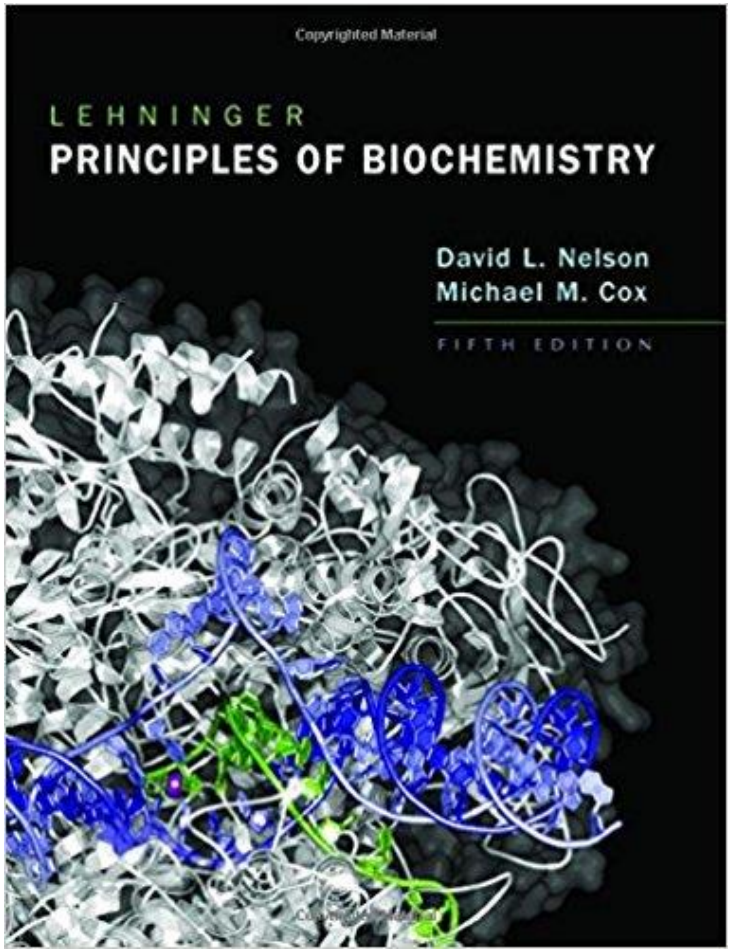
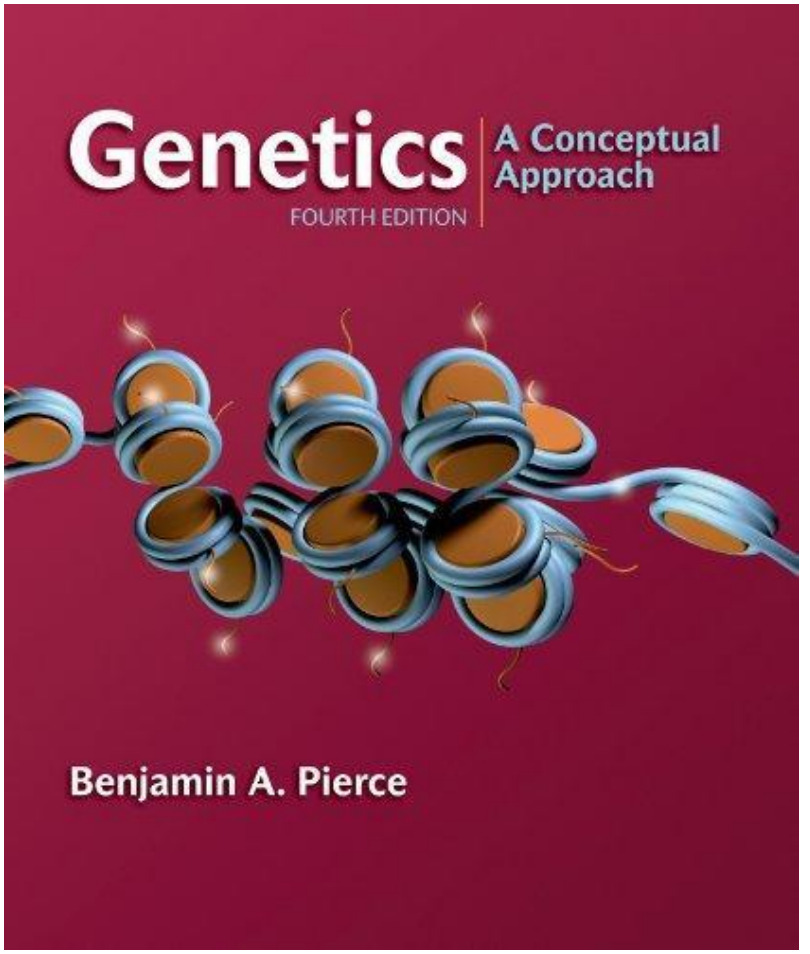
Textbooks



Lehninger Principles of Biochemistry
5th or 6th edition



Genetics A conceptual Approach
Fourth Edition

What is DNA?

- Deoxyribonucleic acid, is a complex molecule that contains all of the information necessary to build and maintain an organism (specify the structure and function of living things)
- The primary unit of heredity in organisms of all types.

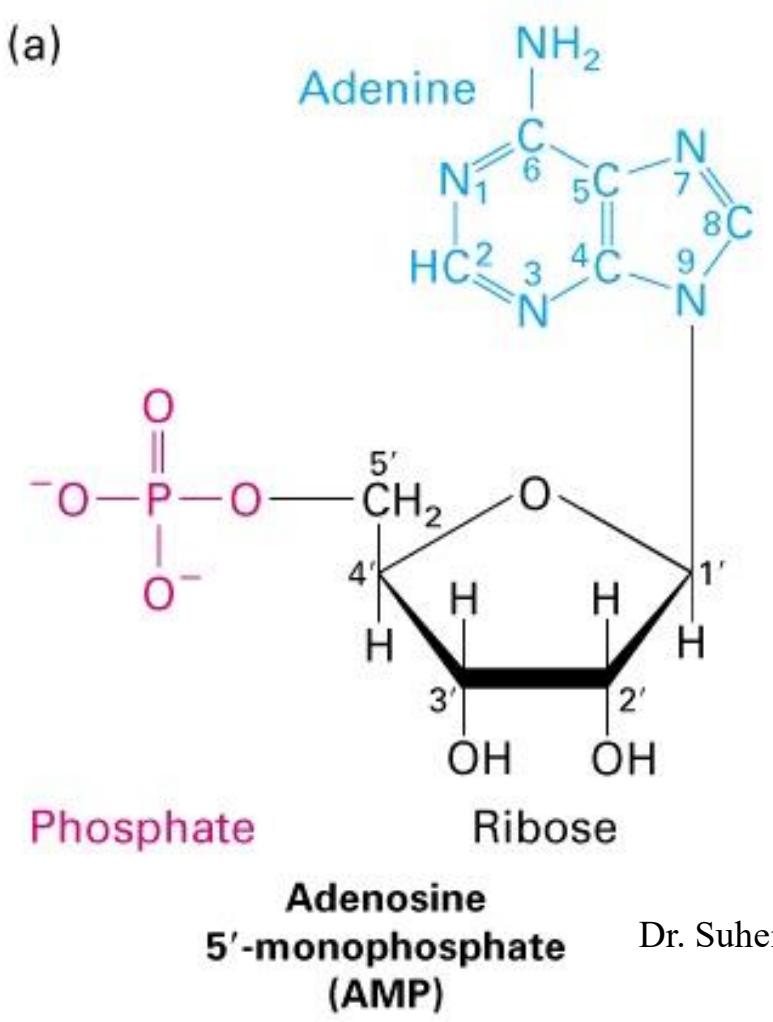
DNA Structure

DNA

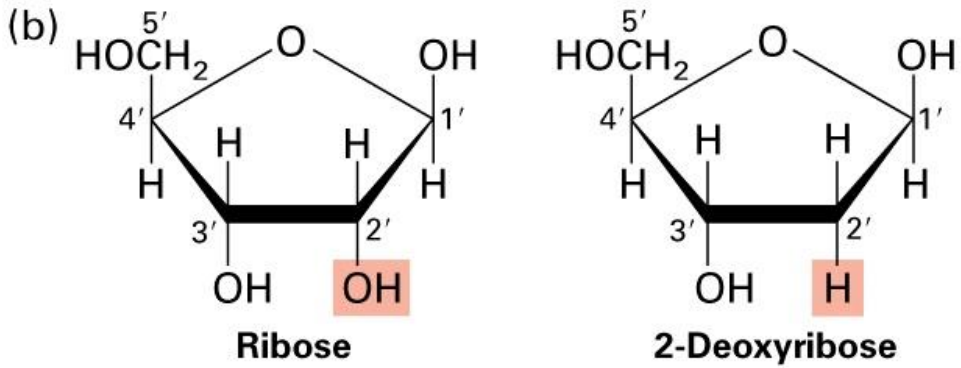
- The structure of DNA can be considered at three hierarchical levels:
- the primary structure of DNA is its nucleotide sequence
- the secondary structure is the double-stranded helix
- the tertiary structure refers to higher-order folding that allows DNA to be packed

All nucleotides have a common structure

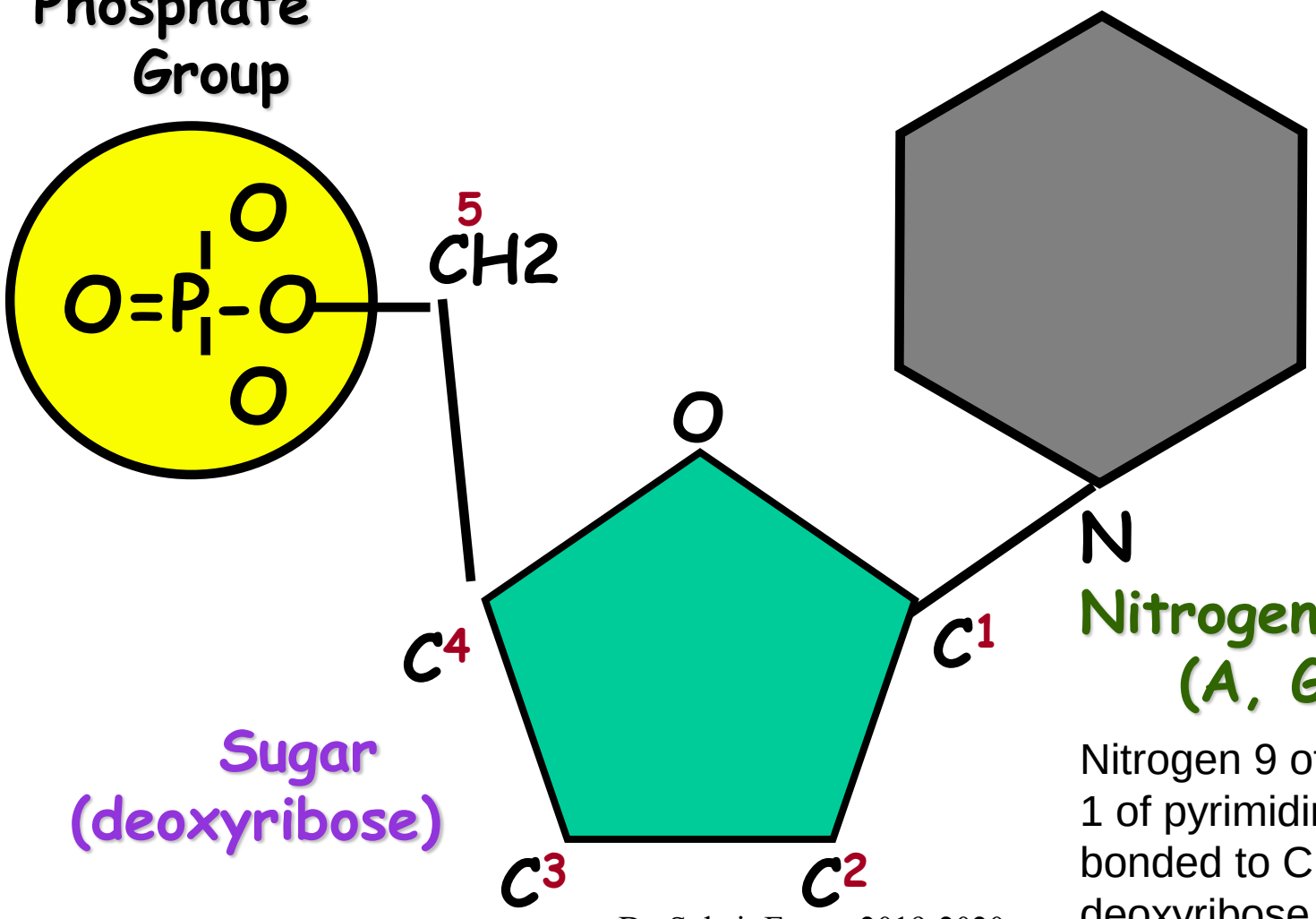
A nucleotide present in RNA



The pentoses in nucleic acids



Remember HOW the Carbons Are Numbered!



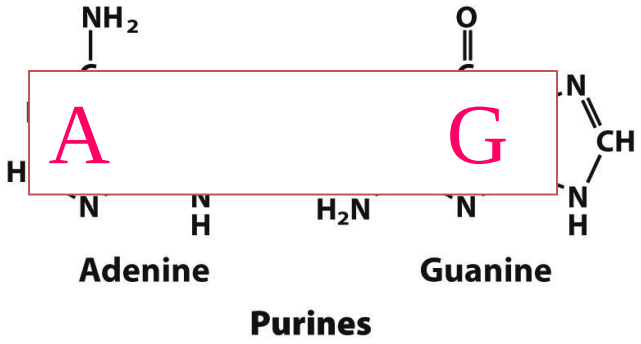
Sugar (deoxyribose)

Nitrogenous base (A, G, C, or T)

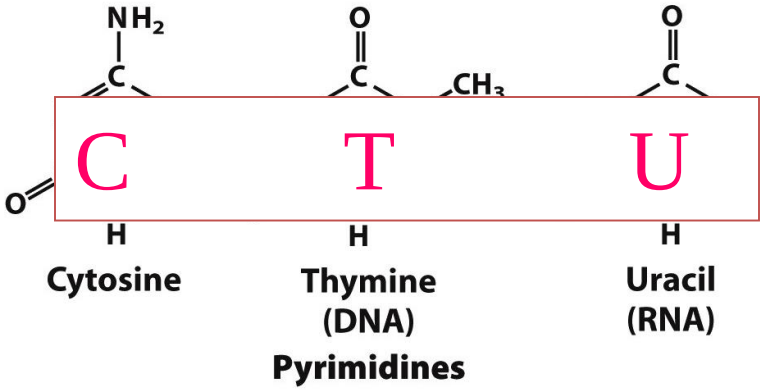
Nitrogen 9 of purines and 1 of pyrimidines are bonded to C1 of ribose or deoxyribose

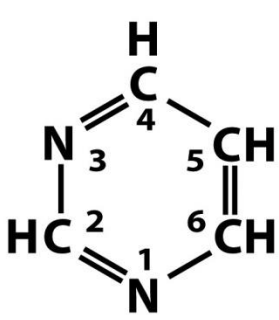
"Bases"

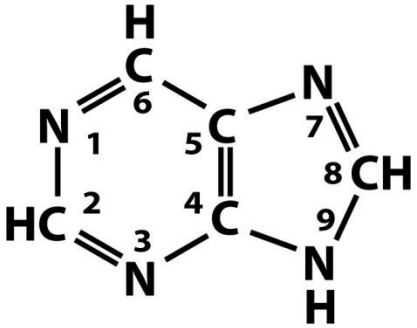
- 2 purine bases
  - Adenine: A
  - Guanine: G



- Bases
  - Pyrimidines
  - Purines







- 2 pyrimidine bases (in DNA)
  - Cytosine: C
  - Thymine: T
    - or Uracil: U

    (in RNA, instead of Thymine)

Figure 8-1b
Lehninger Principles of Biochemistry, Fifth Edition

DNA

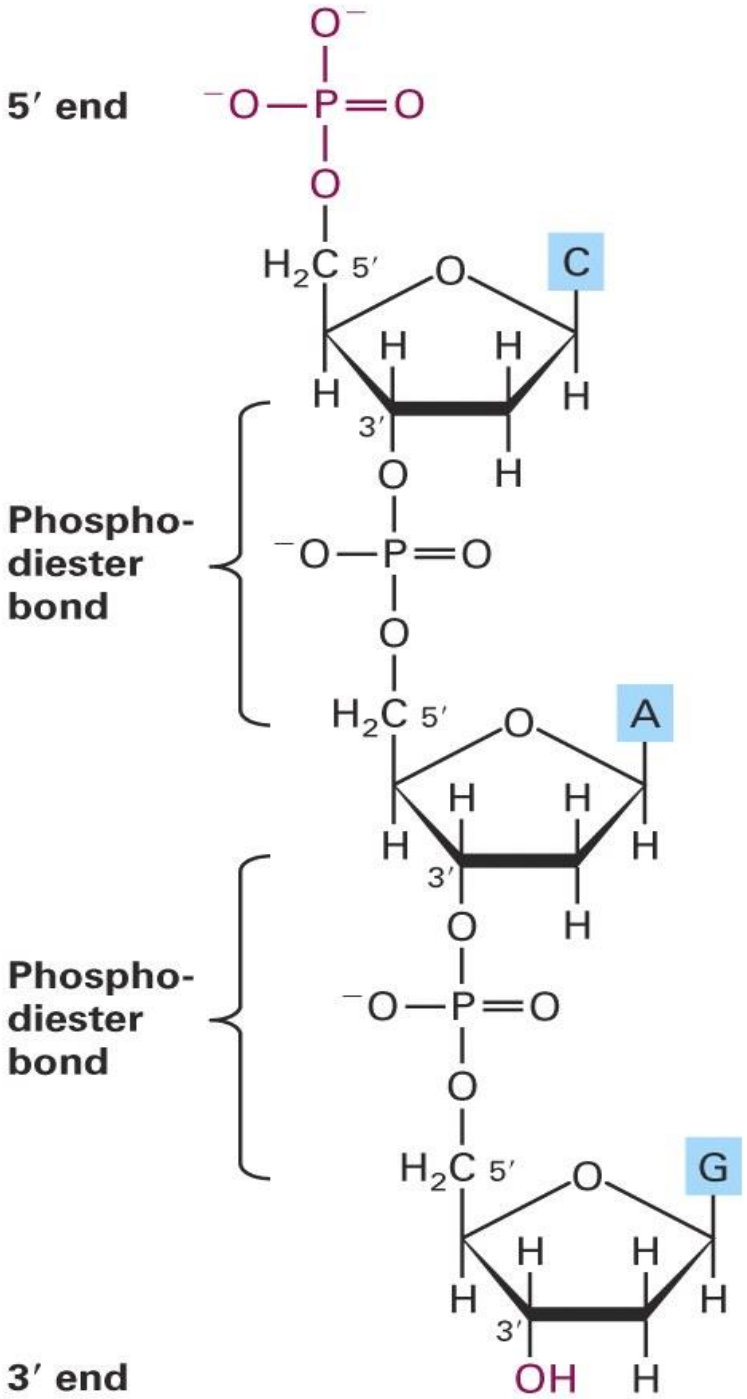
- Two strands coiled called a double helix
- Sides made of a pentose sugar Deoxyribose bonded to phosphate (PO_4) groups by phosphodiester bonds
- Center made of nitrogen bases bonded together by weak hydrogen bonds

Nucleotide subunits are linked together by phosphodiester bonds

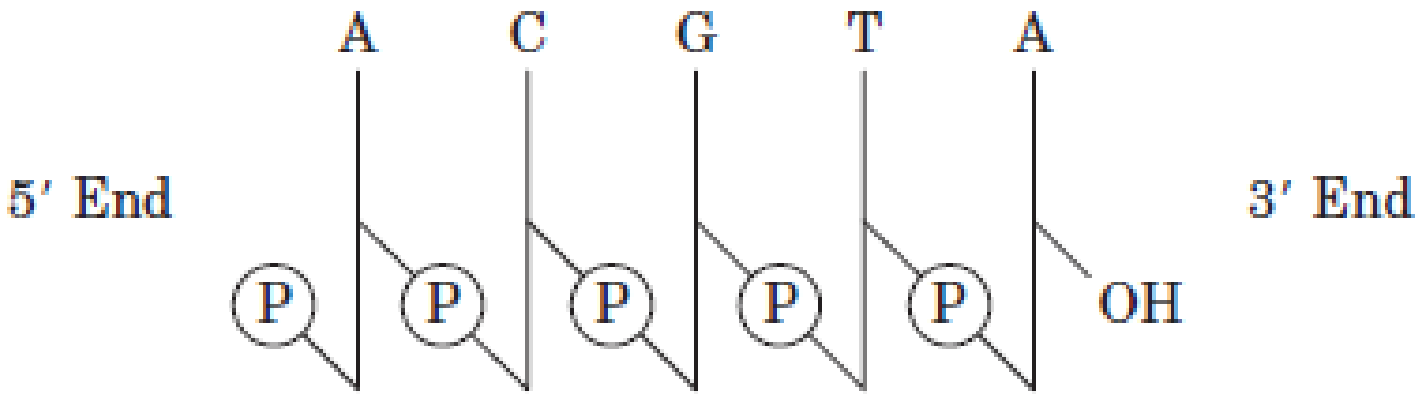
This nucleic acid chain is represented as (5’ C-A-G 3’)

always in 5’ to 3’ direction left to right.

5’ phosphate group on the 5’ of the sugar and a 3’ hydroxyl of the sugar



Polynucleotide



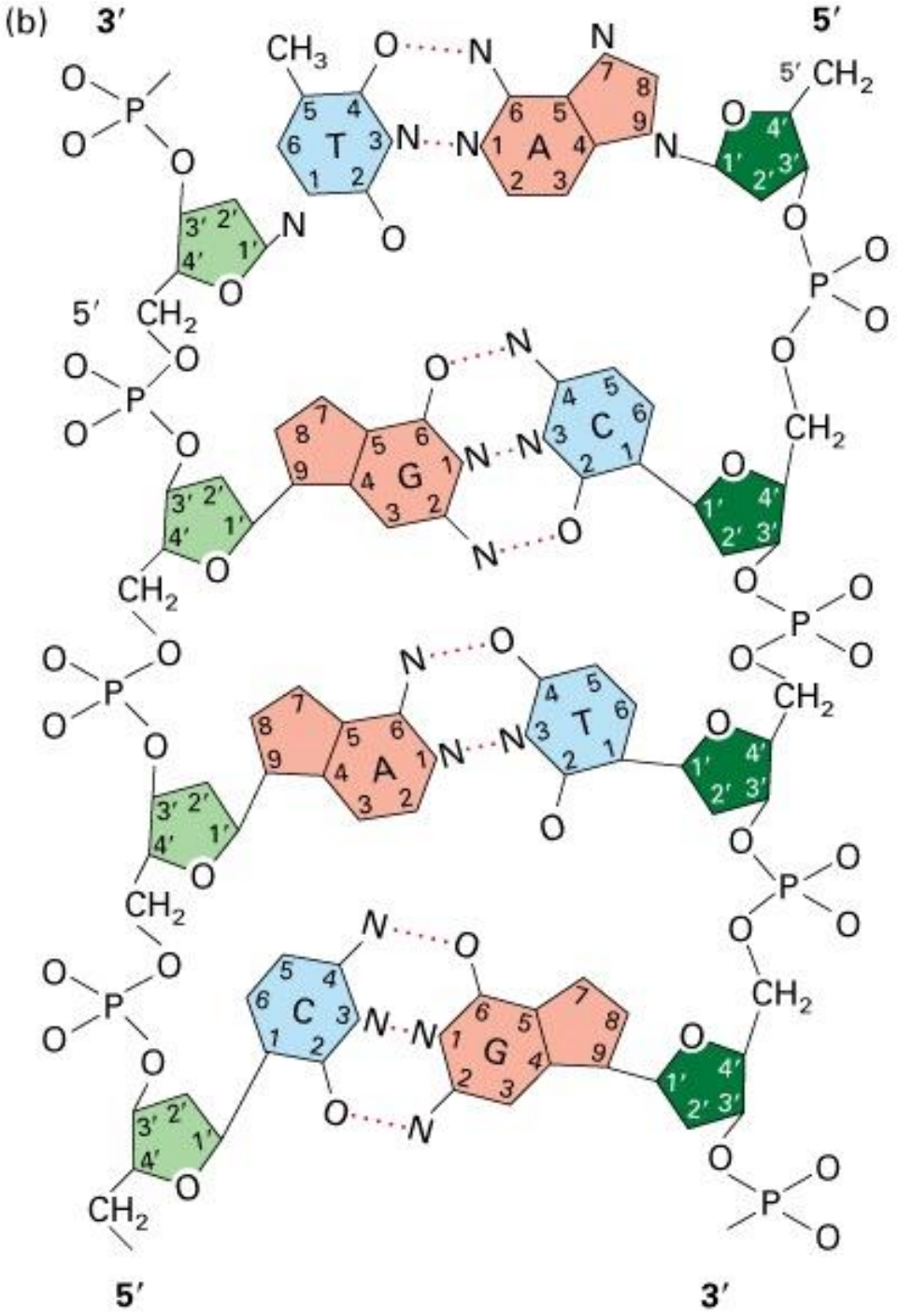
5-phosphate group of one nucleotide unit is joined to the 3-hydroxyl group of the next nucleotide, creating a **phosphodiester linkage**

Native DNA is a double helix of complementary antiparallel chains held together by:

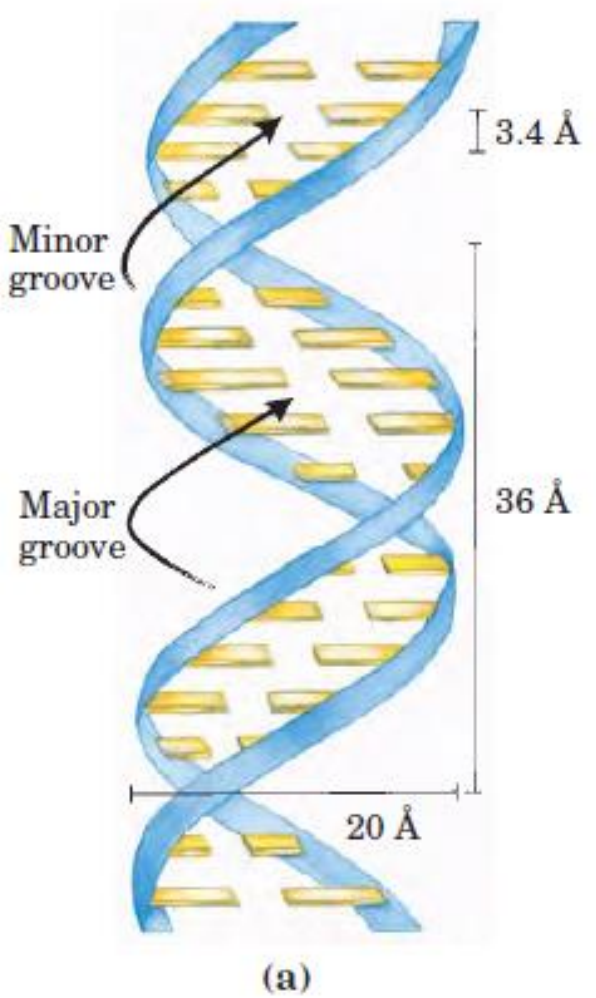
1- Hydrogen bonding between complementary base pairs (A-T or G-C)

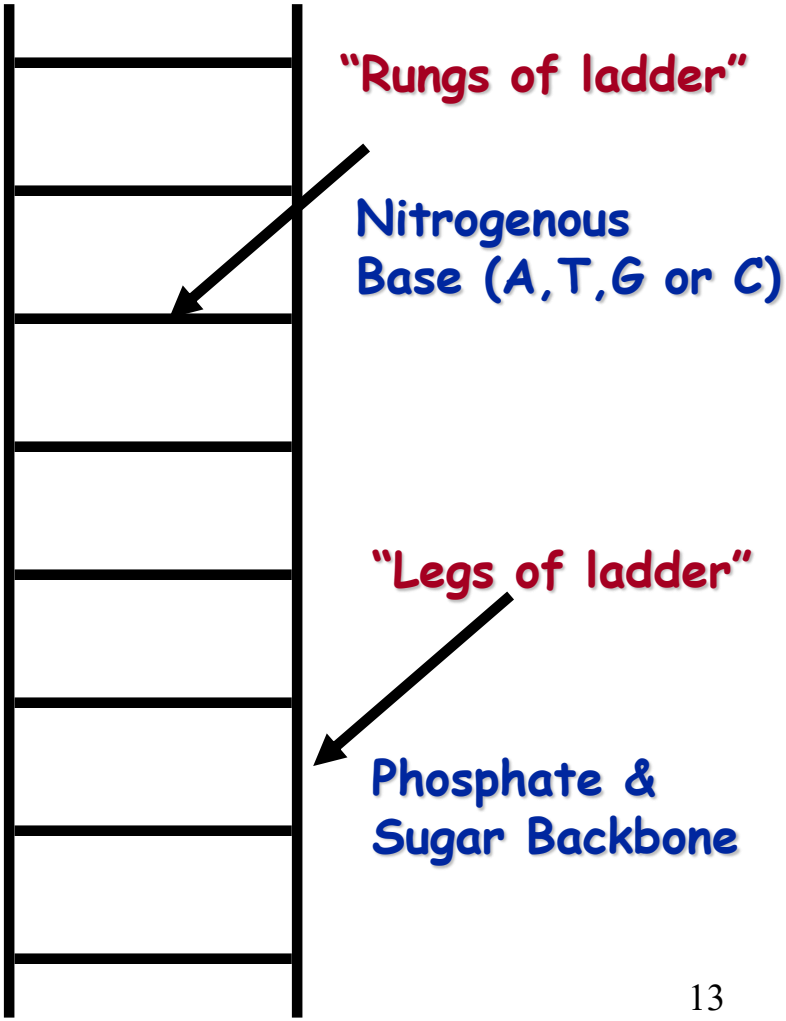
2- Hydrophobic interactions between planar bases/ stacked adjacent bases.

This contributes to the stability of the double helix.
Nitrogen 9 of purines and 1 of pyrimidines are bonded to C1 of ribose or deoxyribose



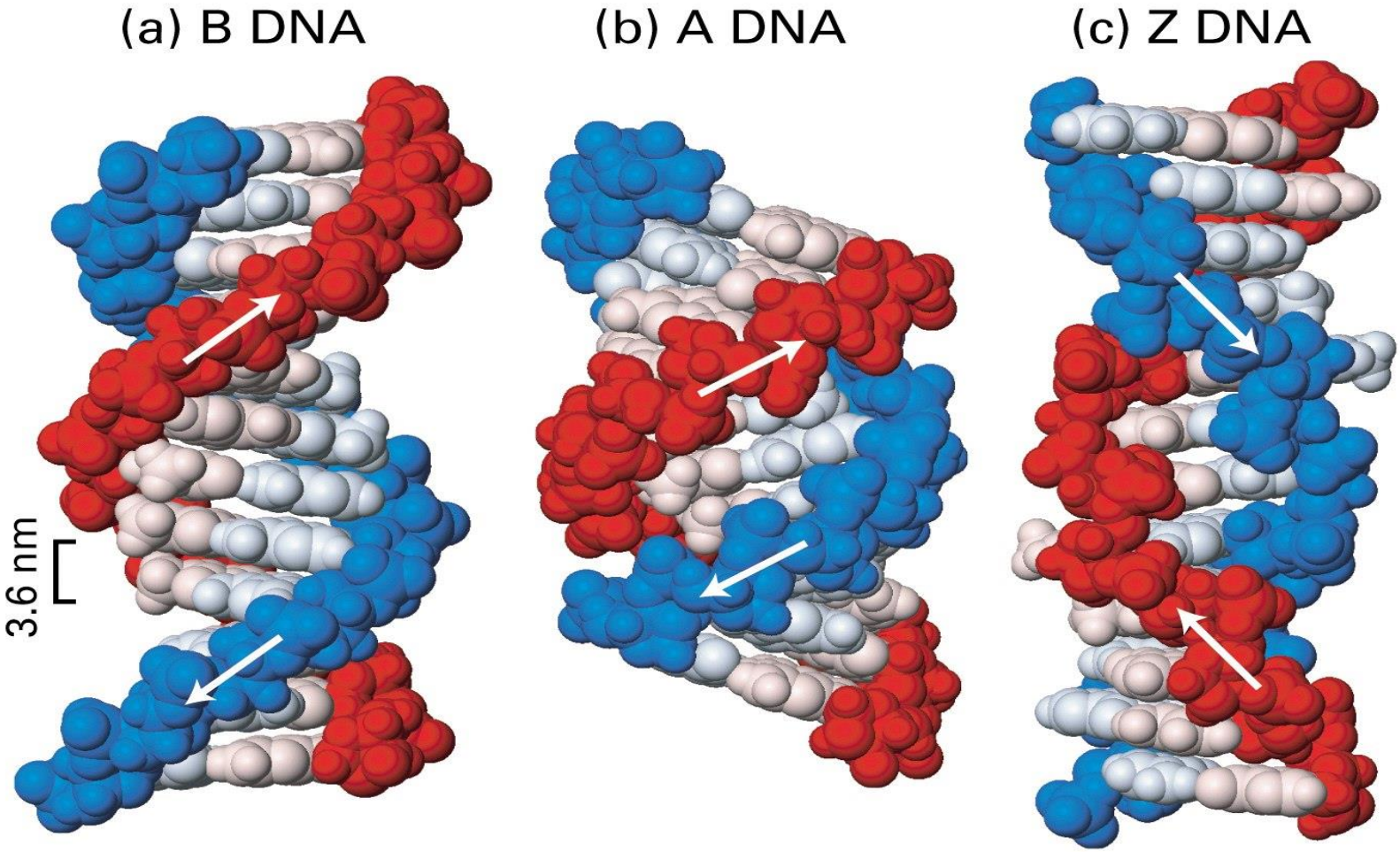
DNA Double Helix



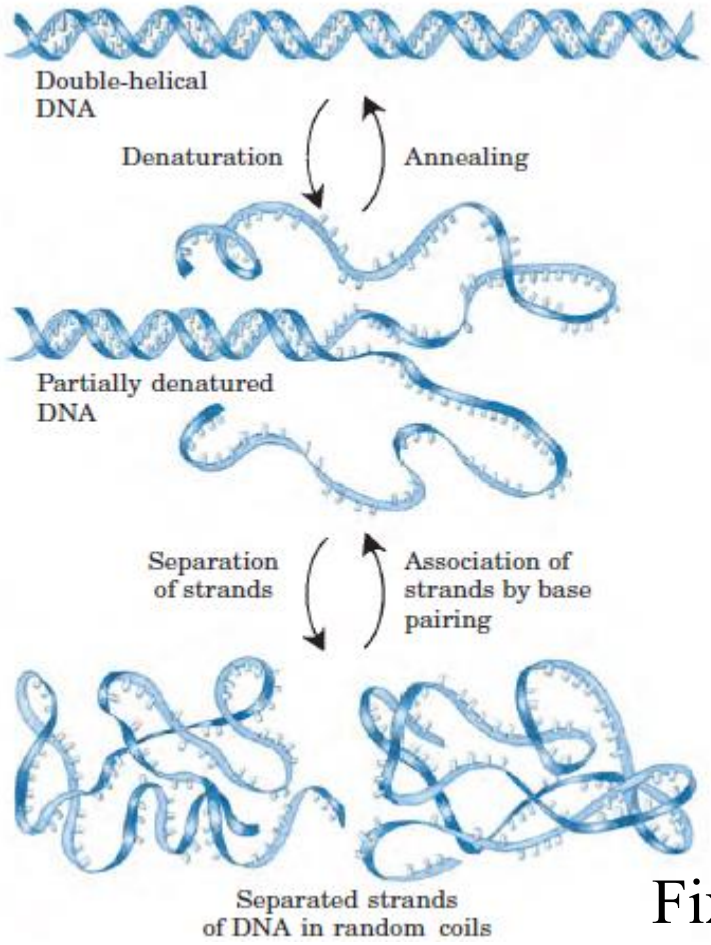


DNA coiled in the form of double helix , with both strands coiling around an axis

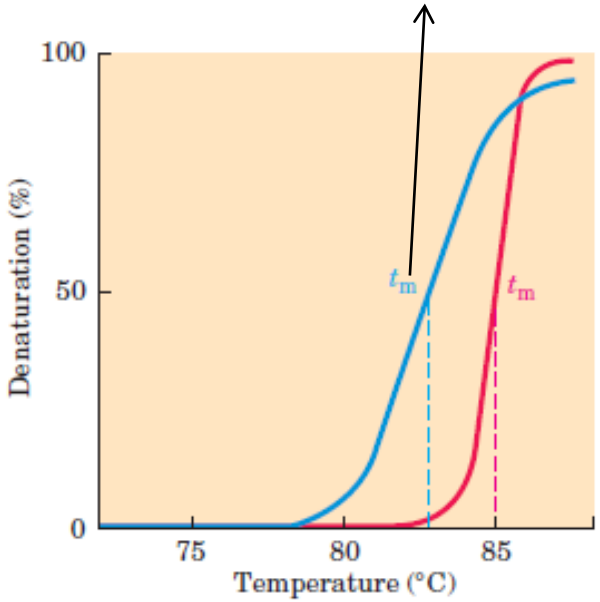
	B DNA	A DNA	Z DNA
Helix sense	Right-handed	right	left
Mean bp/turn	10.5	10	12



Nucleic acid chemistry

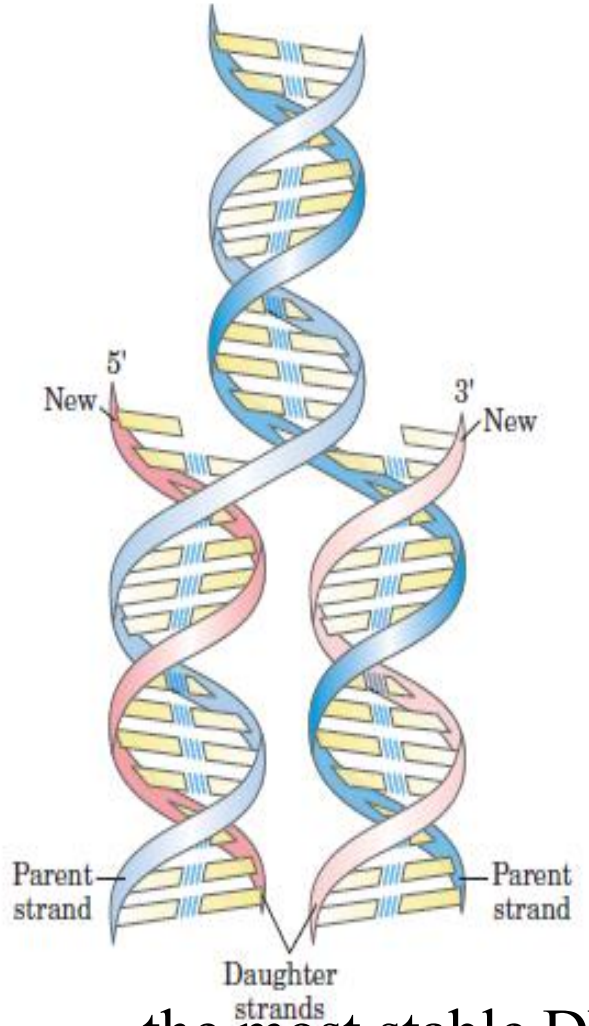


the temperature at which half the DNA is present as separated single strands



Fixed pH and ionic strength → ↑GC=↑ tm

Replication of DNA as suggested by Watson and Crick



DNA could act as a template for the replication and transmission of genetic information

the most stable DNA form=B-form, right handed DNA

- DNA denaturation and renaturation is the

basis of nucleic acid hybridization and PCR = a powerful technique in Molecular studies.

Eukaryotic Vs Prokaryotic DNA

- **Prokaryotic DNA:**
- Is found freely in the cytoplasm (within a region called the nucleoid)
- Is naked (i.e. not bound with proteins and therefore doesn't form chromatin)
- Genomes are compact (contain little repetitive DNA and no introns)
- Contains extra-chromosomal plasmids
- Is circular in shape

Eukaryotic DNA:

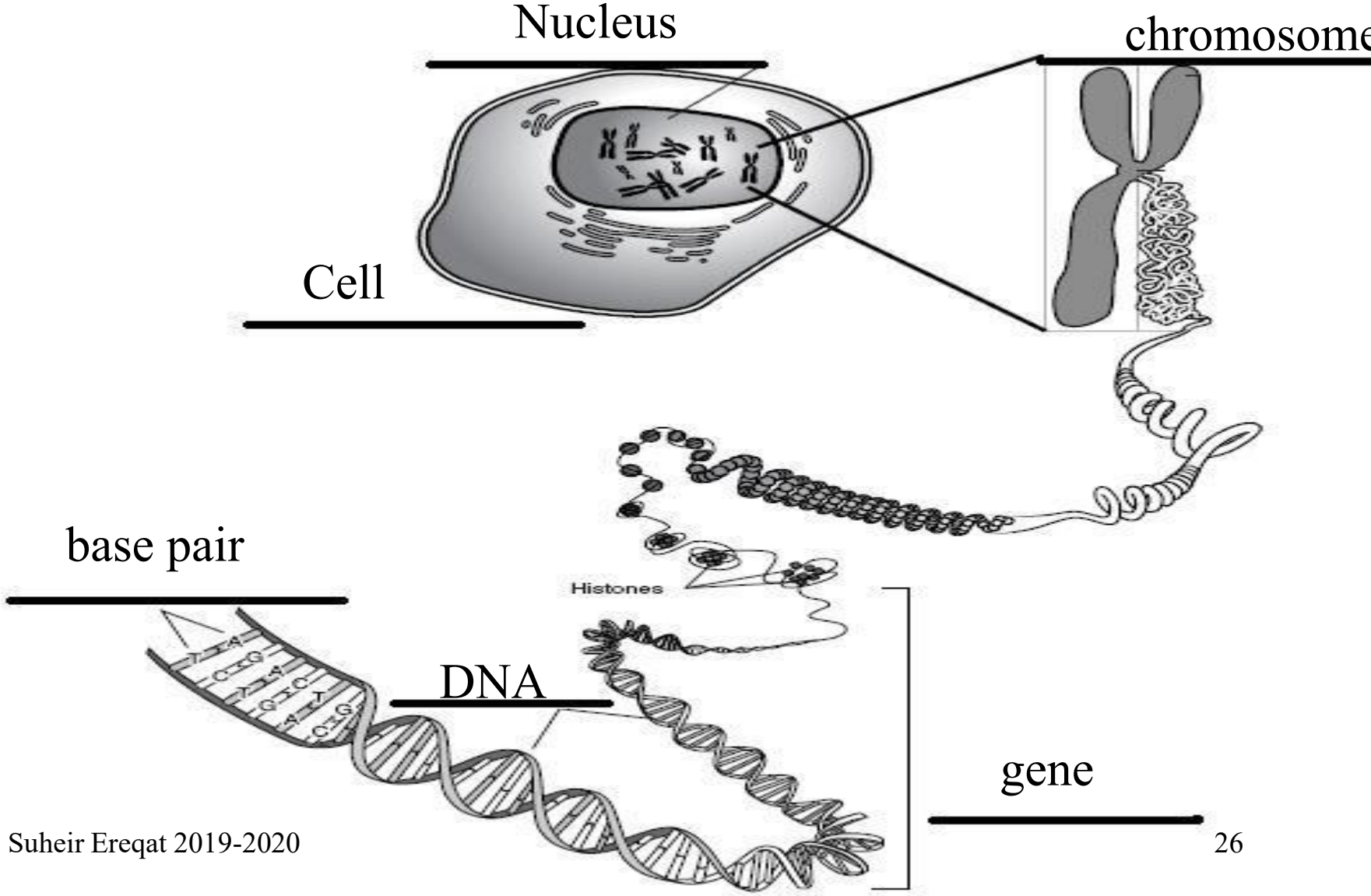
•Is contained within a nucleus
•Is bound to histone proteins
•Genomes contain large amounts of non-coding and repetitive DNA (including introns)
•Do not contain plasmids (but organelles such as the mitochondria may contain their own chromosomes)
•Are linear in shape

3D structure of chromosomes

- Human cell contain about 2m of DNA.
- The human body contain 10 to the power 13 cells. 2x10>13 m.
- This means the DNA can stretch to sun and back 50 times.
- How 2m can fit 0.006 nm diameter in nucleus?????!!!!!!!!!!!!!!!!!!!!!!!!!!!!.

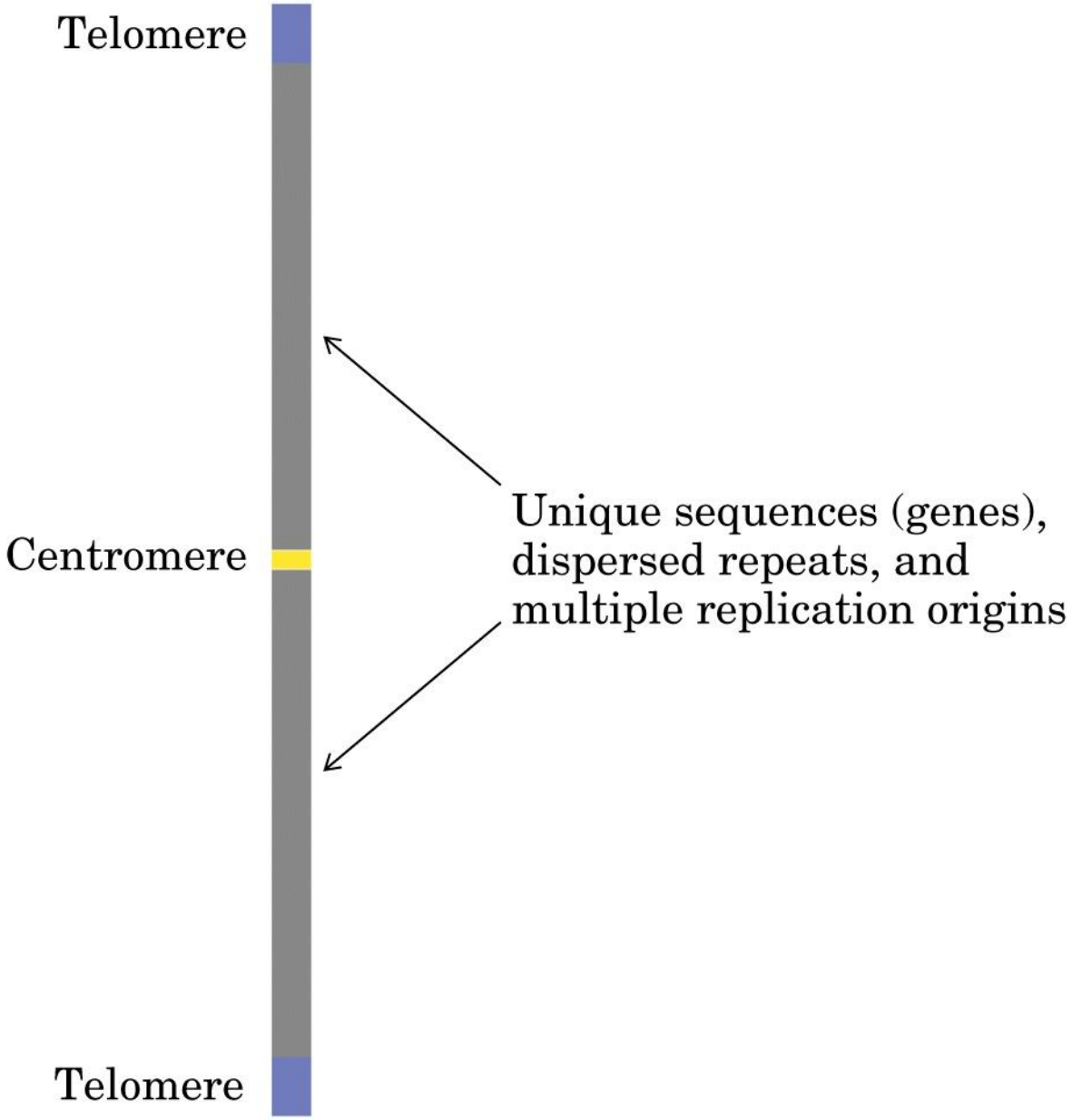
The structure of DNA, genes & chromosomes

Eukaryotic chromosomes have two important special-function repetitive DNA sequences:centromeres, which are attachment points for the mitotic spindle, and telomeres, located at the ends of chromosomes



Centromeres:
a sequence functions during cell division as an attachment point during mitosis,
A=T rich sequence.

Telomeres:
sequence at the end of chromosomes that help stabilize chromosome



Gene =

A portion of chromosome that determines a single specific character / phenotype/ visible property.

A segment of genetic material that determines /codes for a protein / enzyme

One gene one enzyme-------One gene one polypeptide.

Modern biochemical definition:

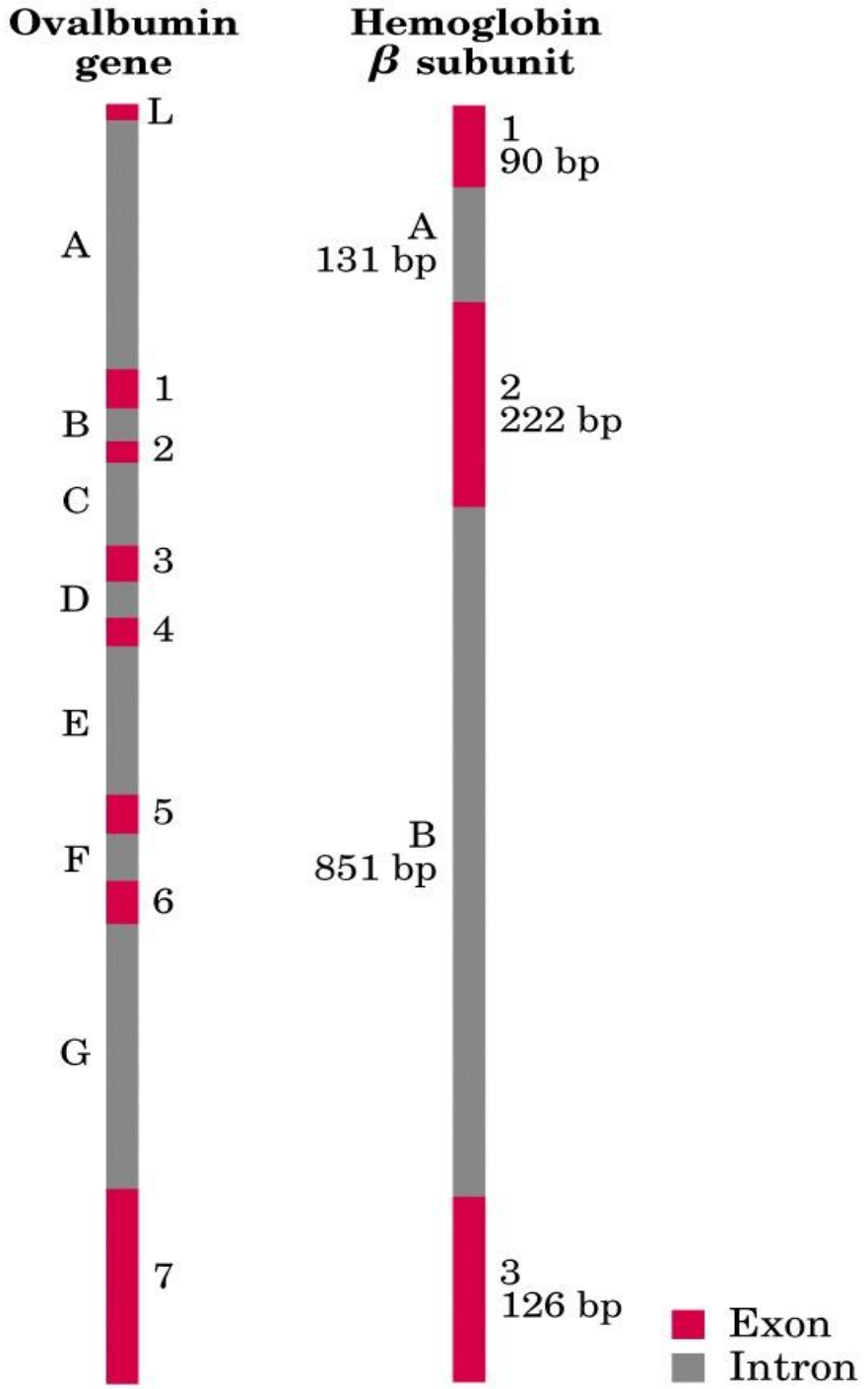
All the DNA that encodes the primary sequence of some final gene product (polypeptide / RNA).

Introns:

intervening sequences = nontranslated DNA segments in genes.

Exons:

a region of DNA within a gene transcribed to final (mRNA) molecule, rather than spliced out from transcribed RNA.

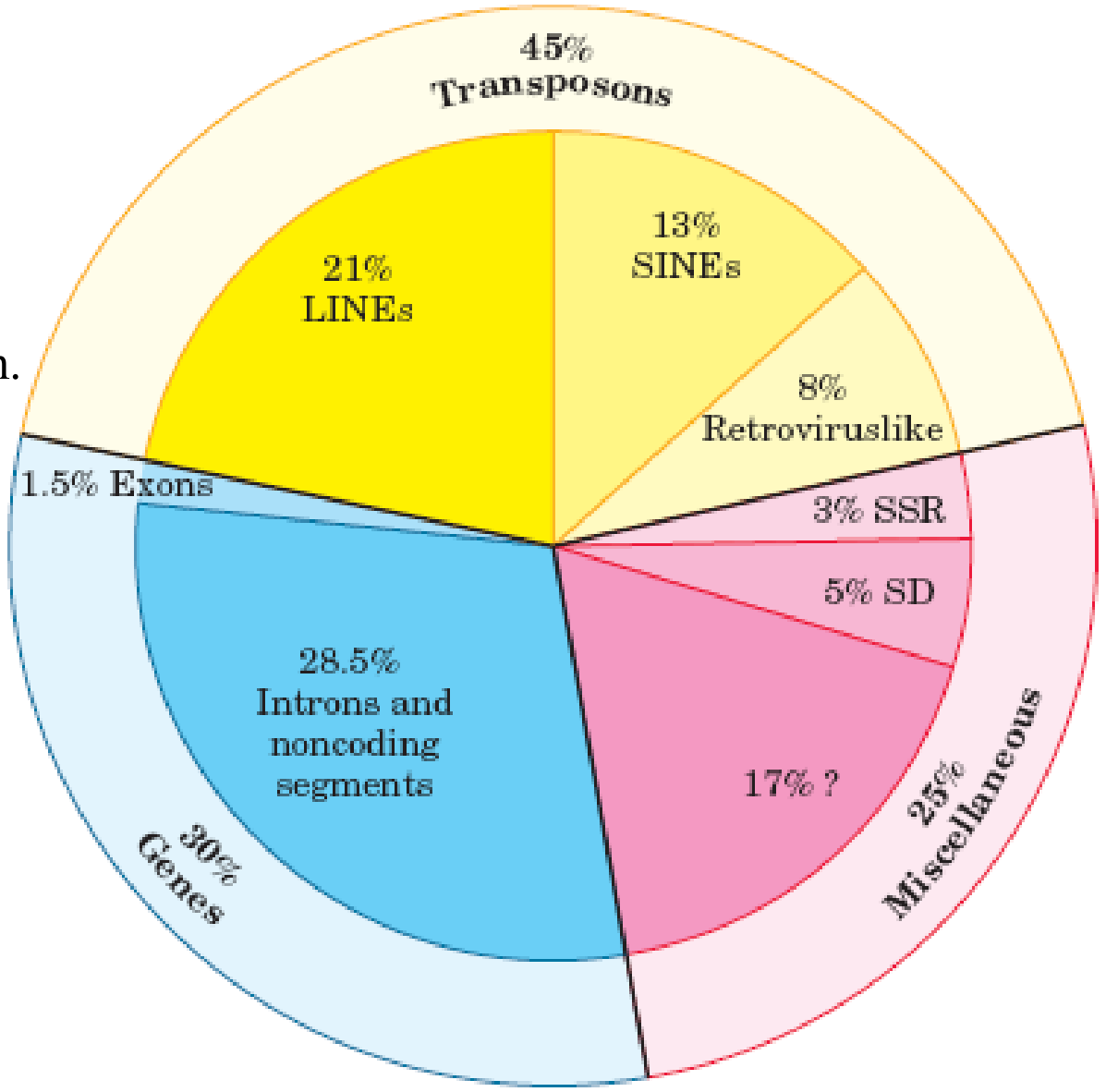


Types of sequences in human genome:

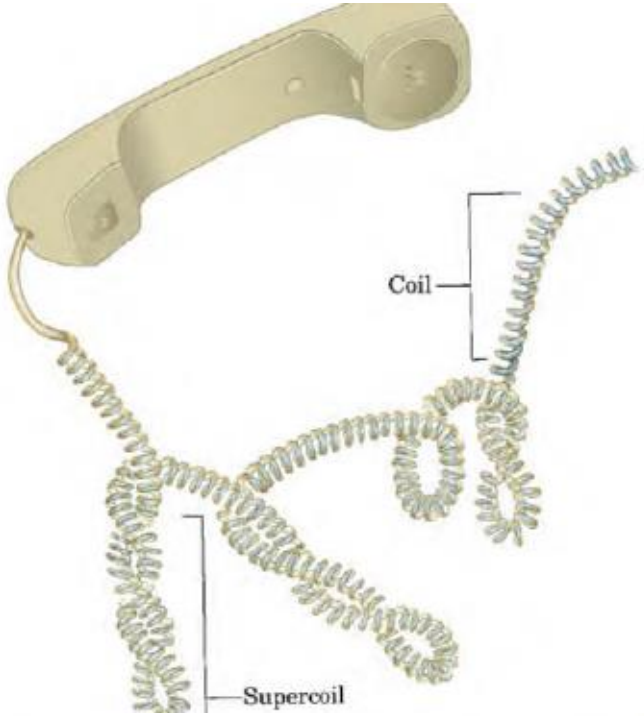
SINEs: Short interspersed elements 100-300bp long

LINEs: Long interspersed elements 6-8kbp, encode a few genes that catalyze transposition.

SSR: simple sequence repeats < 10bp repeated millions times /cell

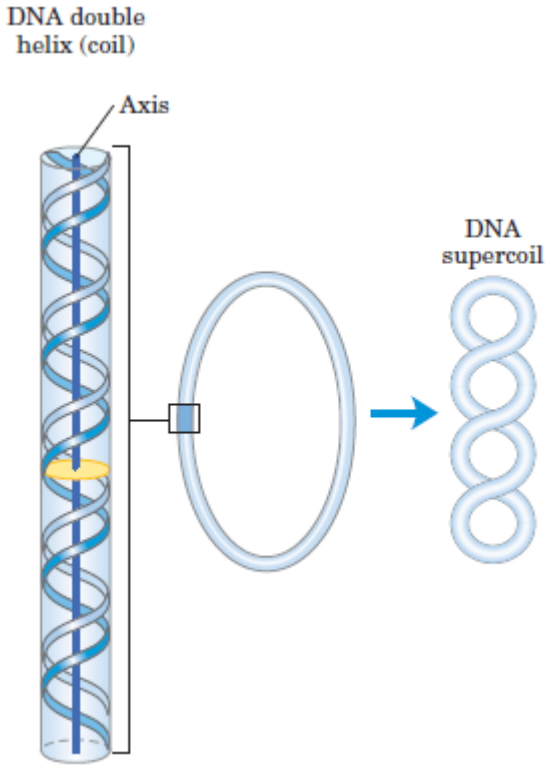


DNA supercoiling



Relaxed

supercoiled



coiling of the axis upon itself= Superheilex=supercoil

Topoisomerases

DNAs that differ only in the linking number (Lk) = **topoisomers.**

Enzymes that underwind/relax DNA = topoisomerases

- The degree of supercoiling in the cell controlled by topoisomerases.

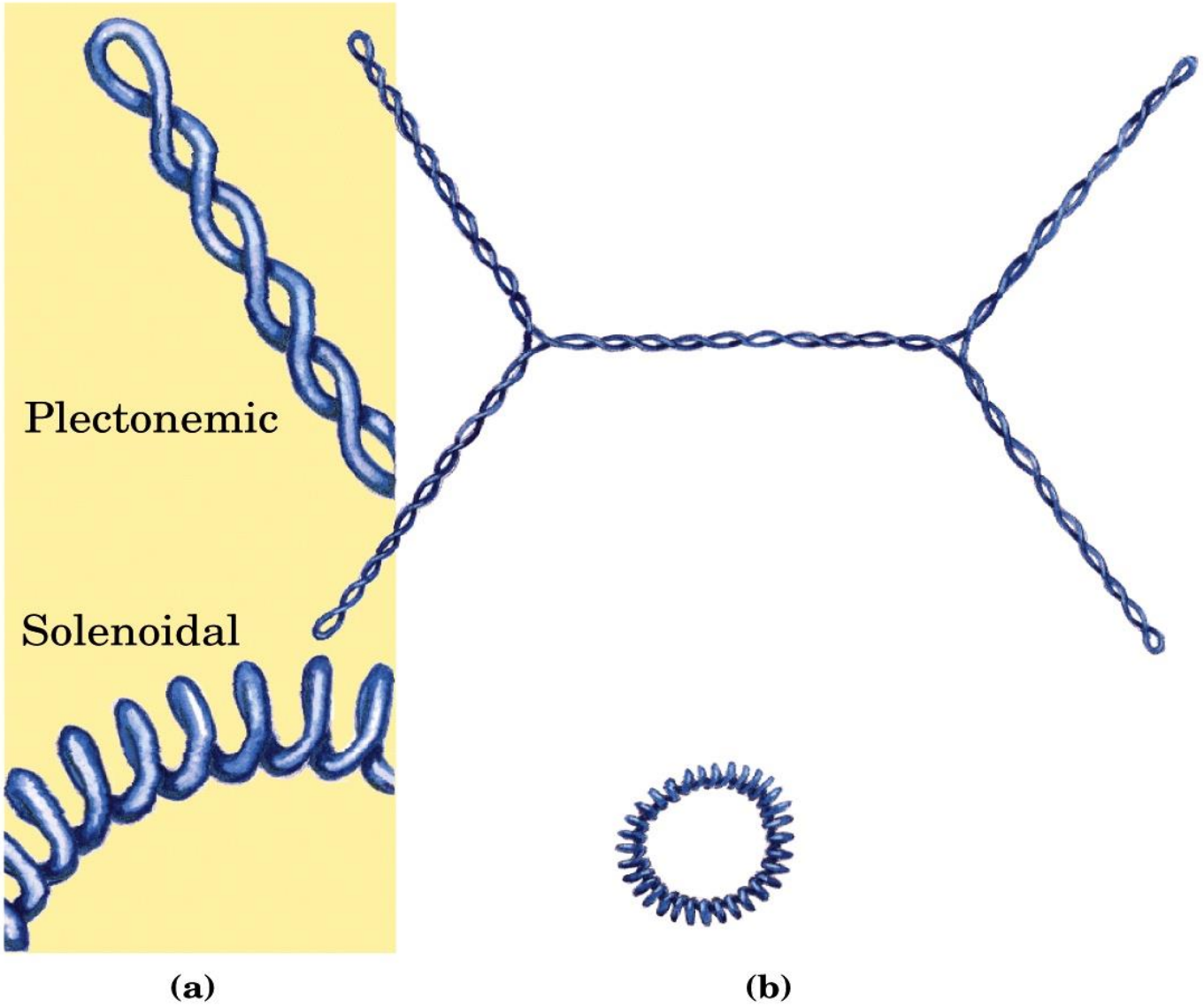
- Advantage: permits DNA to be transiently and locally melted to permit the enzymes of DNA replication and transcription to copy and synthesize new DNA or RNA.

- There are two classes of topisomerase:

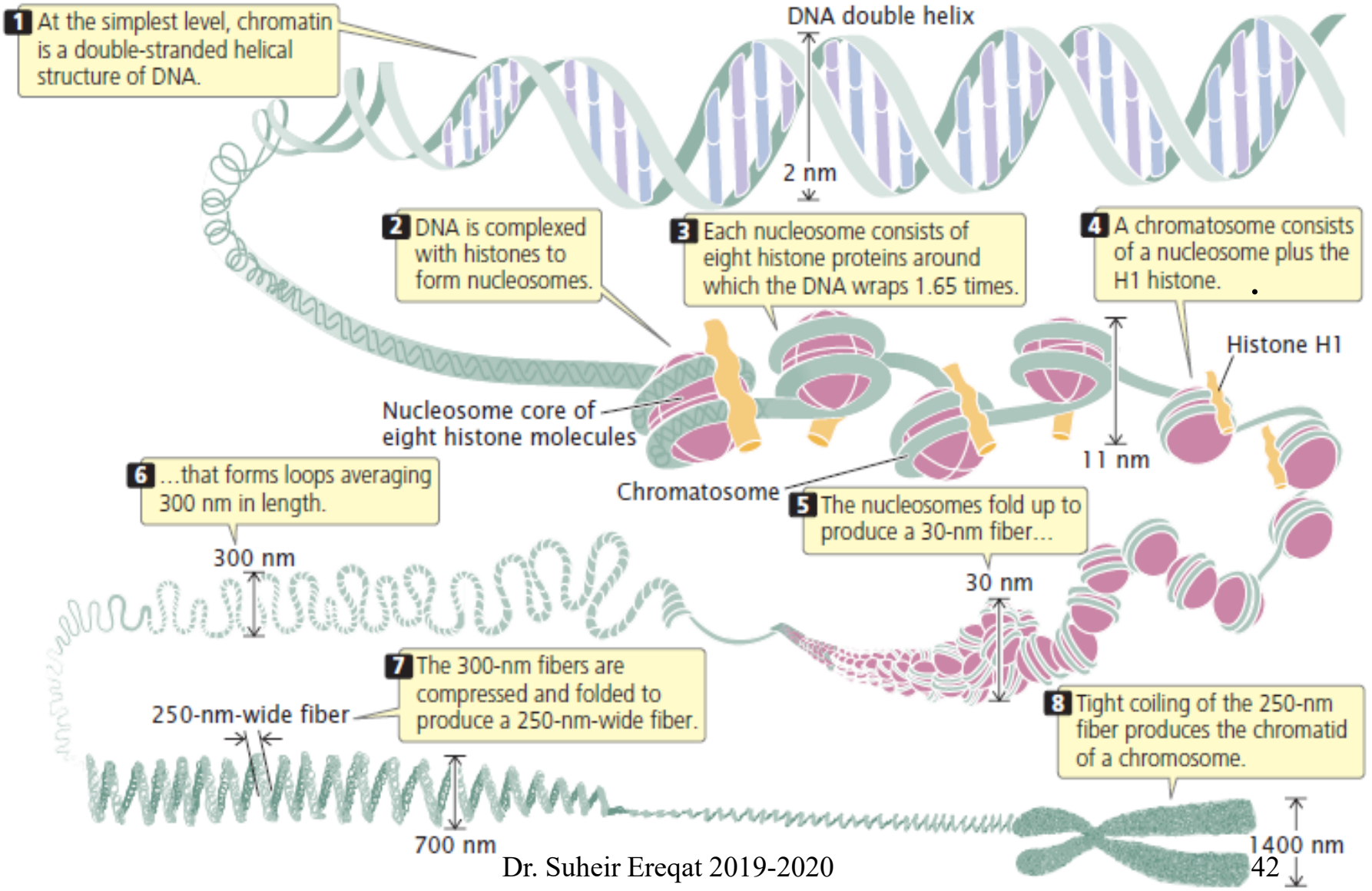
  Type 1 topoisomerases

  Type 2 topoisomerases ((**DNA Gyrase in E coli).**

- **In prokaryotes**, plectonemic supercoils are predominant, because of circular chromosome and small amount of genetic material.
- **In eukaryotes**, both present but solenoidal supercoiling most effective in compacting DNA.

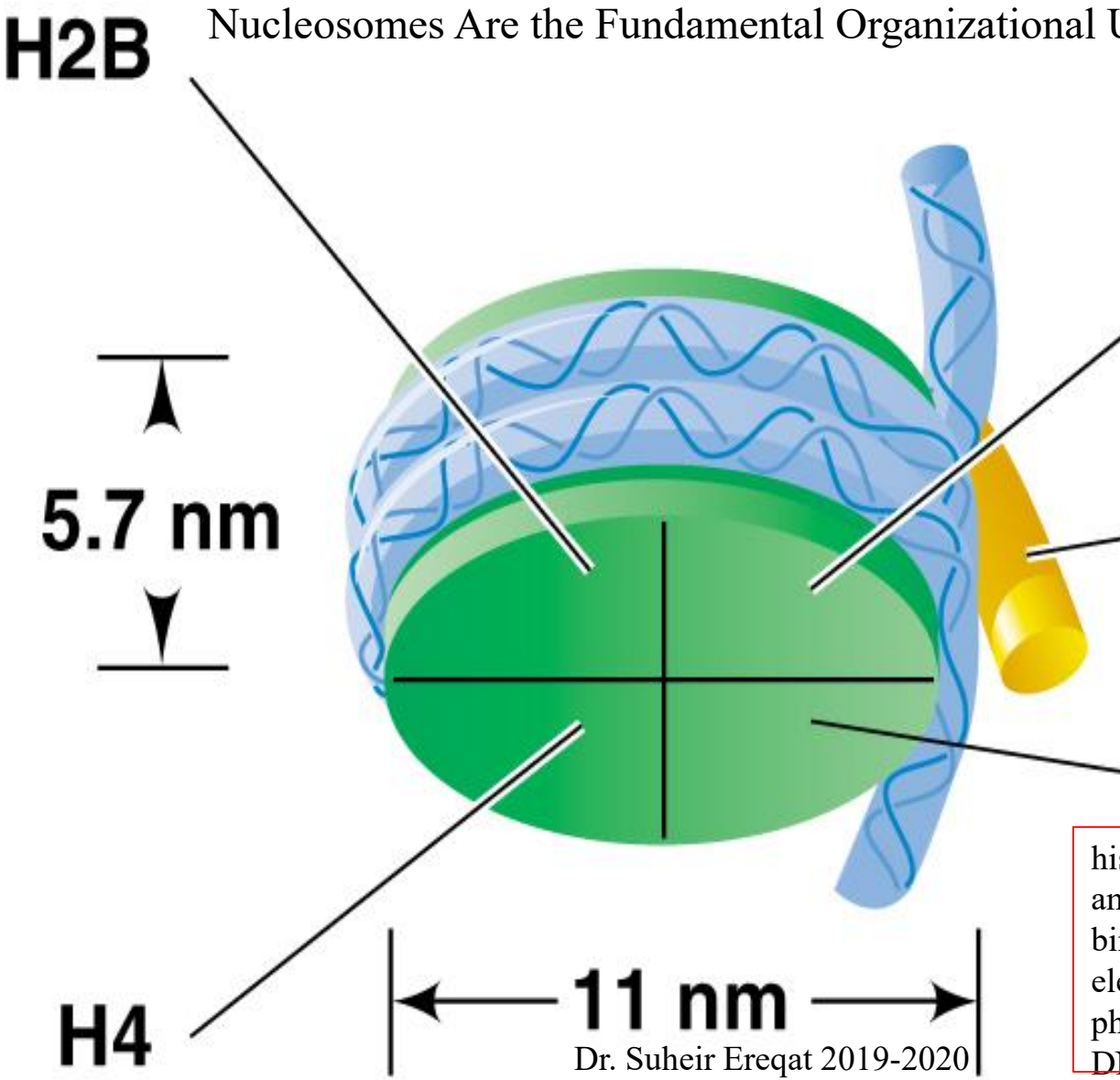


The many different orders of chromatin packing that give rise to the highly condensed metaphase chromosome



11.4 Chromatin has a highly complex structure with several levels of organization.

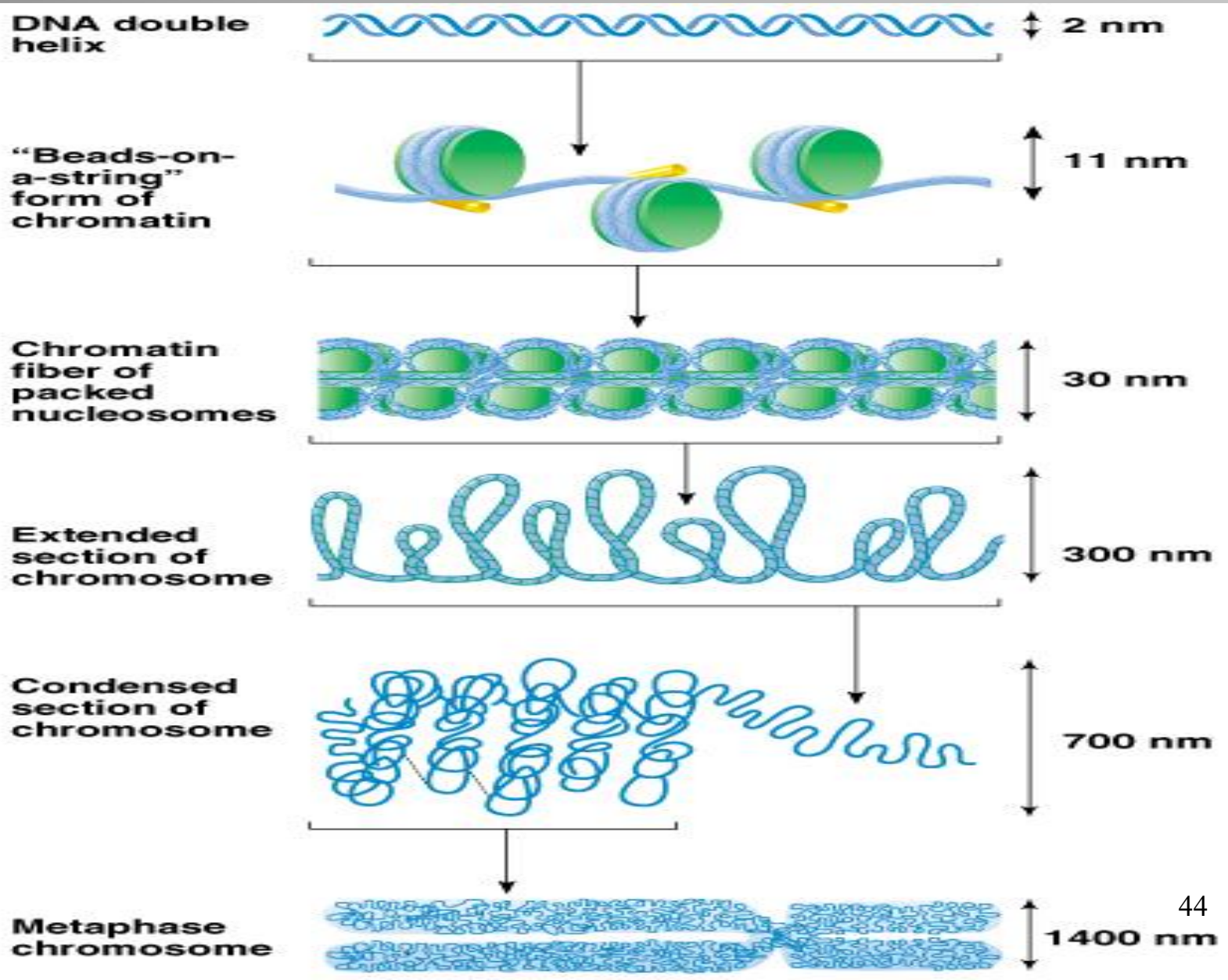
Fig. 8.17 A possible nucleosome structure



acting as a clamp around the nucleosome octamer

histone proteins rich in +ve charged amino acids (20-30% Arg + Lys) bind to DNA mostly via electrostatic interactions with the phosphodiester backbone of the DNA.

The many different orders of chromatin packing that give rise to the highly condensed metaphase chromosome



Histone Acetylation and Deacetylation

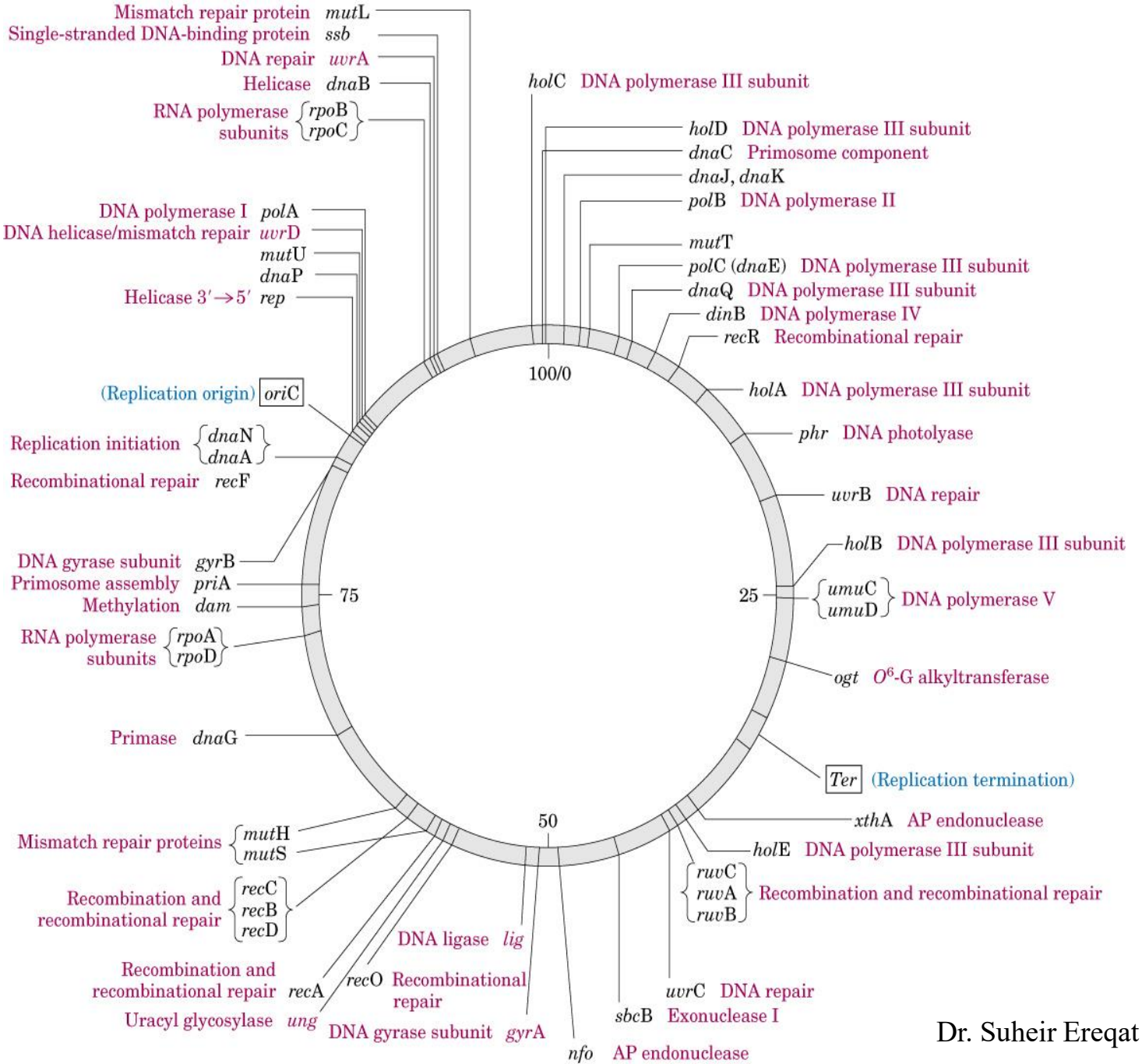
The two basic types of chromatin are **euchromatin,** which undergoes the normal process of condensation and decondensation in the cell cycle, and **heterochromatin, which** remains in a highly condensed state throughout the cell cycle, even during interphase.

•In histone acetylation and deacetylation, the histones are acetylated and deacetylated on lysine residues in the N-terminal tail as part of gene regulation.

•"histone acetyltransferase" **(HAT)** or "histone deacetylase" **(HDAC**). The source of the acetyl group in histone acetylation = Acetyl-CoA, The acceptor of acetyl group in histone deacetylation is CoA.

•Acetylation brings a –ve charge, neutralise the +ve charge on the histones and decreases the interaction of N termini of histones with –ve charged phosphate groups of DNA

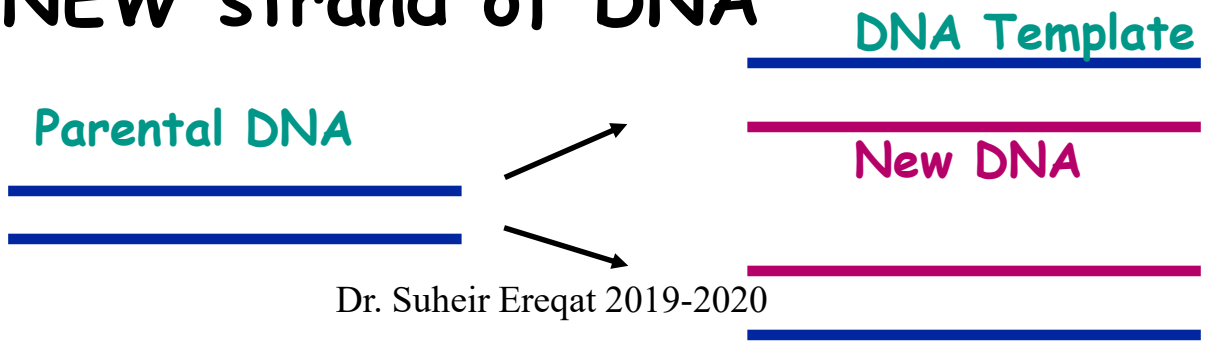
DNA Metabolism


Mismatch repair protein mutL
Single-stranded DNA-binding protein ssb
DNA repair uvrA
Helicase dnaB
RNA polymerase subunits rpoB rpoC
DNA polymerase I polA
DNA helicase/mismatch repair uvrD
mutU
dnaP
Helicase 3′→5′ rep
(Replication origin) oriC
Replication initiation dnaN dnaA
Recombinational repair recF
DNA gyrase subunit gyrB
Primosome assembly priA
Methylation dam
RNA polymerase subunits rpoA rpoD
Primase dnaG
Mismatch repair proteins mutH mutS
Recombination and recombinational repair recC recB recD
Recombination and recombinational repair recA
Uracyl glycosylase ung
recO Recombinational repair
DNA gyrase subunit gyrA
DNA ligase lig
nfo AP endonuclease
sbcB Exonuclease I
uvrC DNA repair
ruvC ruvA ruvB Recombination and recombinational repair
holE DNA polymerase III subunit
xthA AP endonuclease
Ter (Replication termination)
ogt O6-G alkyltransferase
umuC umuD DNA polymerase V
holB DNA polymerase III subunit
uvrB DNA repair
phr DNA photolyase
holA DNA polymerase III subunit
recR Recombinational repair
dinB DNA polymerase IV
dnaQ DNA polymerase III subunit
polC (dnaE) DNA polymerase III subunit
mutT
polB DNA polymerase II
dnaJ, dnaK
dnaC Primosome component
holD DNA polymerase III subunit
holC DNA polymerase III subunit
100/0
25
50
75

DNA Replication Follows a Set of Fundamental Rules

1.DNA Replication Is Semiconservative

- Idea presented by Watson & Crick: DNA could act as a template for the replication and transmission of genetic information
- The two strands of the parental molecule separate, and each acts as a template for a new complementary strand
- New DNA consists of 1 PARENTAL (original) and 1 NEW strand of DNA



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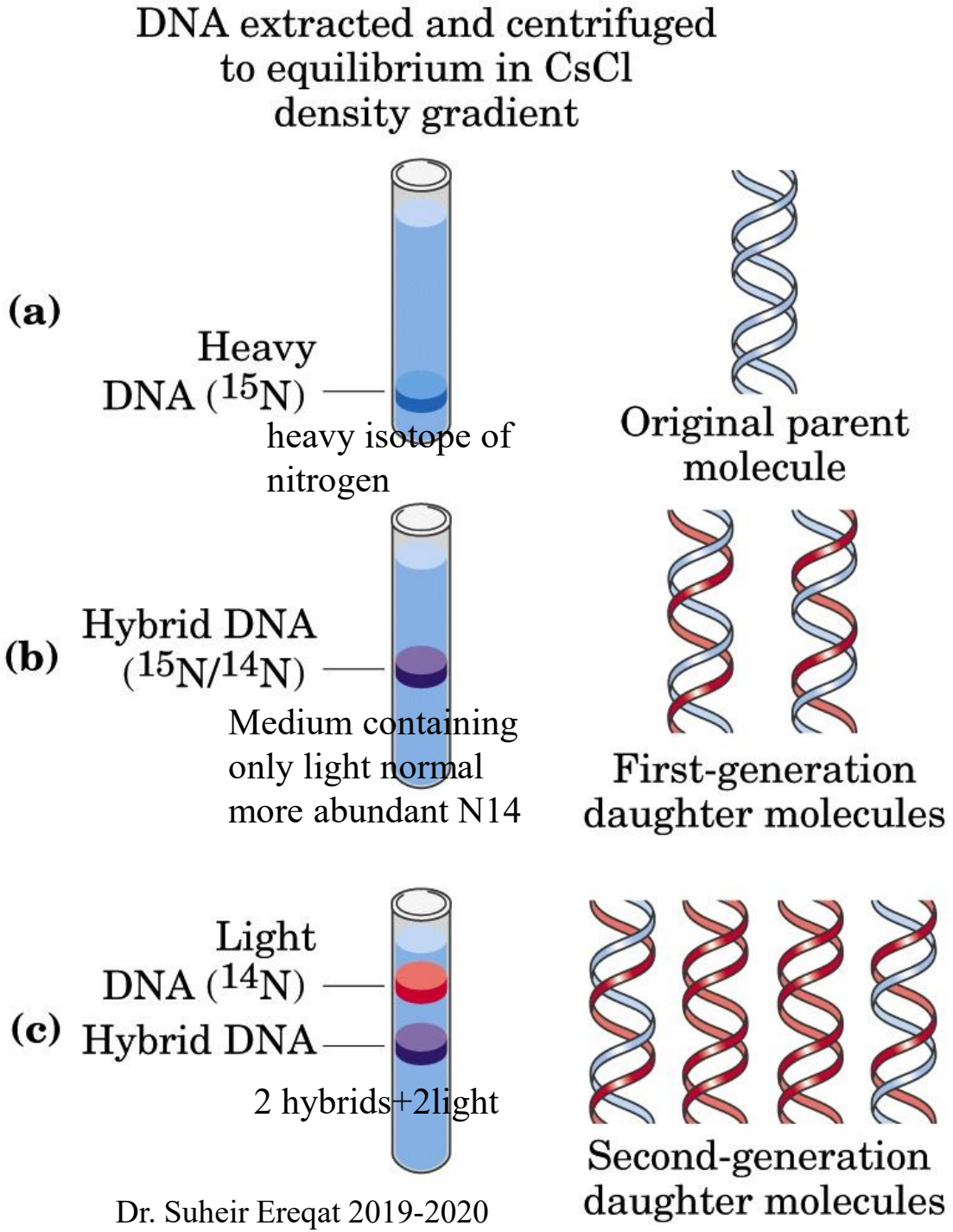
The Meselson-Stahl experiment



The Meselson-Stahl experiment.

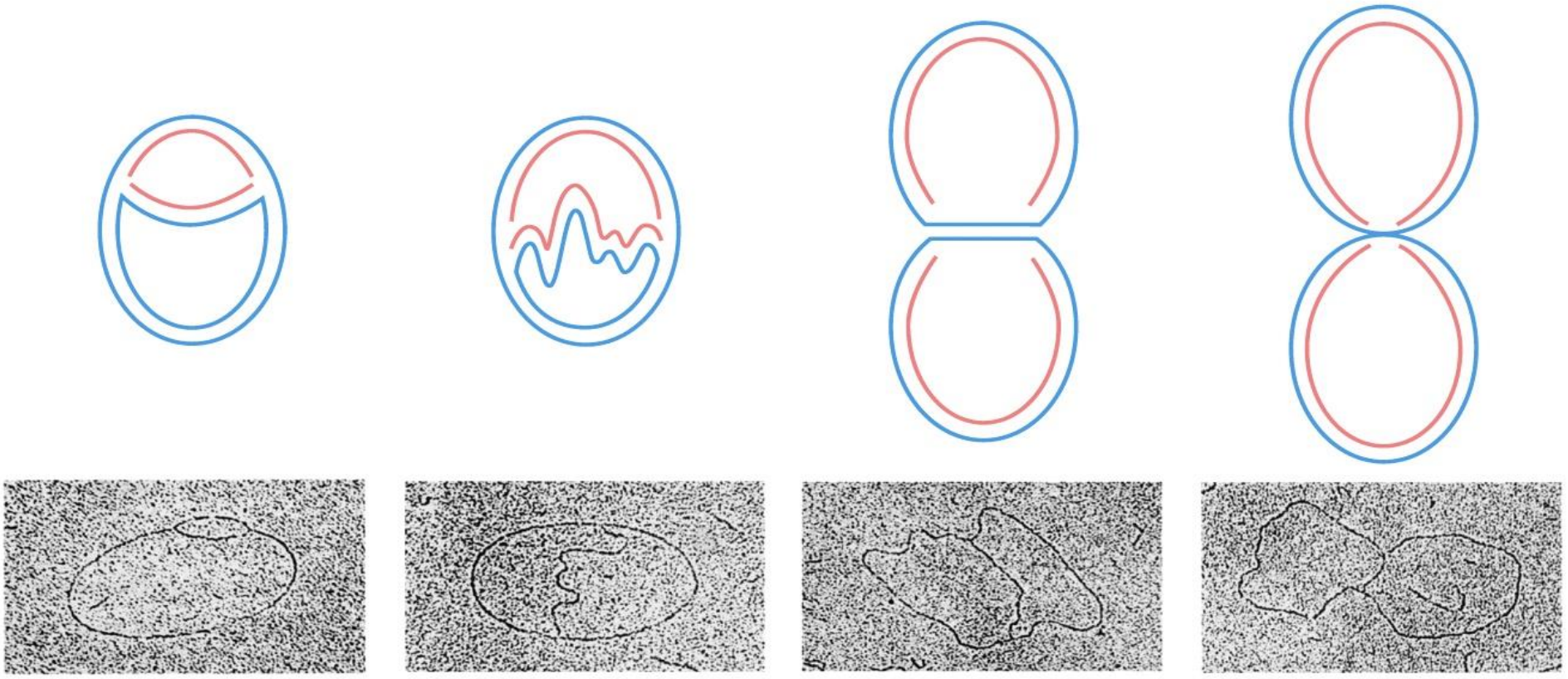
(a) Cells were grown for many generations in a medium containing only heavy nitrogen,15N, so that all the nitrogen in their DNA was 15N, as shown by a single band (blue) when centrifuged in a CsCl density gradient.

(b) Once the cells had been transferred to a medium containing only light nitrogen,14N, cellular DNA isolated after one generation equilibrated at a higher position in the density gradient (purple band).

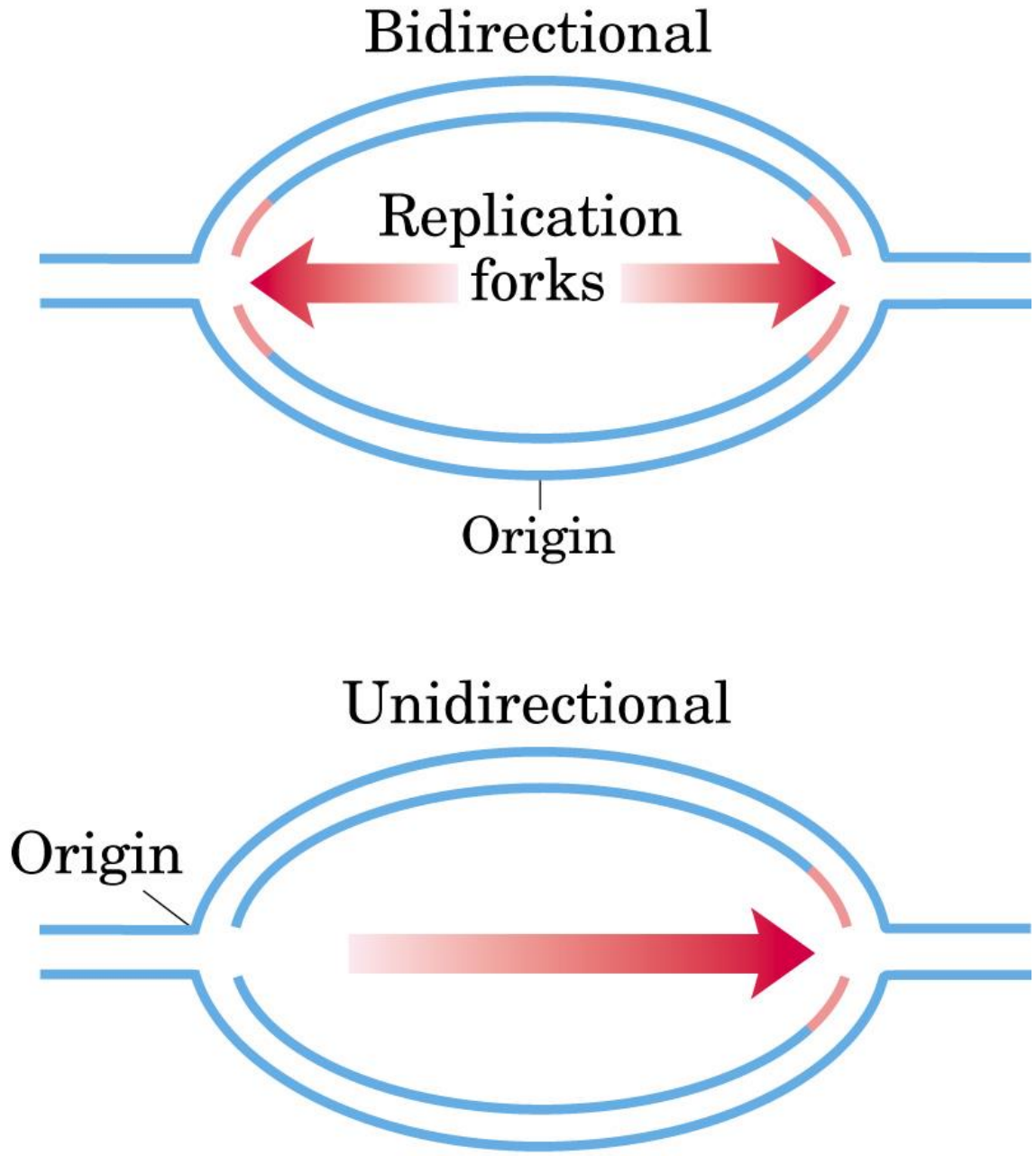
(c) Continuation of replication for a second generation yielded two hybrid DNAs and two light DNAs (red), confirming semiconservative replication

2. Replication begins at an origin and proceeds bidirectionally:

the replication is a highly coordinated process in which the parent strands are simultaneously unwound and replicated



(a)


Bidirectional
Replication forks
Origin
Unidirectional
Origin

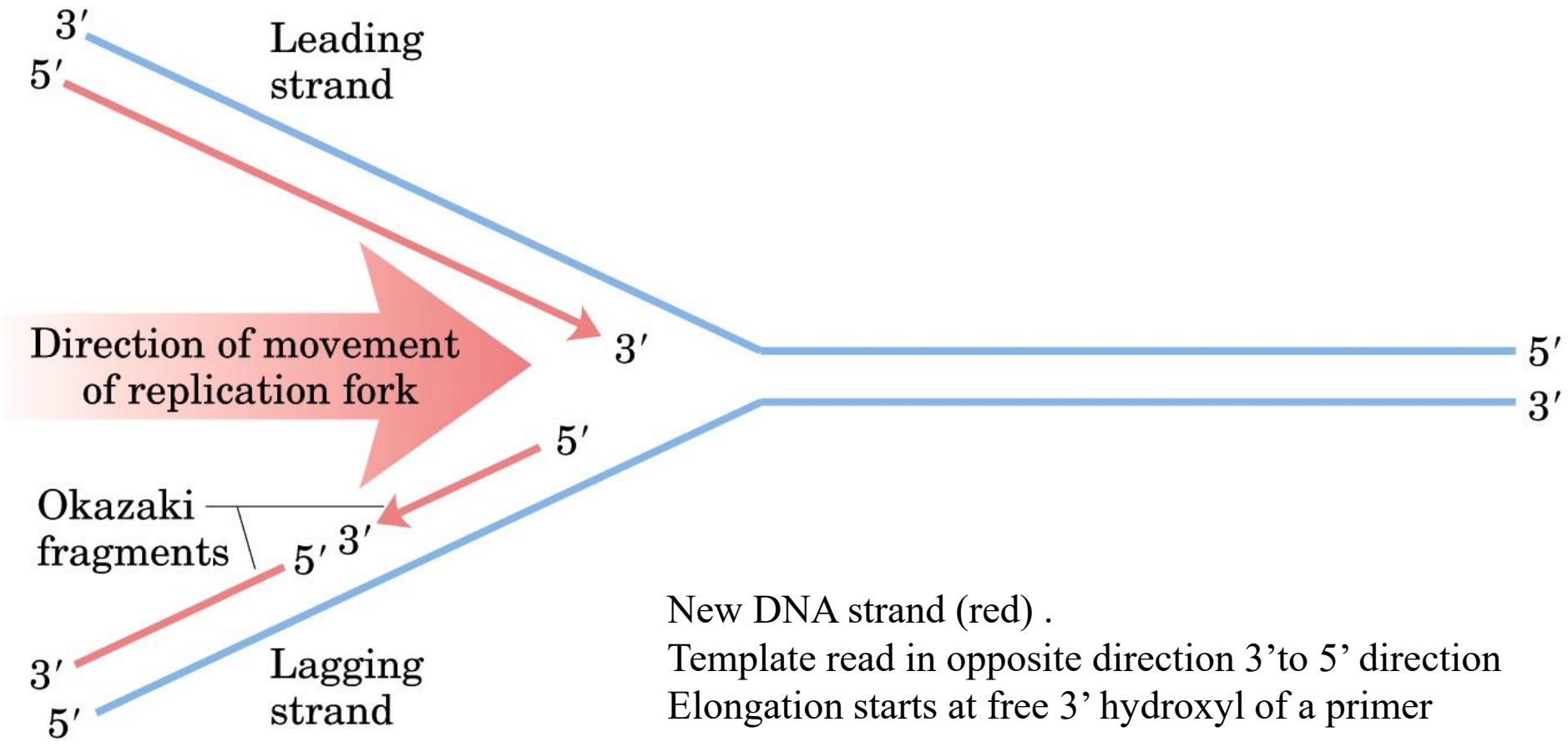
(b)

DNA could be selectively denatured at sequences unusually rich in A=T base pairs, generating a reproducible pattern of single-strand bubbles ; the replication loops always initiate at a unique point, which was termed an **origin**

replication forks, are dynamic points **where parent DNA is being unwound** and the separated strands quickly replicated.

For circular DNA molecules, the two replication forks meet at a point on the side of the circle opposite to the origin.

3. DNA synthesis proceeds 5'→3' direction and is Semicontinous.

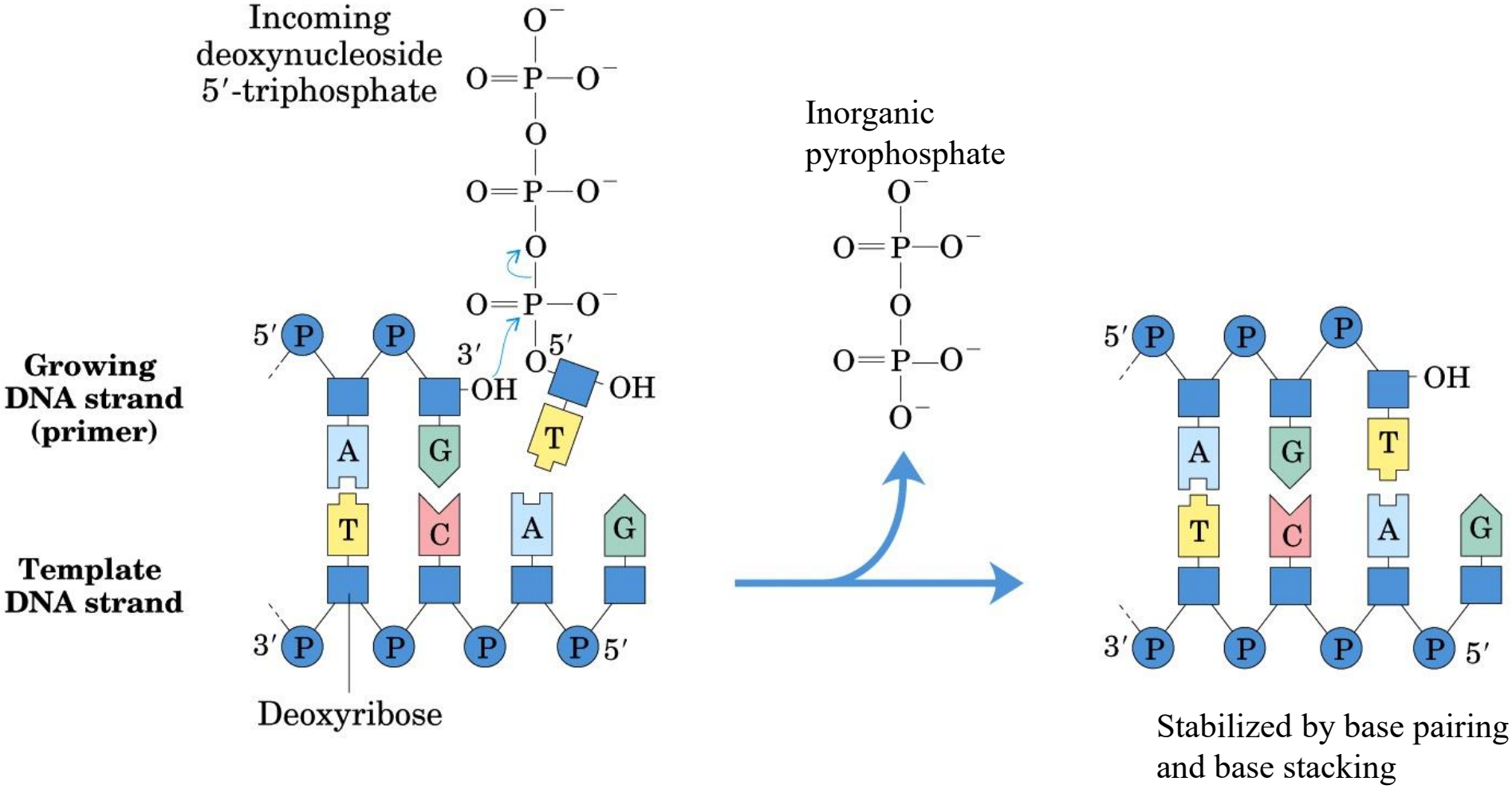


New DNA strand (red) .
Template read in opposite direction 3'to 5' direction
Elongation starts at free 3' hydroxyl of a primer

The Leading Strand is synthesized as a single strand from the point of origin toward the opening replication fork

The Lagging Strand is synthesized discontinuously against overall direction of replication
This strand is made in MANY short segments It is replicated from the replication fork toward the origin

DNA Is Synthesized by DNA Polymerases

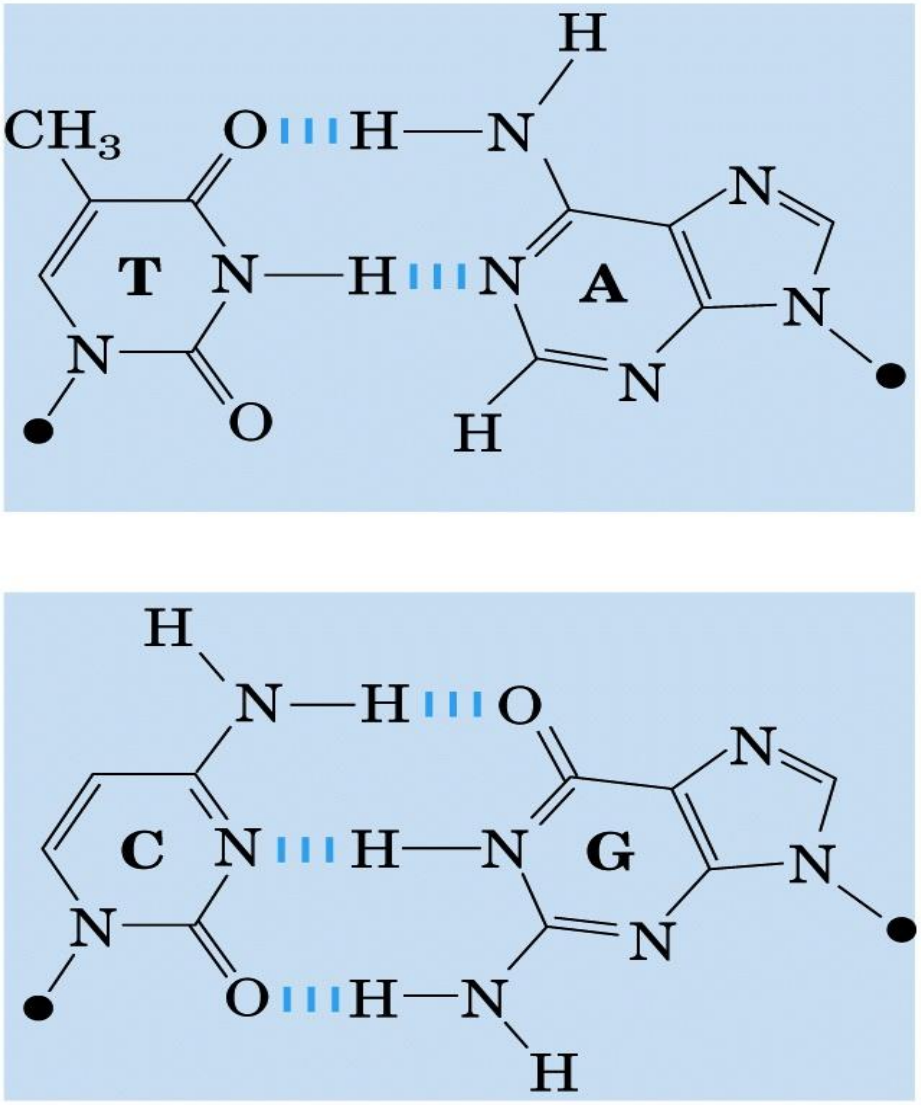


DNA polymerization reaction is guided !

DNA polymerase activity requires a single unpaired strand to act as **template** and **a primer** strand (RNA segment complementary to the template) to provide a free hydroxyl group at the 3 end, to which a new nucleotide unit is added.

Each incoming nucleotide is selected in part by base pairing to the appropriate nucleotide in the template strand. The reaction product has a new free 3 hydroxyl, allowing the addition of another nucleotide.

Nucleotide addition is guided by base pairing:



(a)

Replication is very accurate:

Replication proceeds with high fidelity.

In E coli a mistake occurs / 10^9-10^{10} nucleotides

4.6x 10^6bp.

Discrimination bw correct and incorrect nucleotide:

1- hydrogen bonding (base selection)

2- common geometry of the standard A-T and G-C

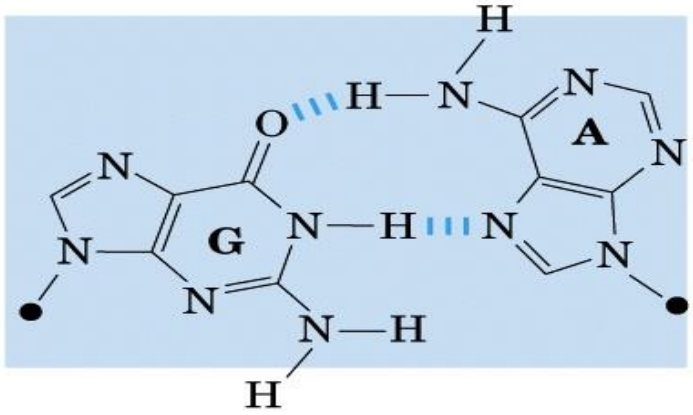
(active site of DNA pol **accommodate correct geometry**

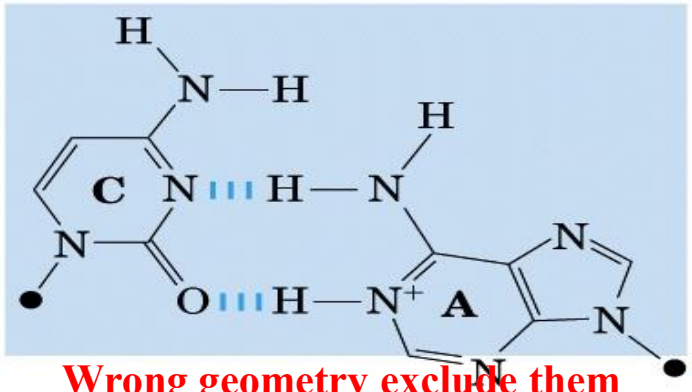
Incorrect bases rejected before phosphodiester bond formed.

However, this doesn't account for high fidelity of pol.

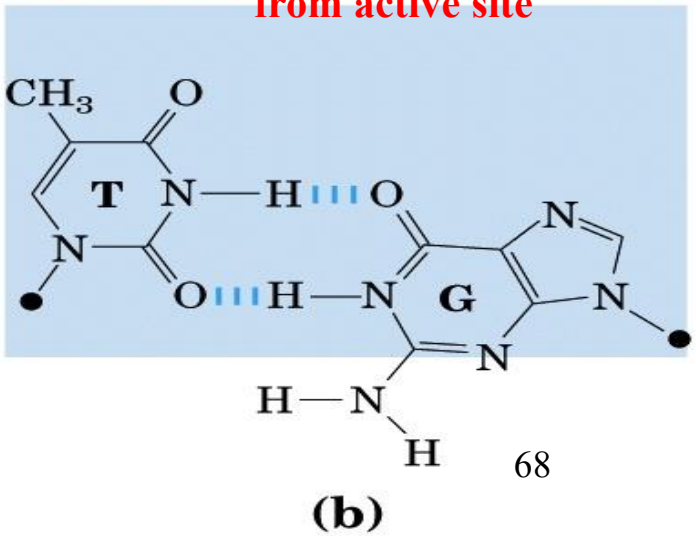
in replication.

Another mechanism!!!

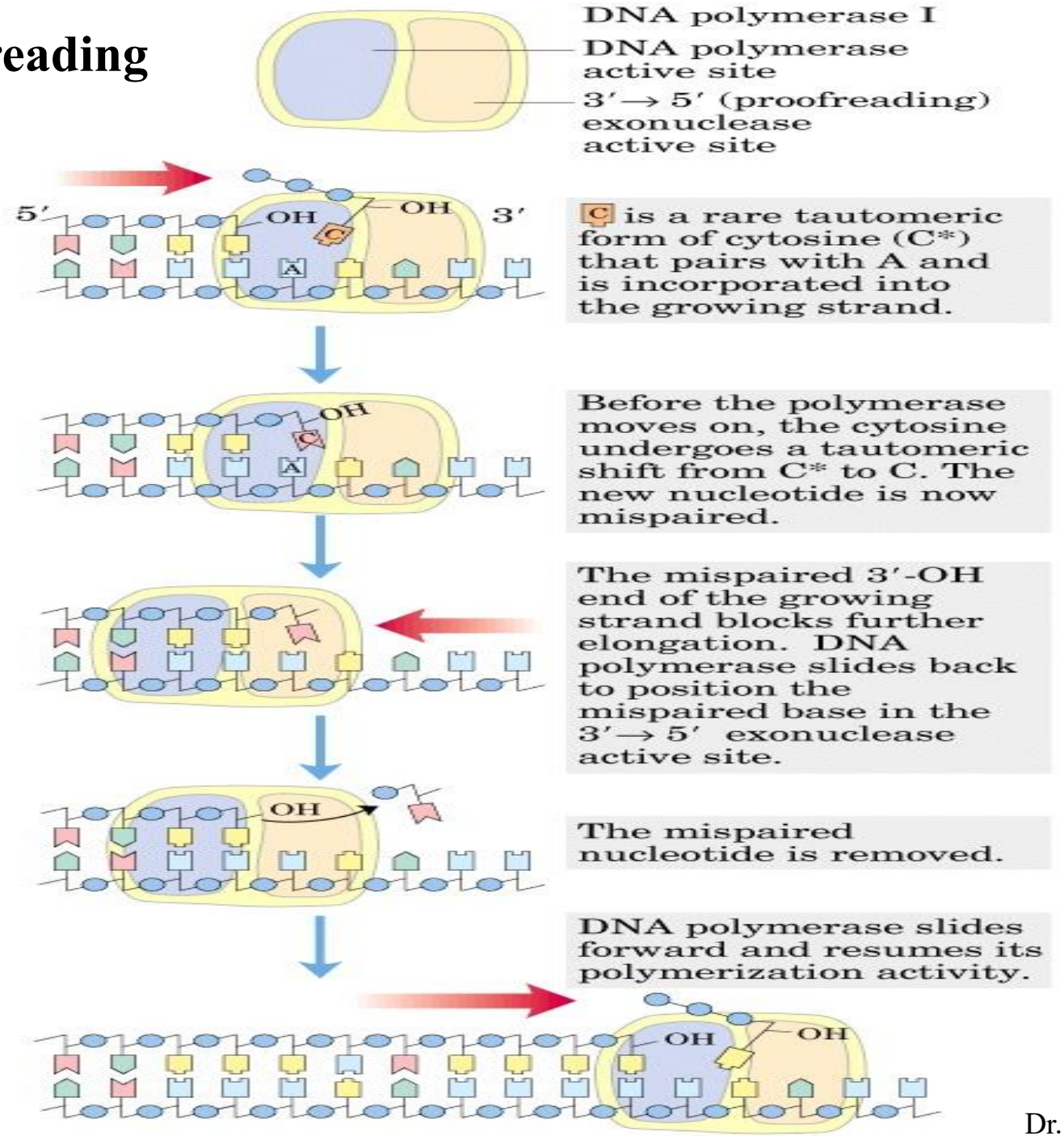




Wrong geometry exclude them from active site



proofreading



Proofreading

An incorrect nucleotide may be able to hydrogen-bond with a base in the template, but it generally will not fit into the active site. Incorrect bases can be rejected before the phosphodiester bond is formed

Proofreading: The 3>5 exonuclease activity of DNA polymerase removes the mispaired nucleotide (double check), and the polymerase begins again.

E. coli Has at Least Five DNA Polymerases

table 25–1

Comparison of DNA Polymerases of *E. coli*

	DNA polymerase		
	I	II	III
Structural gene*	*pol*A	*pol*B	*pol*C (*dna*E)
Subunits (number of different types)	1	≥4	≥10
M_r	103,000	88,000†	830,000
3′→5′ Exonuclease (proofreading)	Yes	Yes	Yes
5′→3′ Exonuclease	Yes	No	No
Polymerization rate (nucleotides/sec)	16–20	40	250–1,000
Processivity (nucleotides added before polymerase dissociates)	3–200	1,500	≥500,000

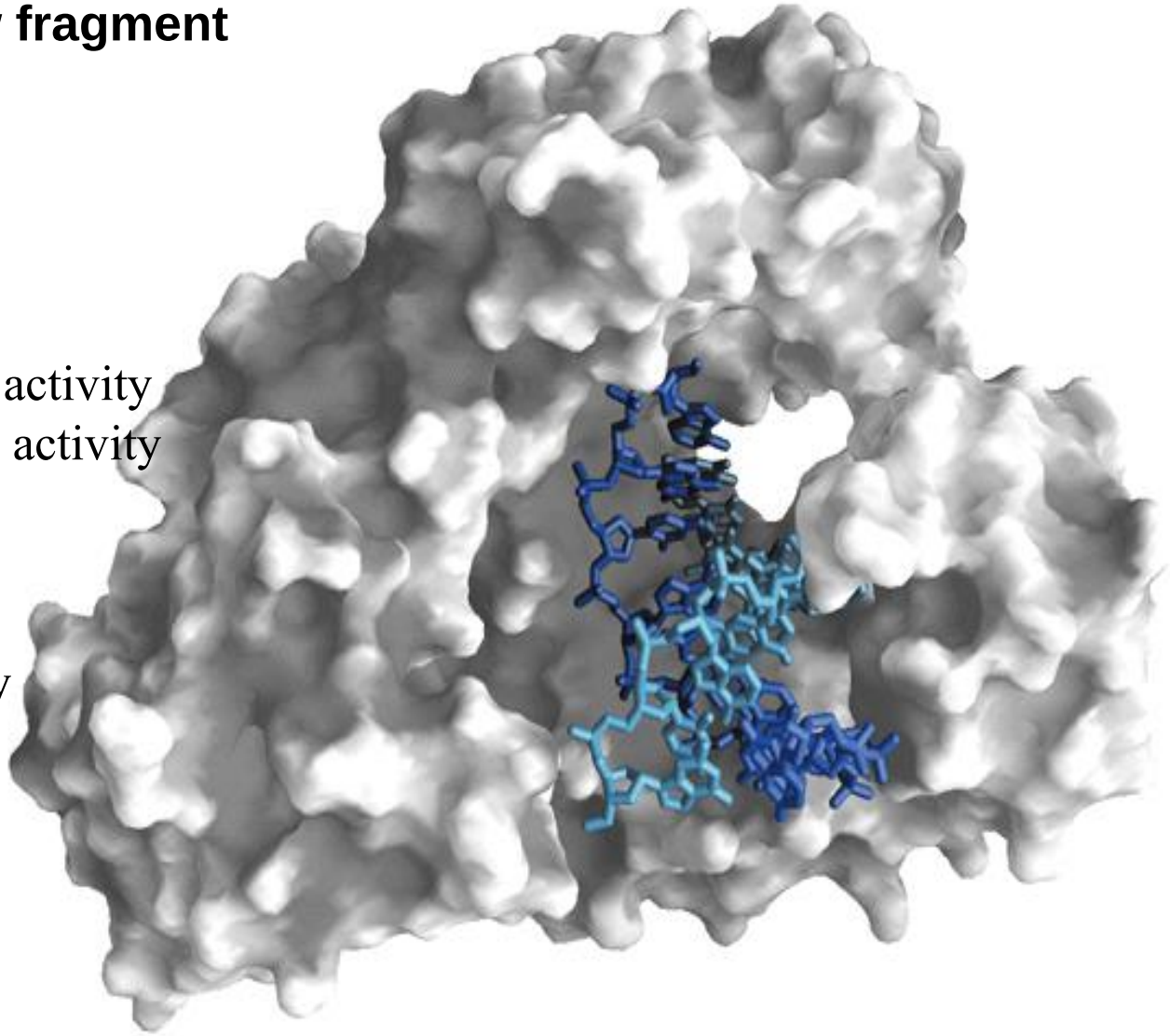
*For enzymes with more than one subunit, the gene listed here encodes the subunit with polymerization activity. Note that *dna*E is an earlier designation of the gene now referred to as *pol*C.

†Polymerization subunit only. DNA polymerase II shares several subunits with DNA polymerase III, including the β, γ, δ, δ', χ, and ψ subunits (see Table 25–2).

DNA pol I: Klenow fragment

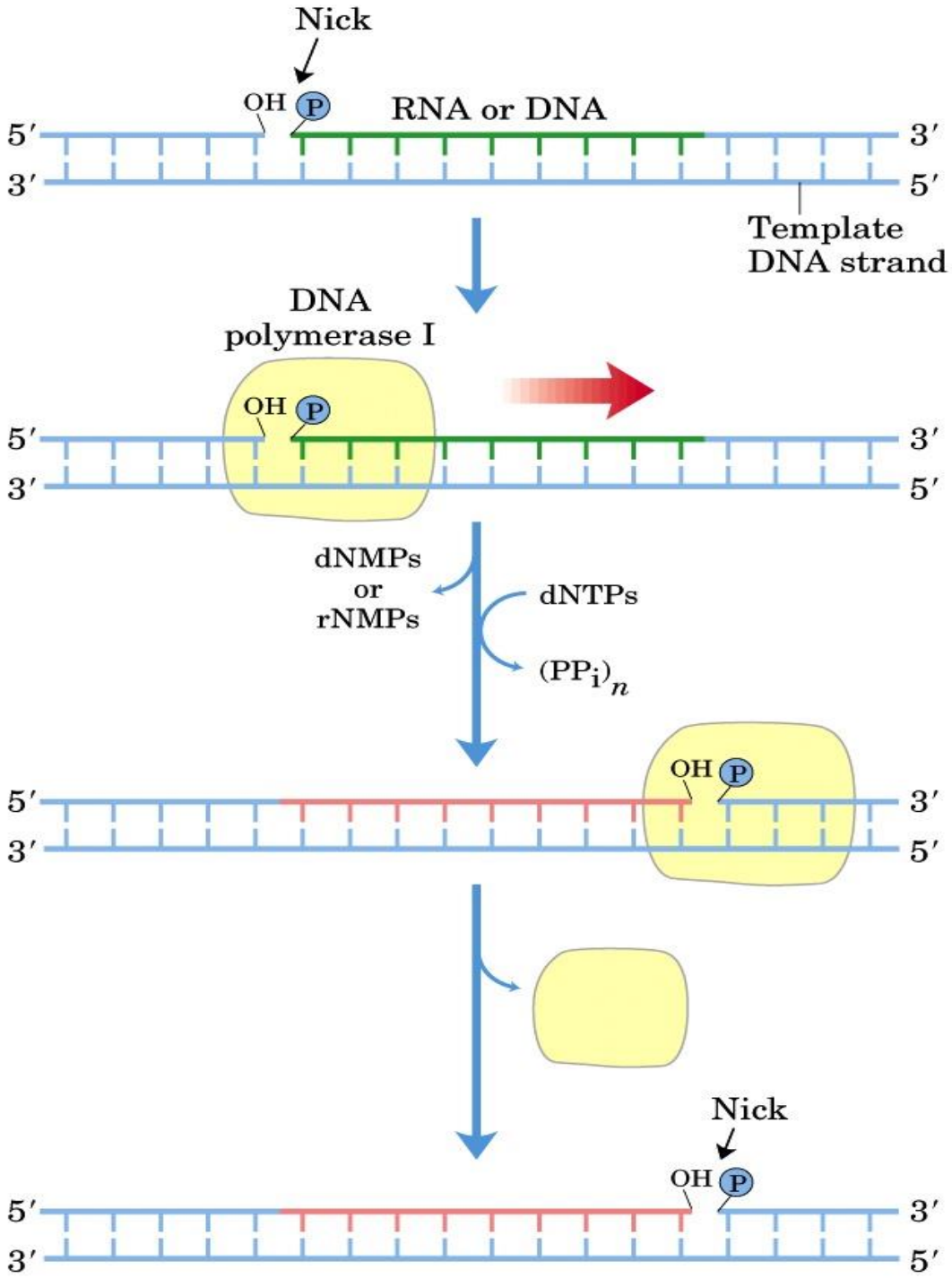
it retains the
5' →3' polymerase activity
3' →5' exonuclease activity
for proofreading,

but loses its 5' → 3'
exonuclease activity



The 5>3 exonuclease activity of intact DNA polymerase I can replace a segment of DNA (or RNA) paired to the template strand, in a process known as nick translation

Nick translation:



Nick translation

In this process, an RNA or DNA strand paired to a DNA template is simultaneously degraded by the 5>3 exonuclease activity of DNA polymerase I and replaced by the polymerase activity of the same enzyme.

These activities have a role in both **DNA repair** and the removal of **RNA primers** during replication (both described later). The strand of nucleic acid to be removed (either DNA or RNA) is shown in green, the replacement strand in red.

DNA synthesis begins at a nick (a broken phosphodiester bond, leaving a free 3 hydroxyl and a free 5 phosphate).
Polymerase I extends the non template DNA strand and moves the nick along the DNA—a process called nick translation. A nick remains where DNA polymerase I dissociates, and is later sealed by another enzyme.

DNA polymerase III.

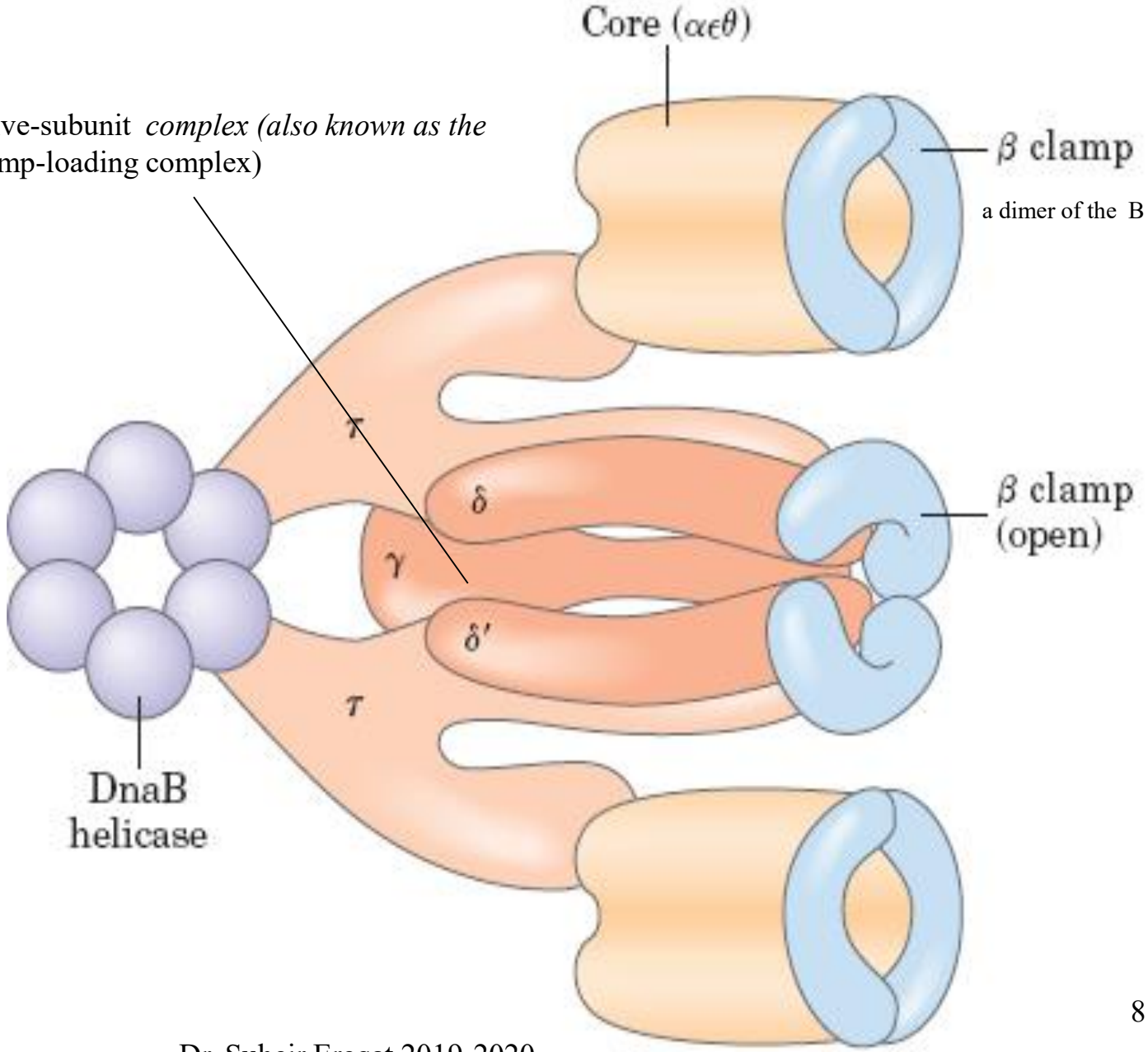


table 25–2

Subunits of DNA Polymerase III of *E. coli*

Subunit	Number of subunits per holoenzyme	M_r of subunit	Gene	Function of subunit	
α	2	132,000	*pol*C (*dna*E)	Polymerization activity	Core polymerase
ϵ	2	27,000	*dna*Q (*mut*D)	3′→5′ Proofreading exonuclease	
θ	2	10,000	*hol*E		
τ	2	71,000	*dna*X	Stable template binding; core enzyme dimerization	Polymerize DNA with minimum processivity
γ	2	52,000	*dna*X*	Clamp-loading complex that loads β subunits on lagging strand at each Okazaki fragment	
δ	1	35,000	*hol*A		
δ'	1	33,000	*hol*B		
χ	1	15,000	*hol*C		
ψ	1	12,000	*hol*D		
β	4	37,000	*dna*N	DNA clamp required for optimal processivity	Increase processivity to >500,000

*The γ subunit is encoded by a portion of the gene for the τ subunit, such that the amino-terminal 80% of the τ subunit has the same amino acid sequence as the γ subunit. The γ subunit is generated by a translational frameshifting mechanism (see Box 28–1) that leads to premature translational termination.

DNA Replication Requires Many Enzymes and Protein Factors

Helicases: move along the DNA and separate the strands, using chemical energy from ATP.

topoisomerases: release the topological stress in the helical DNA structure created by strand separation

Single strand DNA-binding protein(SSB): stabilize the separated strands.

Primases : synthesize segment of RNA (primer)

DNA ligases: seal the nick remains in the DNA backbone in the form of a broken phosphodiester bond.

DNA polymerase I:remove RNA primer and replace it by DNA

The timing of replication initiation is affected by DNA methylation and interactions with the bacterial plasma membrane

Synthesis of DNA divided into 3 stages