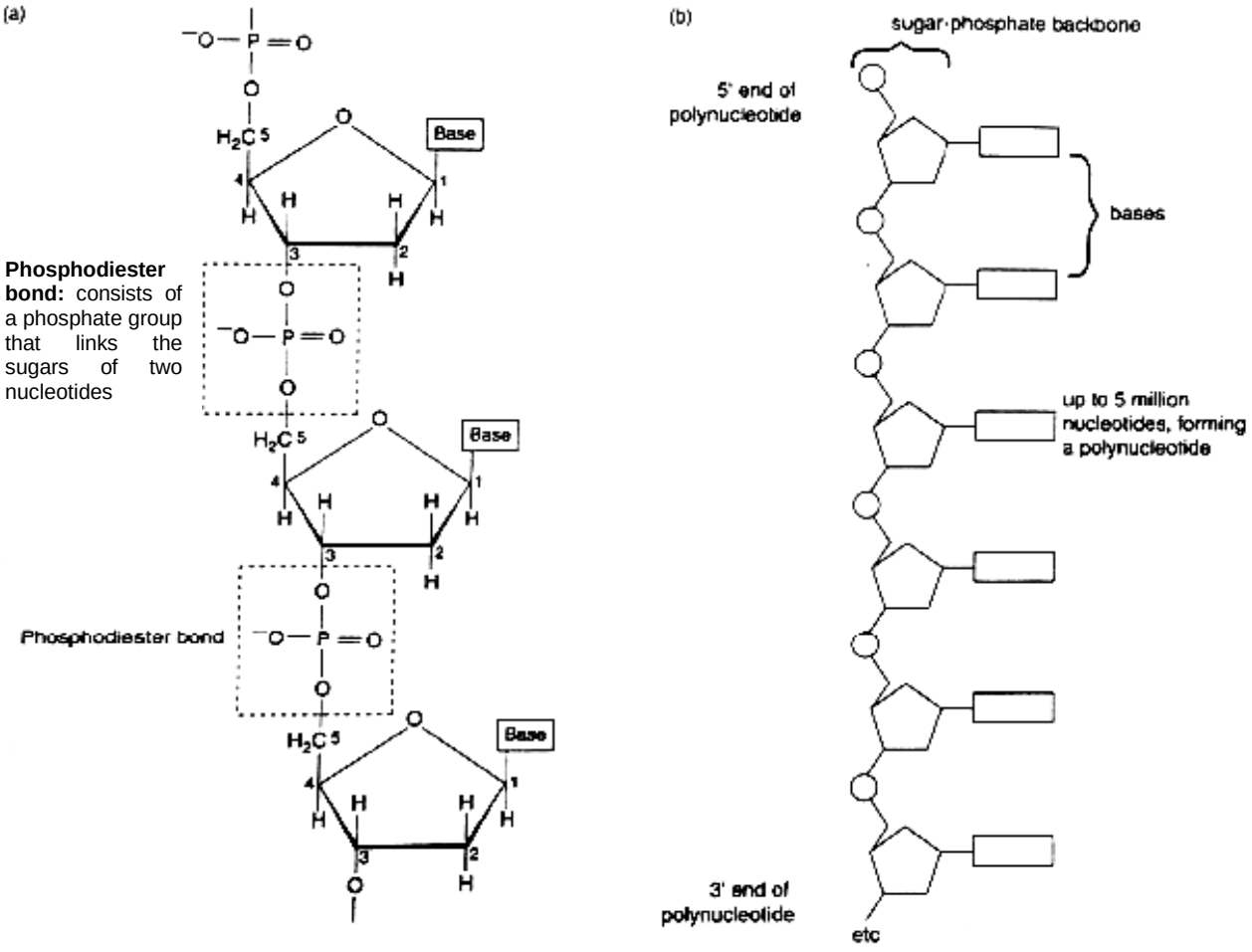
Table 8-1
Lehninger Principles of Biochemistry, Fifth Edition

Formation of Polynucleotide

- Two nucleotides join to form a **dinucleotide** by **condensation reaction** between the phosphate group of one nucleotide and the 3' carbon of pentose of the other nucleotide.
- The two nucleotides are joined by a **phosphoester bond** between the phosphate group of one nucleotide and the 3' carbon of pentose of the other nucleotide.



- Addition of further nucleotides produces a **polynucleotide**, with a backbone consisting of alternating sugar and phosphate groups i.e. **sugar-phosphate backbone**.
- **5' end** of a polynucleotide ends with a **phosphate group** attached to 5' carbon of pentose
- **3' end** of a polynucleotide ends with an **OH group** on 3' carbon of pentose

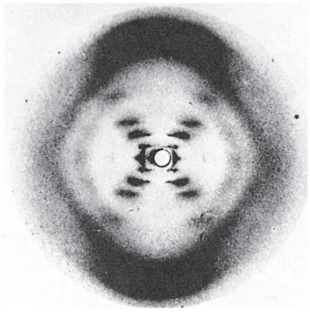


▲ **Figure 6.5** (a) Structure of part of a polynucleotide. (b) Simplified representation of part of a polynucleotide.

B. Physical Structure of DNA

Road to Discovery of Structure of DNA

- In the early 1950s, Nobel prize winning chemist **Linus Pauling** worked out the α-helical structure that is common to many fibrous proteins and was applying himself to the problem of DNA structure, which evidence suggested was also a fibrous molecule.
- At the same time, **Maurice Wilkins** and **Rosalind Franklin** of King's college, London were tackling the same problem using the technique of **X-ray crystallography**. They prepared pure fibres of the salt of DNA, from which they managed to get complete X-ray diffraction patterns.
- Based on the X-ray diffraction data, it is concluded that DNA:
  - is a long, thin molecule, diameter of **2 nm**
  - consists of **2 strands**
  - is coiled in the form of a helix; one complete turn of double helix is **3.4nm** long
  - has **10 bases** to each complete turn of the helix



(b) Franklin's X-ray diffraction photograph of DNA

- In 1951, **Erwin Chargaff** *et al* analysed the DNA from many different organisms. The DNA were hydrolysed and the purines (A,G) and pyrimidines (T,C) released were quantified.

  It was observed that:

  - The base composition of the DNA of an organism is constant throughout all the somatic cells of that organism and is characteristic for a given species.
  - In all the double-stranded DNAs, the molar amount of purines (i.e. A + G) is equal to the molar amount of pyrimidines (i.e. C + T).
  - The amount of adenine (A) is always equal to that of thymine (T)
  - The amount of guanine (G) is always equal to that of cytosine (C)
  - The base composition of DNA varies greatly from one organism to another. This is clearly expressed by the dissymmetry ratio, (A+T) / (G+C).

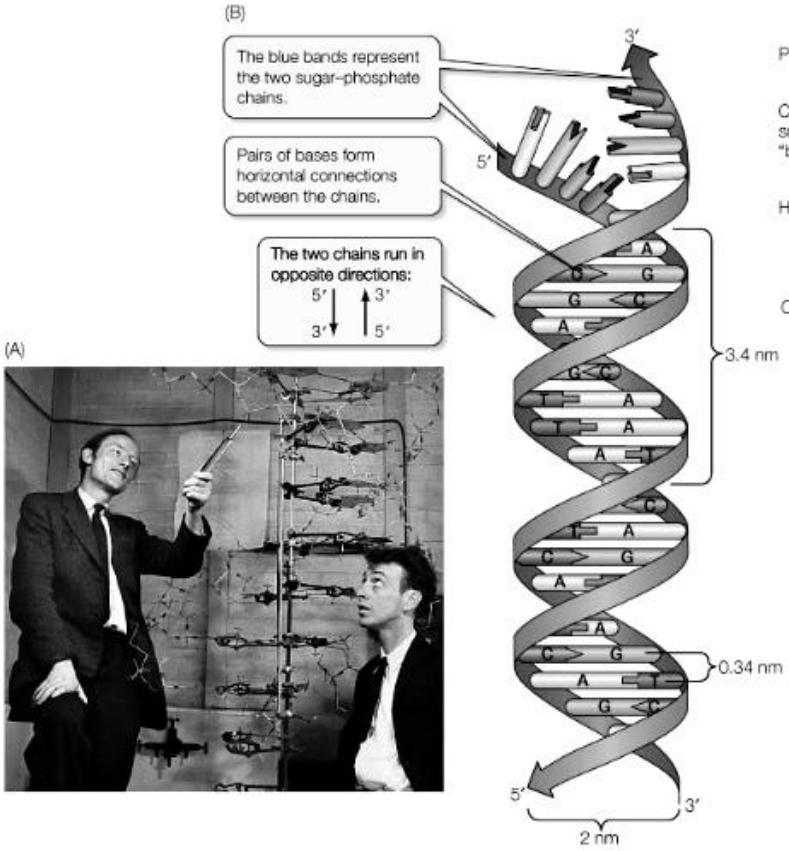
(b) Base compositions of DNAs from various sources

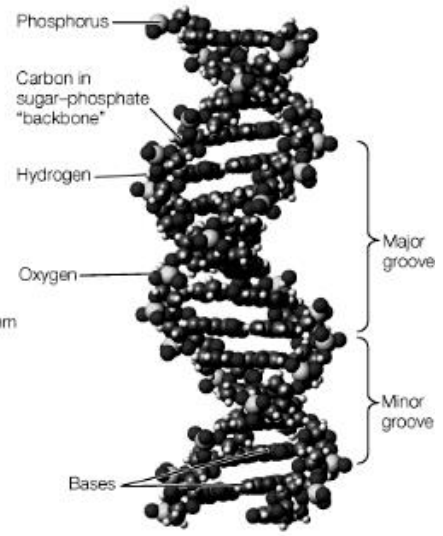
	Base composition				*Base ratio*		*A + T/G + C ratio*	
	1	2	3	4	5	6	7	8
Source	**A**	**T**	**G**	**C**	**A/T**	**G/C**	**(A + G)/(C + T)**	**(A + T)/(C + G)**
Human	30.9	29.4	19.9	19.8	1.05	1.00	1.04	1.52
Sea urchin	32.8	32.1	17.7	17.3	1.02	1.02	1.02	1.58
E. coli	24.7	23.6	26.0	25.7	1.04	1.01	1.03	0.93
Sarcina lutea	13.4	12.4	37.1	37.1	1.08	1.00	1.04	0.35
T7 bacteriophage	26.0	26.0	24.0	24.0	1.00	1.00	1.00	1.08

* *Source*: From Chargaff, 1950.
[a]Moles of nitrogenous constituent per mole of P. (Often, the recovery was less than 100 percent.)

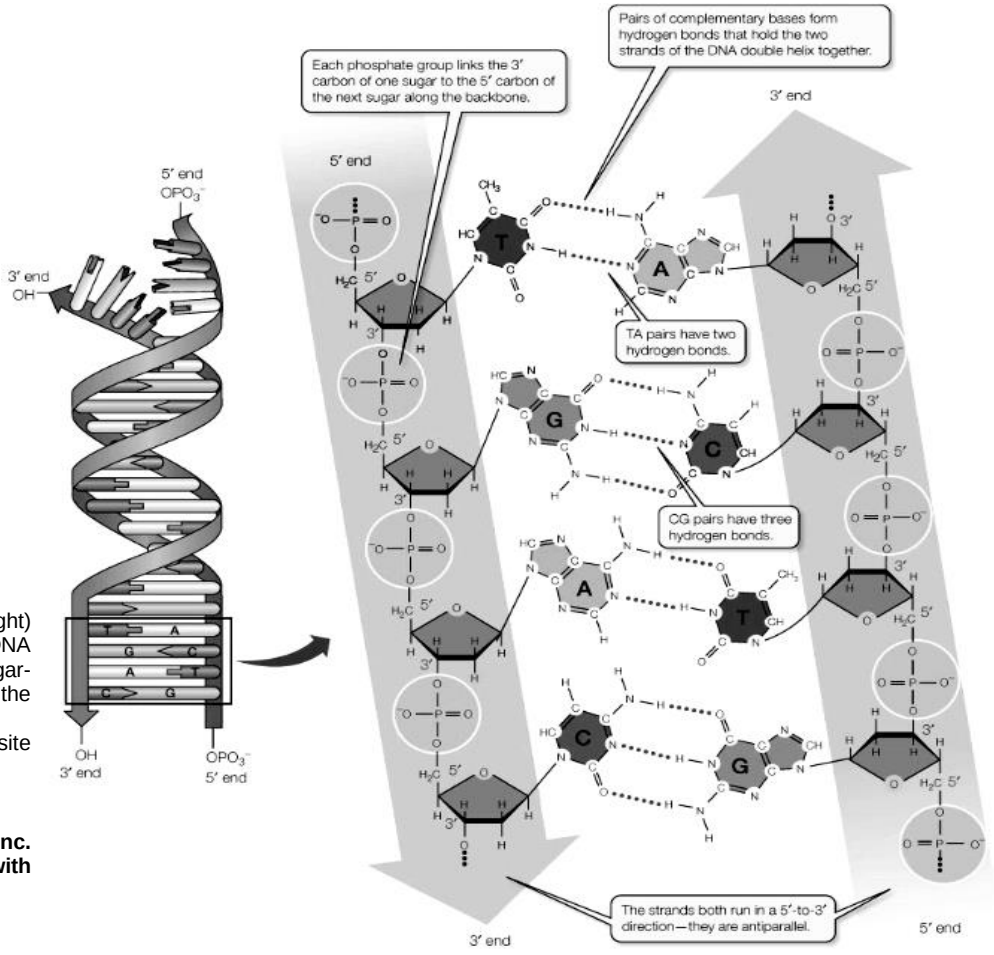
- Based on the above evidences, **James Watson** and **Francis Crick** of the Cavendish Laboratory in Cambridge began building scale models of polynucleotides in the hope that a convincing structure would emerge.
  - According to the proposed model, which was published in 1953, DNA exist as a double helix in which the two polynucleotide strands are coiled about each other in a right-handed (anti-clockwise) helix.

Secondary Structure of DNA – The Double Helix





(A): Francis Crick (left) and James Watson (right)
(B): Diagram (left) and space-filling model (right) of Watson-Crick double helix model of the structure of DNA



(C): Schematic representation (right) of a short segment of a DNA molecule, showing (i) the sugar-phosphate backbones of the polynucleotide strands,
(ii) their antiparallel nature (opposite chemical polarity), and
(iii) complementary base pairing

Main Features of the Double Helix

The double-helical model of DNA proposed by Watson and Crick (1953) has the following main features:

1. DNA consists of <u>two</u> polynucleotide strands.
2. Each polynucleotide strand forms a right-handed (anticlockwise when viewed from top down) helical spiral and the two strands coil around each other to form a **double helix**.
3. The diameter of the double helix is <u>2nm</u>.
4. The two polynucleotide strands ran in <u>opposite directions</u> i.e. they are **antiparallel**. One strand is oriented in the <u>5' to 3' direction</u> while the other is oriented in the <u>3' to 5' direction</u>.
5. Each strand has a <u>sugar-phosphate backbone</u>, with:
    - phosphate groups that project outside the double helix, and
    - nitrogenous bases that orientate inwards towards the central axis at almost right angles
        - This orientation is important because it puts the relatively hydrophobic nitrogenous bases in the molecules interior and thus away from the surrounding aqueous medium.
6. Specific, **complementary base pairing** occurs between adenine (A) and thymine (T) and between cytosine (C) and guanine (G).
7. The bases of the opposite strands are bonded together by relatively weak hydrogen bonds.
    - A -T base pair is held by <u>two</u> hydrogen bonds
    - C-G base pair is held by <u>three</u> hydrogen bonds

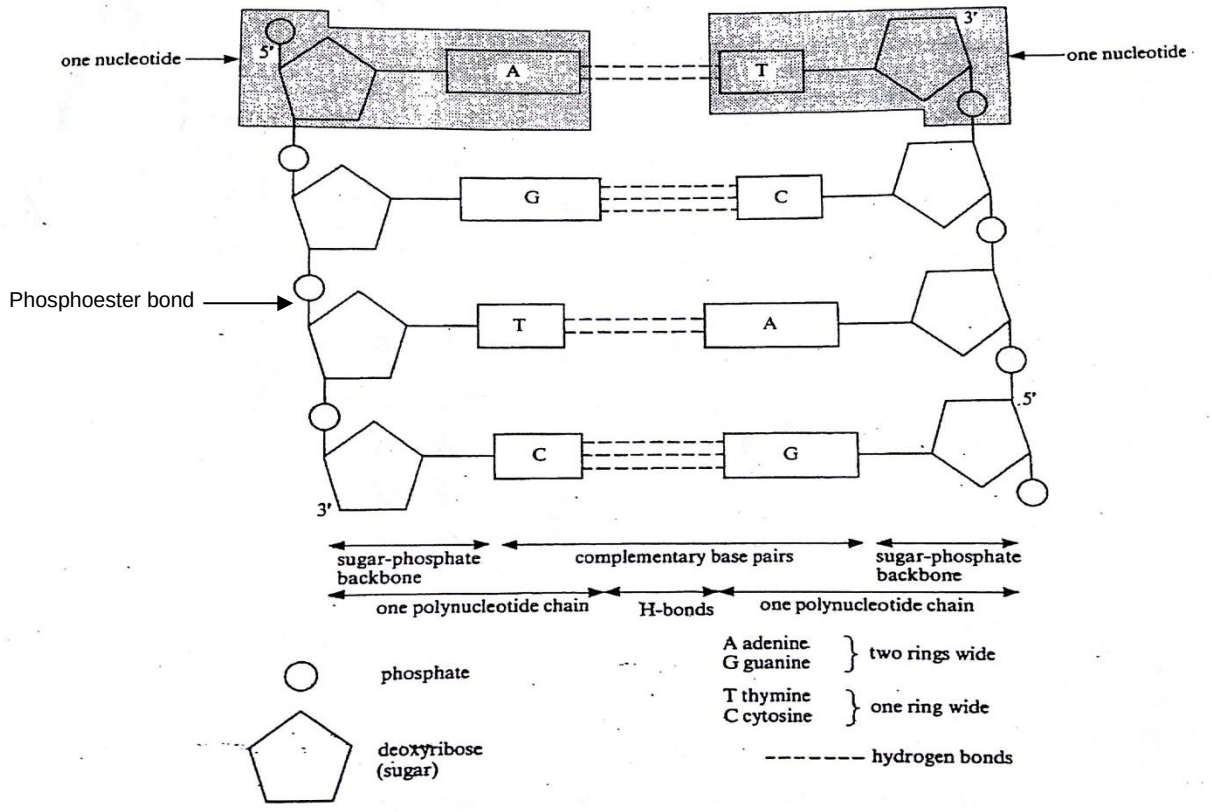
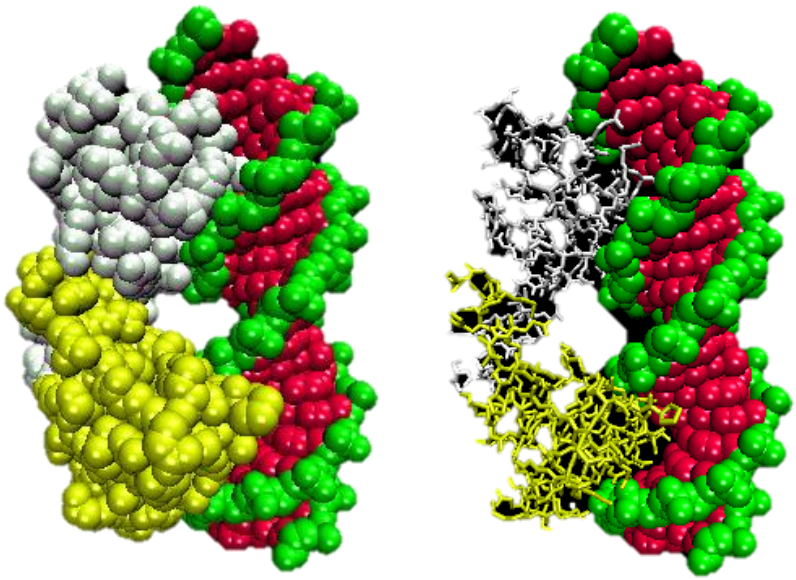


Fig 5.48 *DNA – diagrammatic structure of straightened chains*

8. The number of nucleotides containing adenine is equal to the number of nucleotides containing thymine, while number of nucleotides containing cytosine is equal to the number of nucleotides containing guanine. Thus <u>ratio of A to T is 1:1</u> and <u>ratio of C to G is 1:1</u>
9. Along the central axis of the DNA molecule, the base pairs are stacked <u>0.34nm</u> apart. One complete turn of the double helix has ten base pairs and spans a distance of <u>3.4nm</u>.

10. Because of the way of the bases bond with each other, the two sugar phosphate backbones of the double helix are not equally spaced along the helical axis.
    - This result in grooves of unequal sizes between the backbones called the **major groove** and **minor groove**.
    - Both these grooves are large enough to allow protein molecules to make contact with the bases.



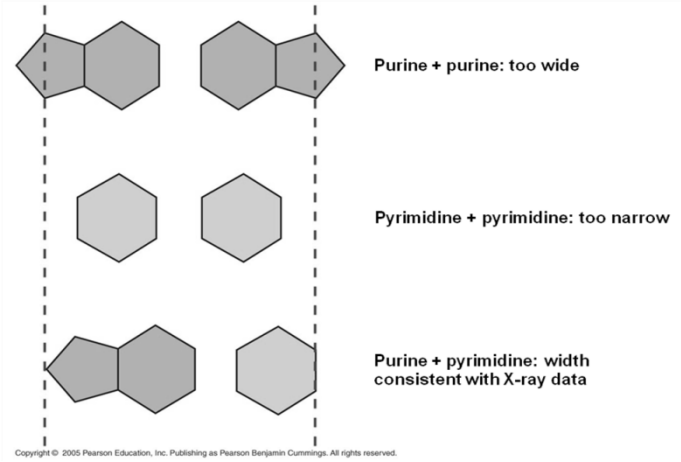
Figures show two proteins binding to major grooves of DNA

Source: DNA binding proteins - BioLibretext

Why do the bases form specific, complementary base pairing?

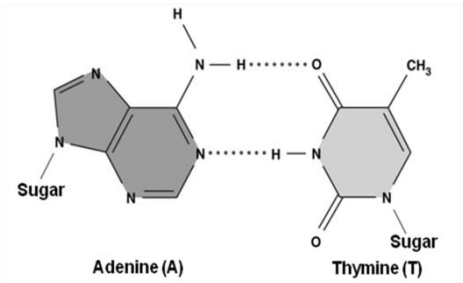
1. Available space within the double helix

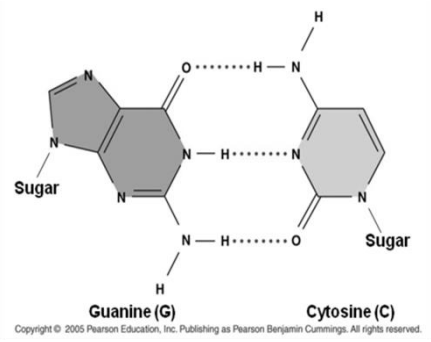
- The DNA double helix has a uniform diameter of 2 nm.
- **T** and **C** are pyrimidines, which have a single ring. **A** and **G** are purines, which are about twice as wide as pyrimidines.
- A purine-purine pair is too wide and a pyrimidine-pyrimidine pair is too narrow to account for the 2nm diameter of the double helix.
  - → Solution: pair a purine with a pyrimidine.



2. Hydrogen-bonding ability of the bases

- Each nitrogenous base has chemical side groups (such as H, N and O) that can form hydrogen bonds with its appropriate partner.
- Such chemical side groups in purines and pyrimidines have well defined positions.
- **A** can form 2 hydrogen bonds with **T**; **G** can form 3 hydrogen bonds with **C**.





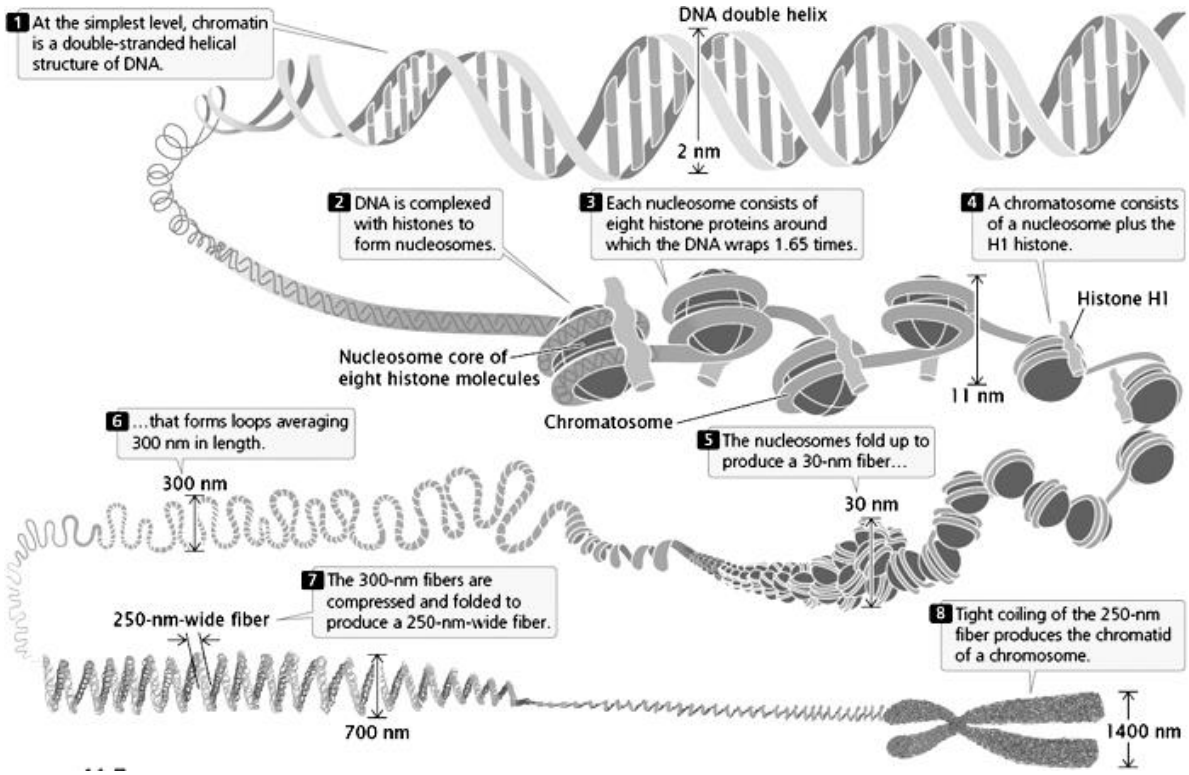
What is the significance of the complementary base pairing?

- Specific, complementary base pairing between **A**-**T** and **G**-**C** meant that the nucleotide sequence in one strand could dictate the nucleotide sequence in the complementary strand.
- For example, if one strand has the sequence 5'-GAATTC-3', the complementary, antiparallel strand would have the sequence 3'- CTTAAG – 5'
- This is necessary in DNA replication and transmission of the information stored in the molecule. If the base sequence in one of the strands is accidentally altered, the cell can repair the damaged strand by using the remaining intact partner as a template.

Stability of the DNA molecule

- In order for DNA to be a <u>stable storage</u> of genetic information, its structure must be relatively <u>resistant to spontaneous changes</u>.
- The high degree of stability of the DNA double helix results from:
  1. <u>Phosphoester bonds</u> between nucleotides are strong covalent bonds.
     - They confer strength and stability on the polynucleotide strand, preventing breakage of the strand during DNA replication.
  2. A large number of <u>hydrogen bonds</u> between base pairs. (note: each hydrogen bond, by itself is weak)
  3. <u>Hydrophobic interactions</u> between the stacked base pairs.
  4. The exposure to outside influences of only the sugar-phosphate backbone.
     - The potentially reactive side-groups of deoxyribose are already involved in various different bonds such as glycosidic and phosphoester bonds.

5. The involvement of nitrogenous bases in hydrogen bonds.
    - The nitrogenous bases which form the repository of genetic information are safely tucked inside the double helix and are rather immobilised by the geometric tightness of the molecule.
6. [ONLY in eukaryotes] The DNA double helix is tightly wound around an equal mass of histones (positively-charged proteins) to form a repeating array of nucleosomes.
    - The histones form ionic bonds with the negatively charged phosphate groups of DNA.
    - The nucleosomes are eventually folded into higher order structures such as chromosomes, in which DNA is prevented from thermal and physical damage.



Annunziato, A. (2008) DNA packaging: Nucleosomes and chromatin. *Nature Education*

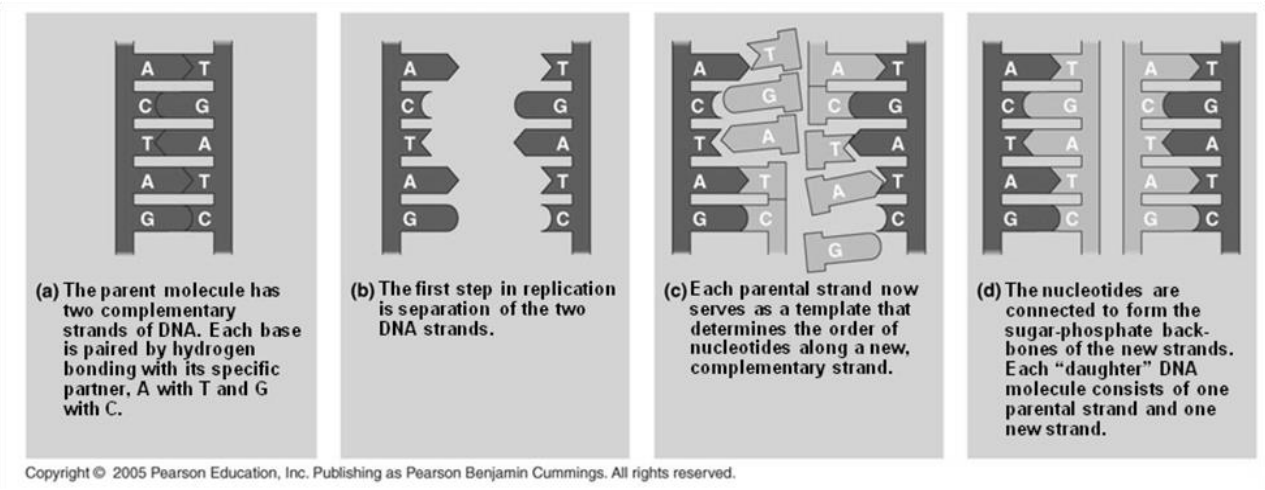
III. Replication of DNA

A. DNA Strands as Templates for Replication

- The idea that there is specific pairing of nitrogenous bases in DNA led Watson and Crick to the correct double helix. At the same time, they saw a functional significance of the base-pairing rules:

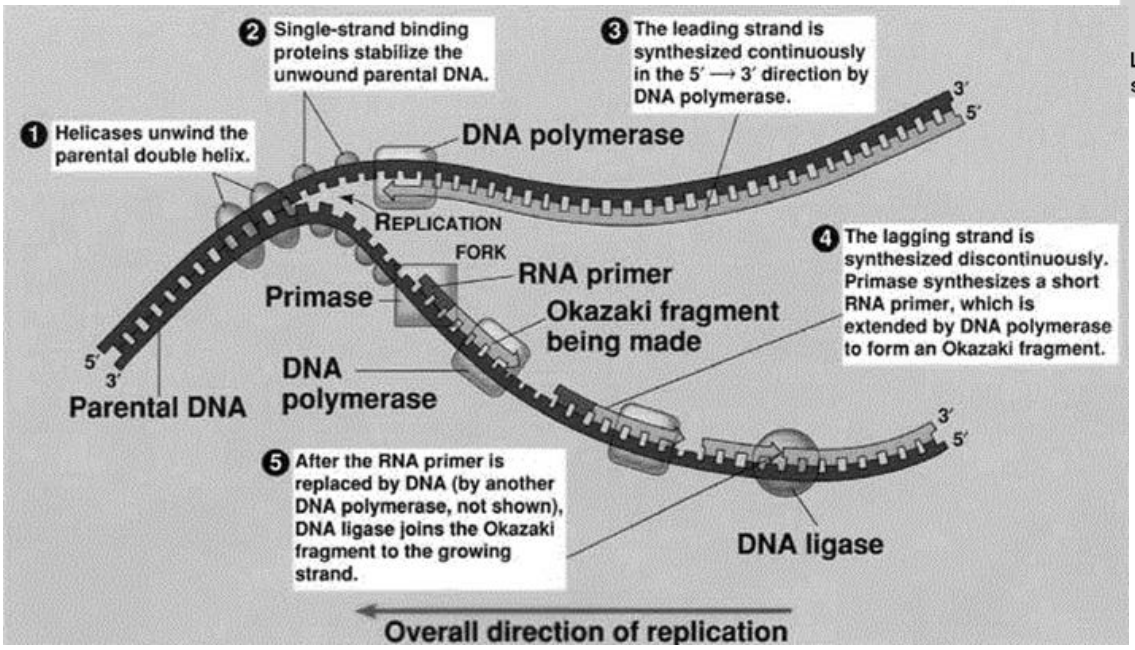
  "It has not escaped our notice that the specific pairing that we have postulated immediately suggests a possible copying mechanism for the genetic material."

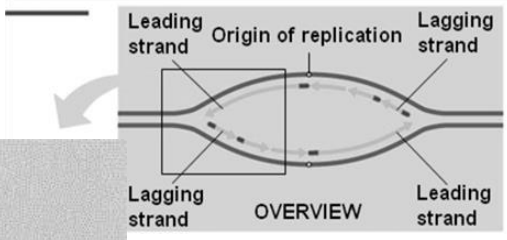
- The two strands of DNA are **complementary** – each stores the information necessary to reconstruct the other.
- When a cell copies a DNA molecule, each strand serves as a **template** for the synthesis of new, complementary strand. Nucleotides line up along the template strand according to the base-paring rules and are linked to form a new strand.
- Where there was one double-stranded DNA molecule at the beginning of the process, there are now two DNA molecules – each an exact replicate of the 'parent' molecule to ensure faithful transmission of genetic information.



B. Mechanism of DNA Replication

- The mechanism of DNA replication as suggested by Watson and Crick's semi-conservative model is conceptually simple. This involves separation of parental DNA strands, whereby each acts as a template for the synthesis of a new daughter strand. The daughter molecule therefore comprises one parental strand and a new daughter strand.
- The mechanism of DNA replication involves the following steps:
  1. **Location of origin of replication**
  2. **Separation of parental DNA strands**
  3. **Synthesis of RNA primer**
  4. **Synthesis of daughter DNA strands**





A summary of DNA replication.
The detailed diagram (left) shows one replication fork, but as indicated in the overview diagram (right), replication usually occurs simultaneously at two forks, one at either end of a replication bubble. Notice in the overview diagram is initiated by a RNA primer, as is each Okazaki fragment in a lagging strand. Viewing each daughter strand in its entirety, you can see that half of it is made continuously as a leading strand, while the other half (on the other side of the origin) is synthesised in fragments as a lagging strand.

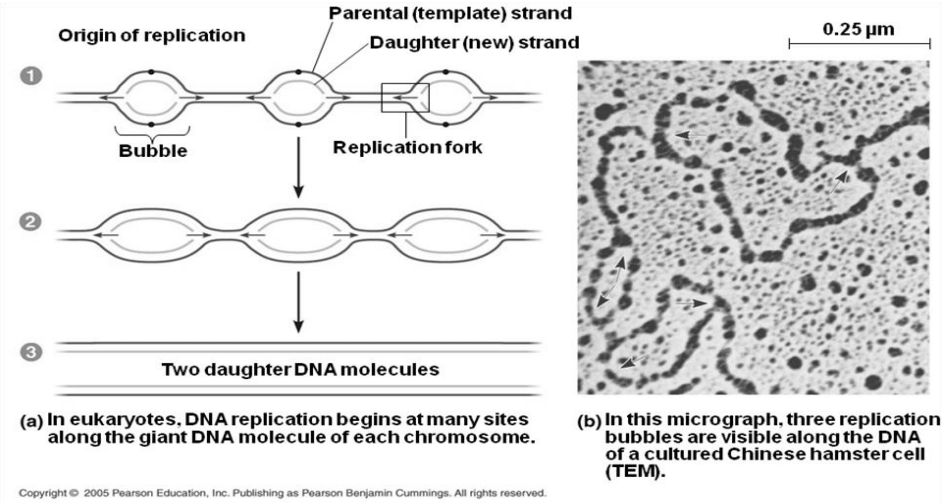
1. Location of Origin of Replication

1. Replication of a DNA molecule begins at specific sites called **origins of replication**.
2. The two polynucleotide strands separate and form a **replication bubble**. At each end of a replication bubble is a **replication fork** (a Y-shaped region where the new strands of DNA are elongating).
3. Replication of DNA proceeds in <u>both</u> directions, until the entire molecule is copied.

1) Replication begins at specific sites where the two parental strands separate to form replication bubbles.

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

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



(a) In eukaryotes, DNA replication begins at many sites along the giant DNA molecule of each chromosome.

(b) In this micrograph, three replication bubbles are visible along the DNA of a cultured Chinese hamster cell (TEM).

2. Separation of Parental Strands

During replication, there is a continual need for the separation of the base pairs of the parental DNA molecule so that DNA strands can act as templates for the synthesis of daughter DNA strands. Three types of proteins are involved in the separation of parental DNA strands: (i) Helicase, (ii) Single-strand DNA-binding proteins, and (iii) Topoisomerase.

1. **Helicase** catalyses the breakage of hydrogen bonds, thus separating the two parental DNA strands.
    - The parental DNA strands have the tendency to bind together via hydrogen bonds. Hence, helicase activity requires energy (in the form of ATP) to break the bonds and keep the strands apart.
2. **Single-strand DNA-binding proteins (SSB proteins)** bind tightly to single-stranded regions of DNA to help maintain the stability of the replication fork.
    - Single-stranded DNA is very labile → binding of SSB proteins keeps it from being degraded.
    - Binding of SSB proteins also keeps the single-stranded DNA in an extended configuration and prevents it from folding back on itself. This ensures that the DNA is still "readable" by DNA polymerase.

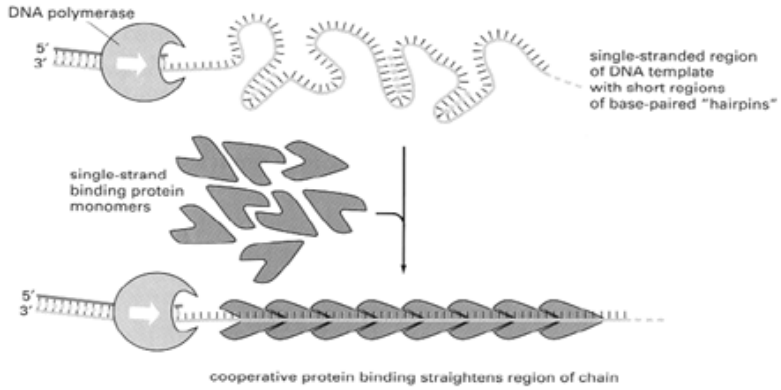
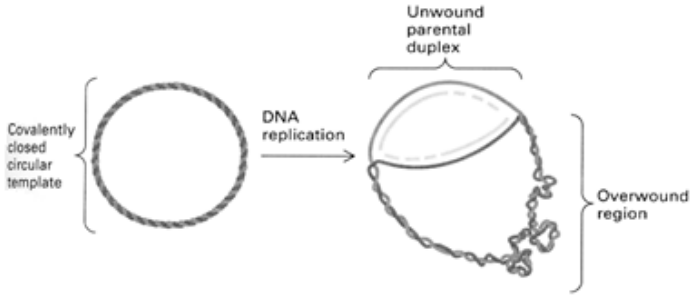


Figure 5–17 The effect of single-strand DNA-binding proteins (SSB proteins) on the structure of single-stranded DNA. Because each protein molecule prefers to bind next to a previously bound molecule, long rows of this protein form on a DNA single strand. This *cooperative binding* straightens out the DNA template and facilitates the DNA polymerization process. The "hairpin helices" shown in the bare, single-stranded DNA result from a chance matching of short regions of complementary nucleotide sequence; they are similar to the short helices that typically form in RNA molecules (see Figure 1–6).

3. **Topoisomerase** create a transient break by nicking a strand of DNA. This helps to **unwind** the double helix ahead of the replication fork for initiation of replication.
    - The tension holding the DNA double helix in its coiled and supercoiled structure can thus be relieved.



◀ FIGURE 10-21 Movement of the growing fork during DNA replication induces formation of positive supercoils in the duplex DNA ahead of the fork. In order for extensive DNA synthesis to proceed, the positive supercoils must be removed (relaxed). This can be accomplished by *E. coli* DNA gyrase and by eukaryotic type I and type II topoisomerases. [Adapted from A. Kornberg and T. Baker, 1992, *DNA Replication*, 2d ed., W. H. Freeman and Company, p. 380.]

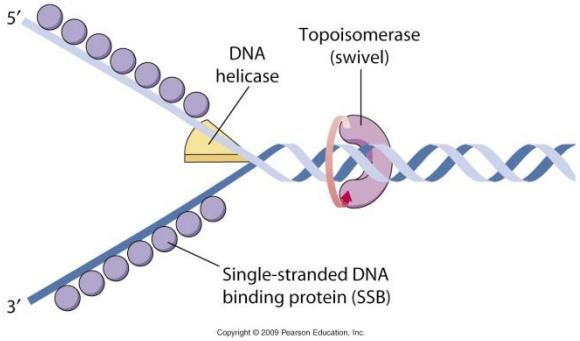
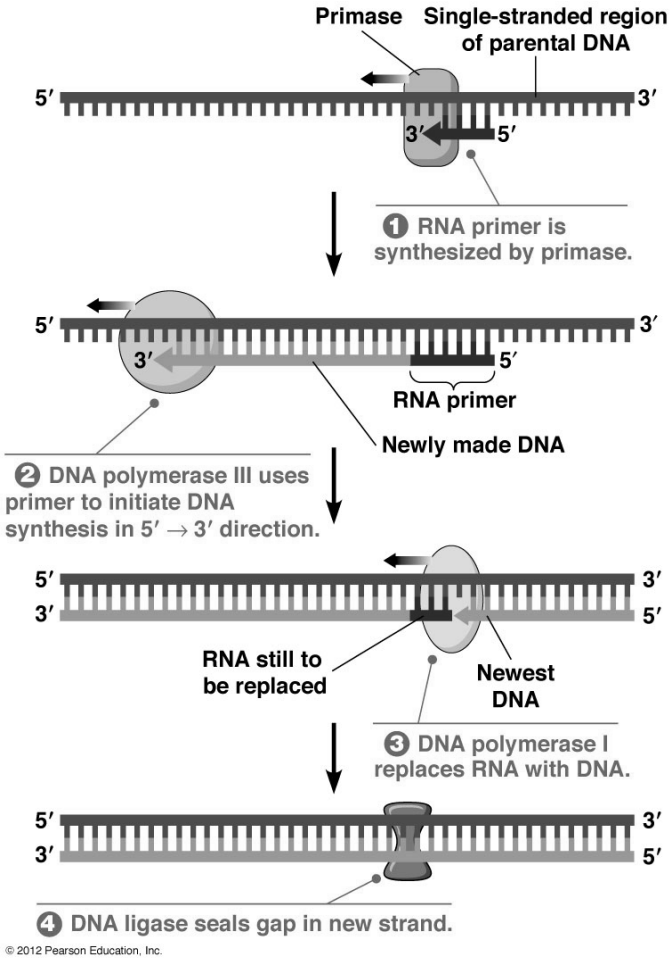


Figure 15-10 Proteins Involved in Unwinding the DNA at the Replication Fork. Three types of proteins are required for this aspect of DNA replication. The actual unwinding proteins are the helicases; the principal one in *E. coli*, which is part of the primosome, operates 5′ → 3′ along the template for the lagging strand, as shown here. Single-strand binding proteins (SSB) stabilize the unwound DNA in an extended position. A topoisomerase forms a swivel ahead of the replication fork; in *E. coli*, this topoisomerase is DNA gyrase.

3. Synthesis of RNA Primers

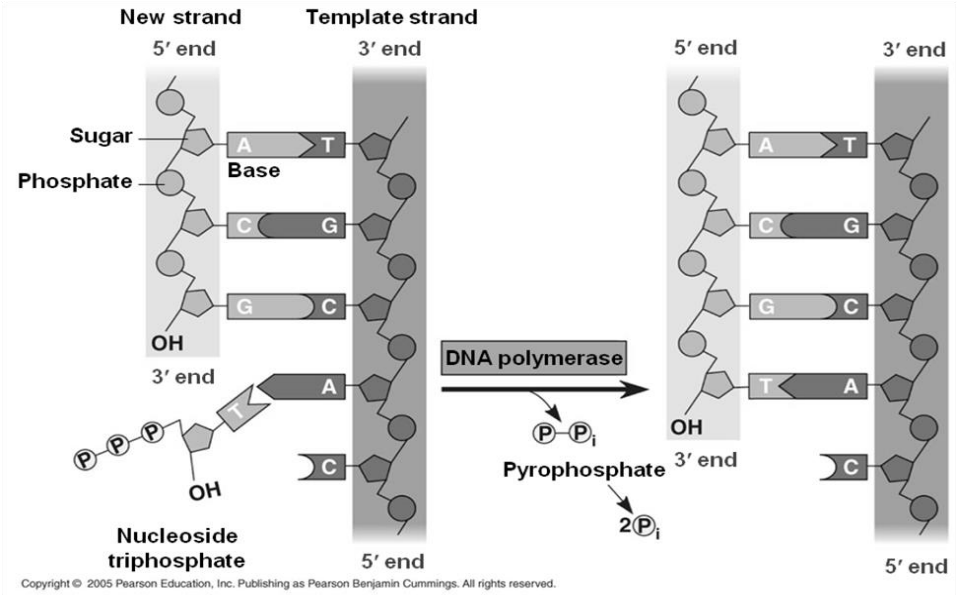
Enzymes that synthesise DNA cannot initiate the synthesis of a polynucleotide; they **can only add nucleotides to the 3' OH end of an already existing strand** that is base-paired with the template strand. The initial oligonucleotide – known as a primer - is actually a short stretch of RNA, not DNA.

1. A portion of each parental DNA strand serves as a template for making the RNA primer with complementary base sequence.
2. An enzyme called **primase** catalyses synthesis of RNA primer in 5' to 3' direction.
    - RNA primer is about 10 nucleotides long in eukaryotes.
3. **DNA polymerase (III)** can now elongate the strand by adding the next dNTP to the free 3' hydroxyl group of the primer.
4. **DNA polymerase (I)** will later replace RNA nucleotides of the primers with DNA nucleotides.

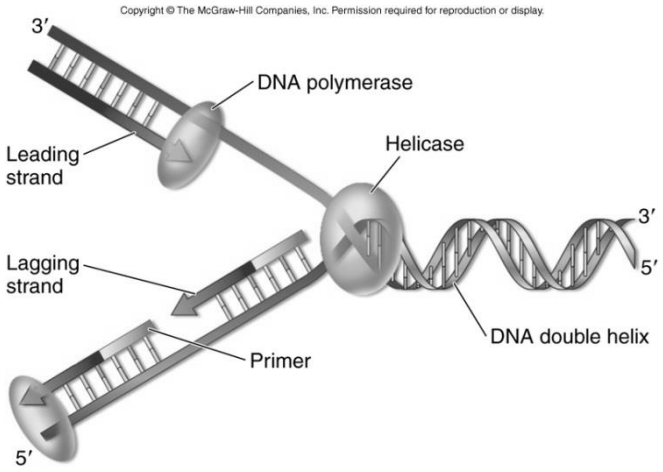


4. Synthesis of Daughter DNA Strands

1. The separated parental DNA strands form the template along which **deoxyribonucleoside triphosphates** (dNTPs) align themselves by complementary base pairing i.e. adenine to thymine and guanine to cytosine.
2. **DNA polymerase (III)** catalyses the formation of a phosphoester bond between the 3' hydroxyl group of the last nucleotide in the growing strand and the 5' phosphate group of the incoming dNTP.
    - In this process, the incoming dNTP loses two of its phosphates as a pyrophosphate molecule when they form the covalent phosphoester bond with the growing strand. The energy released from pyrophosphate bond breakage is coupled to phosphoester bond formation.
3. DNA polymerases (III) thus catalyses the polymerisation of the new DNA strand in 5' to 3' direction.



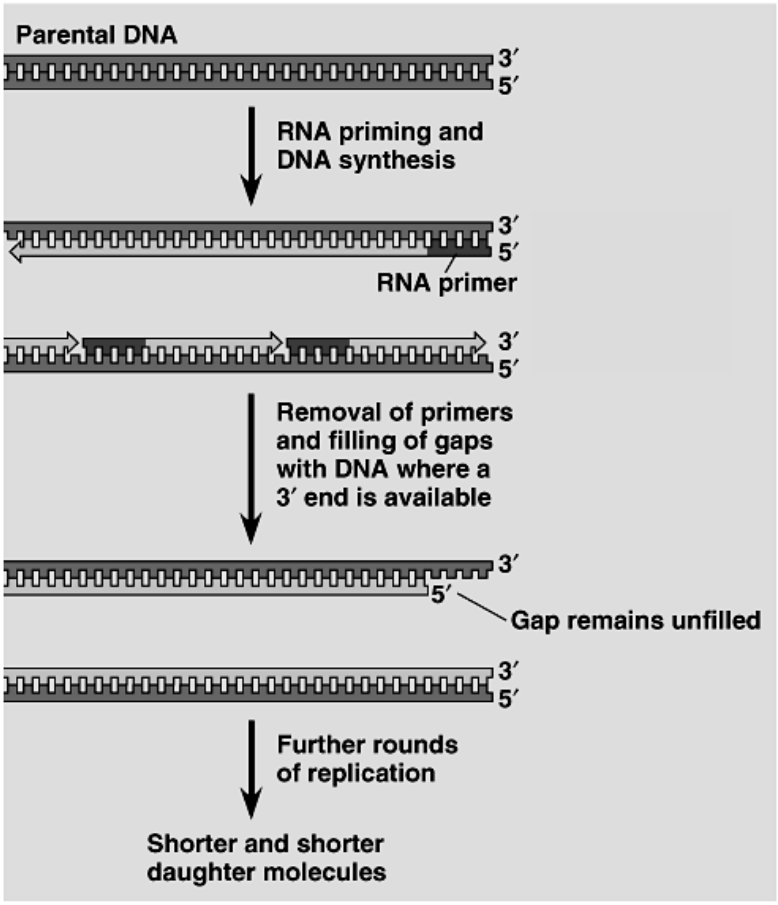
4. Each growing new DNA strand is antiparallel to its parental template strand.
5. The **leading strand** is synthesised continuously as a single polymer along the template strand.
    - The leading strand is polymerised in the mandatory 5' to 3' manner towards replication fork.
6. The **lagging strand** is synthesised discontinuously as a series of short fragments called **Okazaki fragments** along the template strand.
    - Each Okazaki fragment requires an RNA primer for strand initiation.
    - Each Okazaki fragment is polymerised in the mandatory 5' to 3' manner against the overall direction of the replication fork. About 100 to 200 nucleotides per fragment in eukaryotes.



7. **DNA ligase** catalyses the formation of a **phosphoester bond** between the 3’ end of each new Okazaki fragment and the 5’ end of the growing strand to form a continuous strand.
8. Each daughter DNA molecule now consists of a newly synthesised strand and a parental strand.

End-Replication Problem

Upon the completion of DNA replication of linear DNA in eukaryotes, the RNA primers complementary to the 3’ ends of both daughter DNA molecules cannot be replaced after their removal. This results in the newly synthesised strands being shorter than the template strands. This is known as the **end replication problem**.



1. The end replication problem arises **due to the specificity of DNA polymerase**. DNA polymerase can only add deoxyribonucleotides to the free 3’-OH end of a pre-existing polynucleotide, not to the 5’ end. Without a 3’ hydroxyl group at the 5’ ends of the newly synthesised strand, the addition of the DNA nucleotides is not possible, resulting in a gap.
2. This progressive shortening of DNA strands with each replication limits the number of cell divisions to approximately 40–60 times, a phenomenon known as the **Hayflick limit**, before apoptosis (programmed cell death) is triggered.
3. Telomeres found at the ends of DNA molecules are used to buffer the loss of important genetic information as a result of this end replication loss.

C. DNA Repair and Maintenance

DNA Repair during Replication:

- The accuracy of DNA replication cannot be attributed solely to the specificity of base pairing. DNA repair mechanisms do contribute to the accuracy.
    - errors in the completed DNA molecule amount to only one in a billion nucleotides
    - initial pairing errors between nucleotides and those in the template strand are 100 000 times more common – an error rate of one in 10 000 base pairs.
- During DNA replication, DNA polymerases proofread each nucleotide against its template as soon as the nucleotide is added to the growing strand. Such proofreading is achieved by the 3' to 5' exonuclease activity of the DNA polymerase.

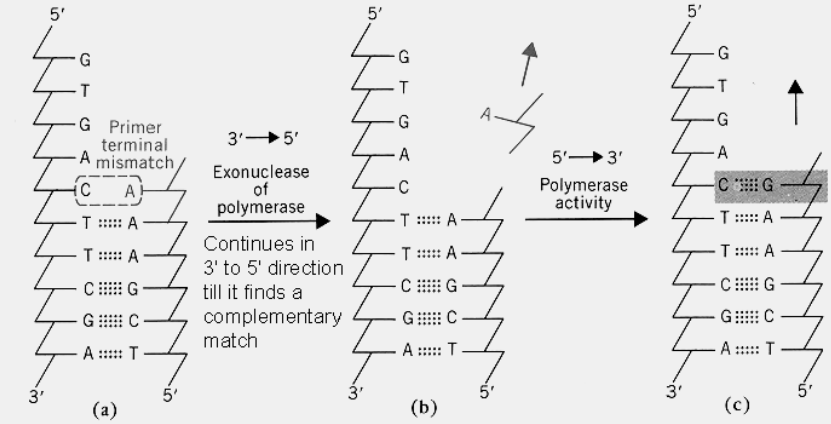


Figure 5.27 "Proofreading" by the 3′⟶5′ exonuclease activity of DNA polymerases during DNA replication. If DNA polymerase is presented with a template and primer containing a 3′ primer terminal mismatch (a), it will not catalyze covalent extension ("polymerization"). Instead, the 3′⟶5′ exonuclease activity, an integral part of many DNA polymerases, will cleave off the terminal mismatched nucleotide (b). Then, presented with a correctly base-paired primer terminus, DNA polymerase will catalyze 5′⟶3′ covalent extension of the primer strand (c).

DNA Repair and Maintenance of Existing DNA:

- Maintenance of the genetic information encoded in DNA requires frequent repair of various kinds of damage to existing DNA.
- DNA molecules are constantly subjected to potentially harmful chemical and physical agents, such as:
    - Reactive chemicals such as free radicals (in the environment and occurring naturally in cells)
    - Radioactive emissions
    - X-rays
    - Ultraviolet light

  which can change nucleotides in ways that can affect encoded genetic information, usually adversely.
- Fortunately, changes in DNA are usually corrected before they become self-perpetuating mutations
    - Each cell continuously monitors and repairs its genetic material
    - Because repair of damaged DNA is so important to the survival of an organism, it is no surprise that many DNA repair enzymes have evolved.
- Most mechanisms for repairing DNA damage take advantage of the base-paired structure of DNA.
    - Usually, a segment of the strand containing the damage is excised by a DNA cutting enzyme – a **nuclease**.
    - The resulting gap is filled in with nucleotides properly paired with nucleotides in the undamaged strand.
    - This type of repair is called nucleotide excision repair.

- One function of the DNA repair enzymes in our skin cells is to repair genetic damage caused by ultraviolet rays of the sunlight
  - One such type of damage is the covalent linkage of thymine bases that are adjacent on a DNA strand.
  - Such thymine dimers cause the DNA to buckle and interfere with DNA replication.
  - Inherited genetic defect in the nucleotide excision repair enzymes – xeroderma pigmentosum. Individuals with this disorder are hypersensitive to sunlight. Mutations in their skin cells caused by ultraviolet light are left uncorrected and cause skin cancer.