Genetics

L2 Natural science and life

Provided by::

Sabri Bousbia, *Ph.D.*

Centre Universitaire Abdelhafid Boussouf, Mila, Algérie

Genetics: a little history

✓Definition:

- The word genetics comes from the ancient Greek "γενετικός" meaning "genitive" or "generative", which in turn derives from the word "genesis" (γένεσις) meaning "origin".

- Genetics is the science that studies heredity and genes in living beings, it is a sub-discipline of biology.

Genetics: a little history

✓Definition:

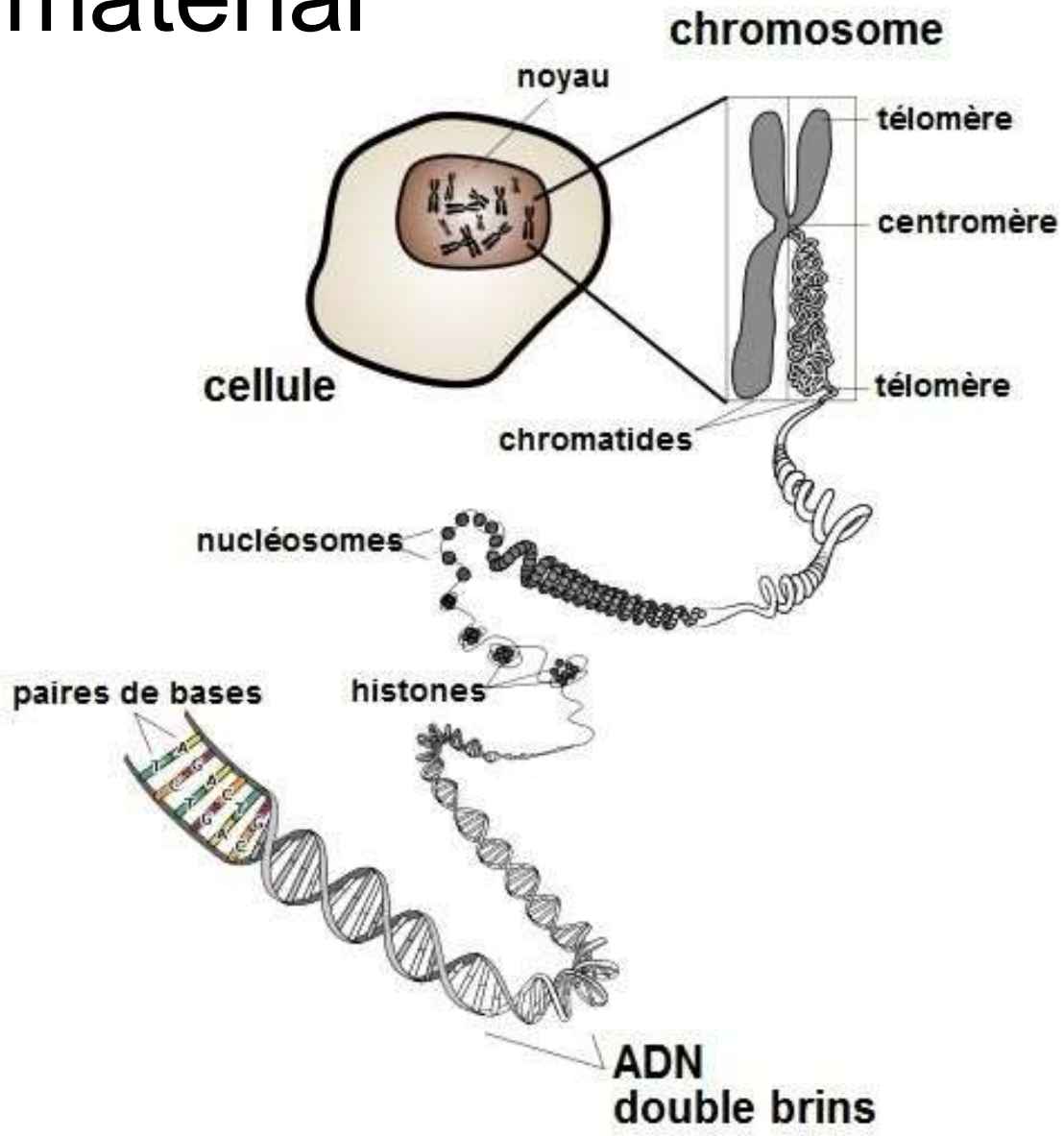
- Today, genetics has diversified into several different branches such as:
- developmental genetics
- medical genetics
- genomics
- quantitative genetics
- genetics of populations
- ……etc.

1. Chemical nature of genetic material

- 1.1. Structure of nucleic acids:
  - Genetic material consists of nucleic acids (DNA and RNA)
  - Nucleic acids are polymers of very large size of nucleotides (macromolecules).

1. Chemical nature of genetic material

DNA in eukaryotes



1. Chemical nature of genetic material

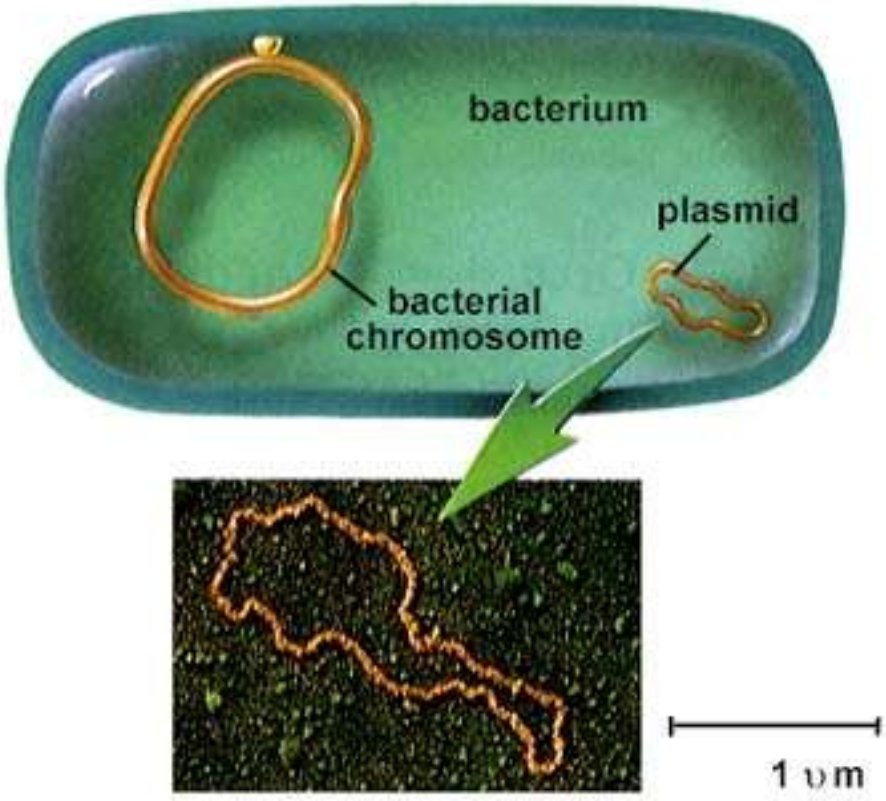
Bacterial genome:
chromosome + plasmid

- Haploid genome: only 1 copy of each chromosome per bacterium

- Structural organization: The bacterial chromosome is often circular and present in the nucleoid

- Contain significantly fewer genes than eukaryotic genomes

- Almost the entire bacterial genome is coding!



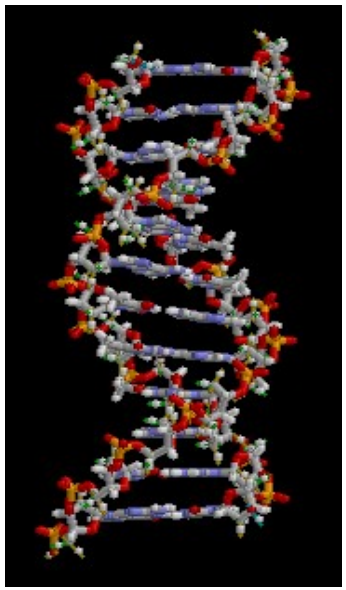
1. Chemical nature of genetic material

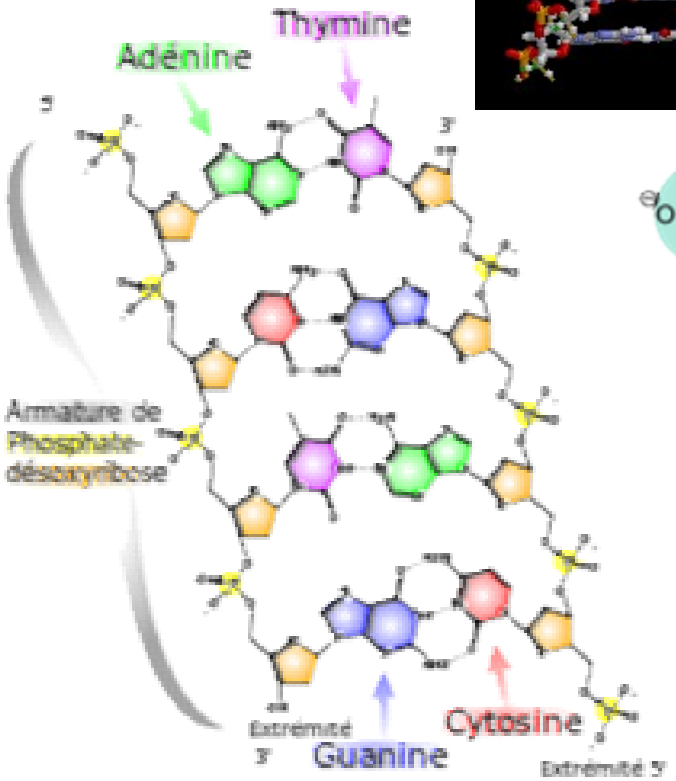
DNA

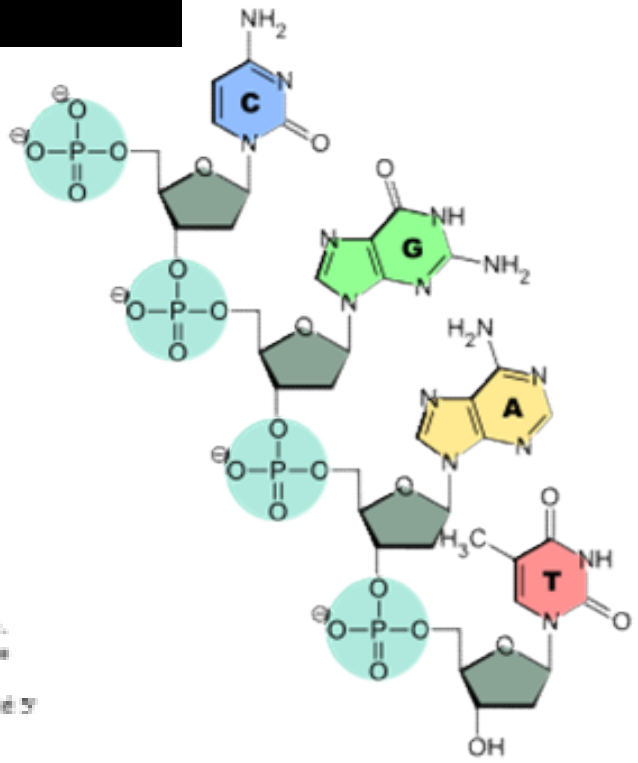
-DNA is shaped like a double helix.

-The sides are anti-parallel.

-Each side (or end) therefore has a 5'phosphorylated end and a 3'hydroxylated end.







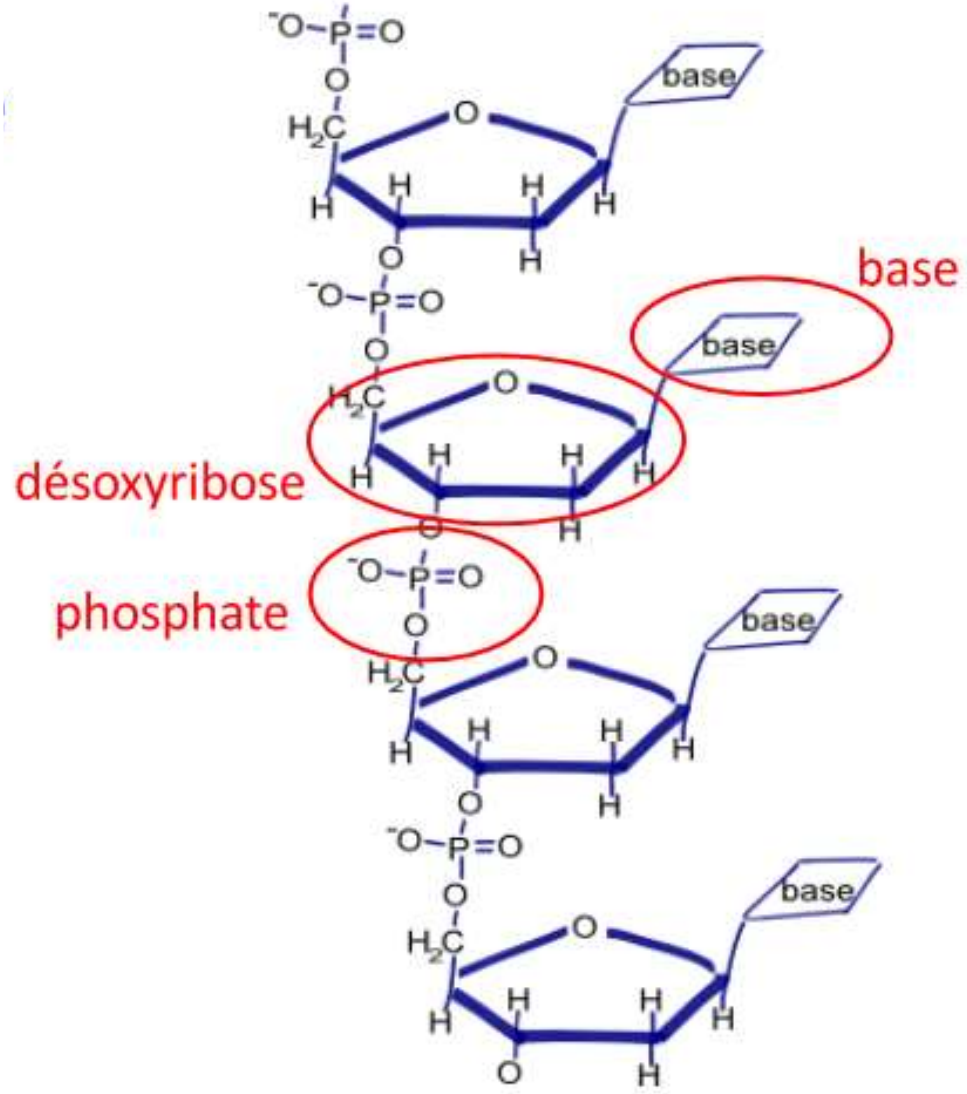
1. Chemical nature of genetic material

DNA:

-DNA is a polymer (made up of several monomers).

-The DNA monomer is called a nucleotide (sugar + base + phosphate).

-Nucleotides are linked together by **phosphodiester bonds** to form the sides of the DNA molecule.



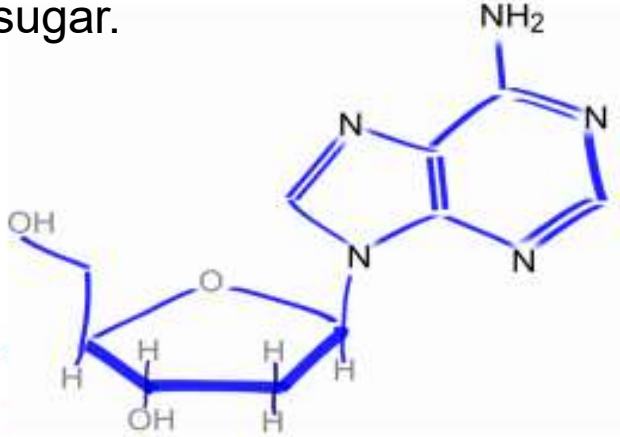
1. Chemical nature of genetic material

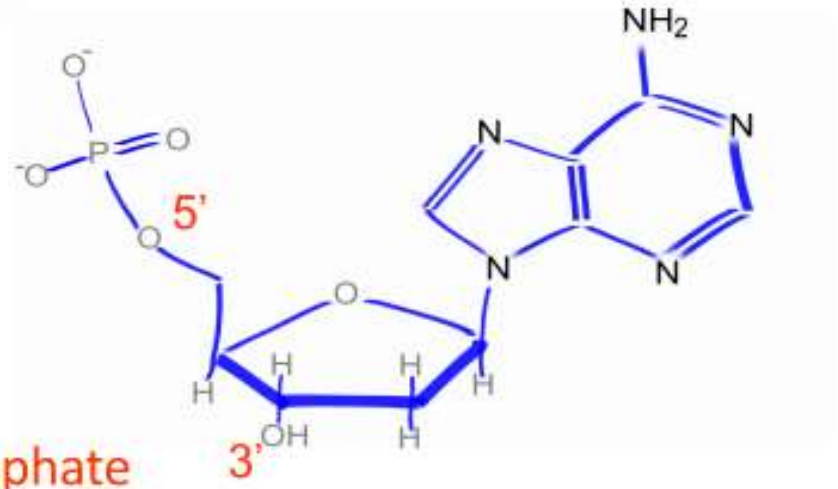
Deoxyribonucleotide:

- A phosphate group attached to carbon 5 of deoxyribose (sugar) and a nitrogenous base attached to carbon 1 in the sugar.

Base

Nucléoside =
sucre + base



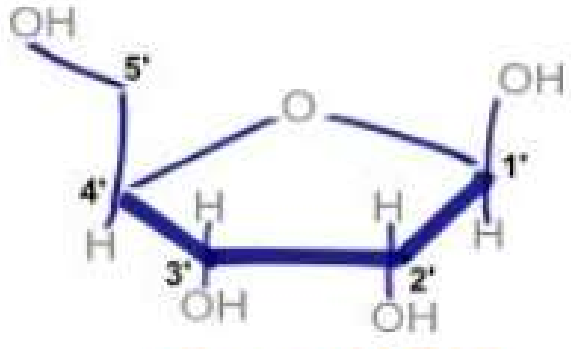


Nucléotide =
sucre + base + phosphate

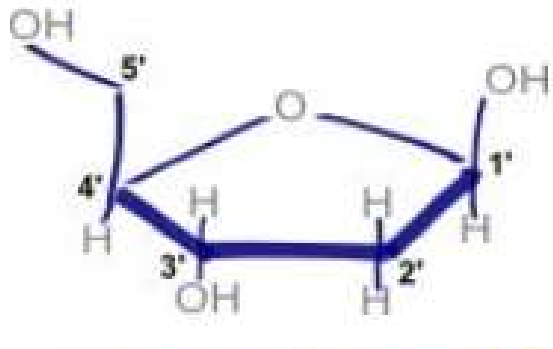
1. Chemical nature of genetic material

DNA and RNA sugars

- Knowing the carbon numbering (3’ and 5’)!
- In DNA, evolution has selected the deoxyribose to transmit information
- In RNA it is a ribose



RNA Ribose

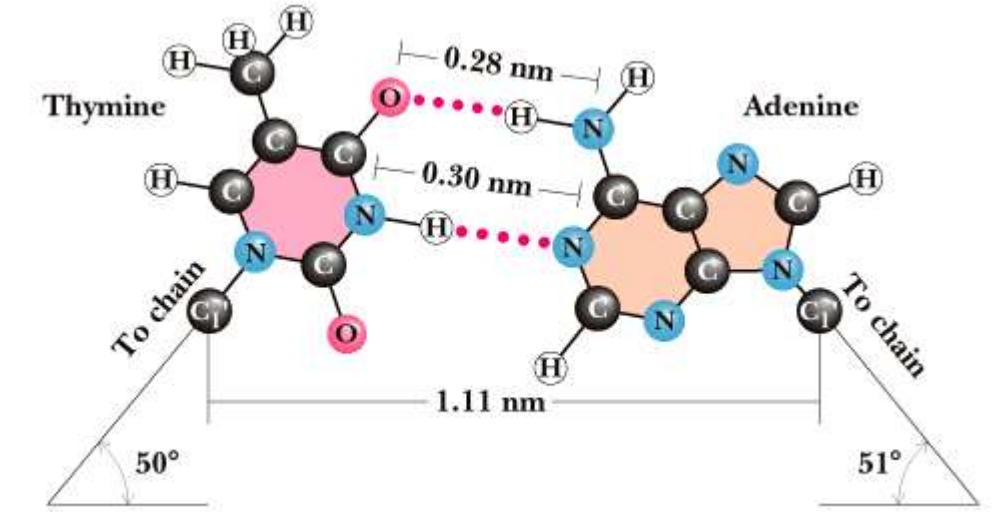


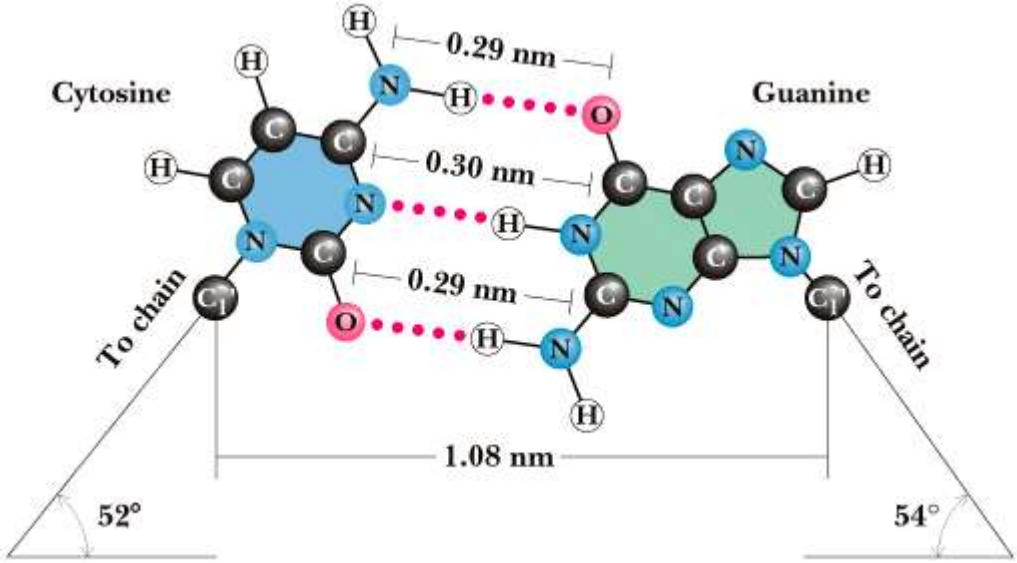
DNA Ribose

1. Chemical nature of genetic material

DNA

- The secondary structure of nucleic acids is imposed by base pairing, that is, the formation of hydrogen bonds between two organic bases.
- Adenine is bonded with thymine using 2 hydrogen bonds. (A-T)
- Guanine is bonded with Cytosine using 3 hydrogen bonds. (G-C)





1. Chemical nature of genetic material

DNA and RNA

Purine bases

adenine

guanine

Pyrimidine bases

cytosine

thymine

thymine is replaced by uracil

uracil

Nomenclature of Nucleosides and Nucleotides

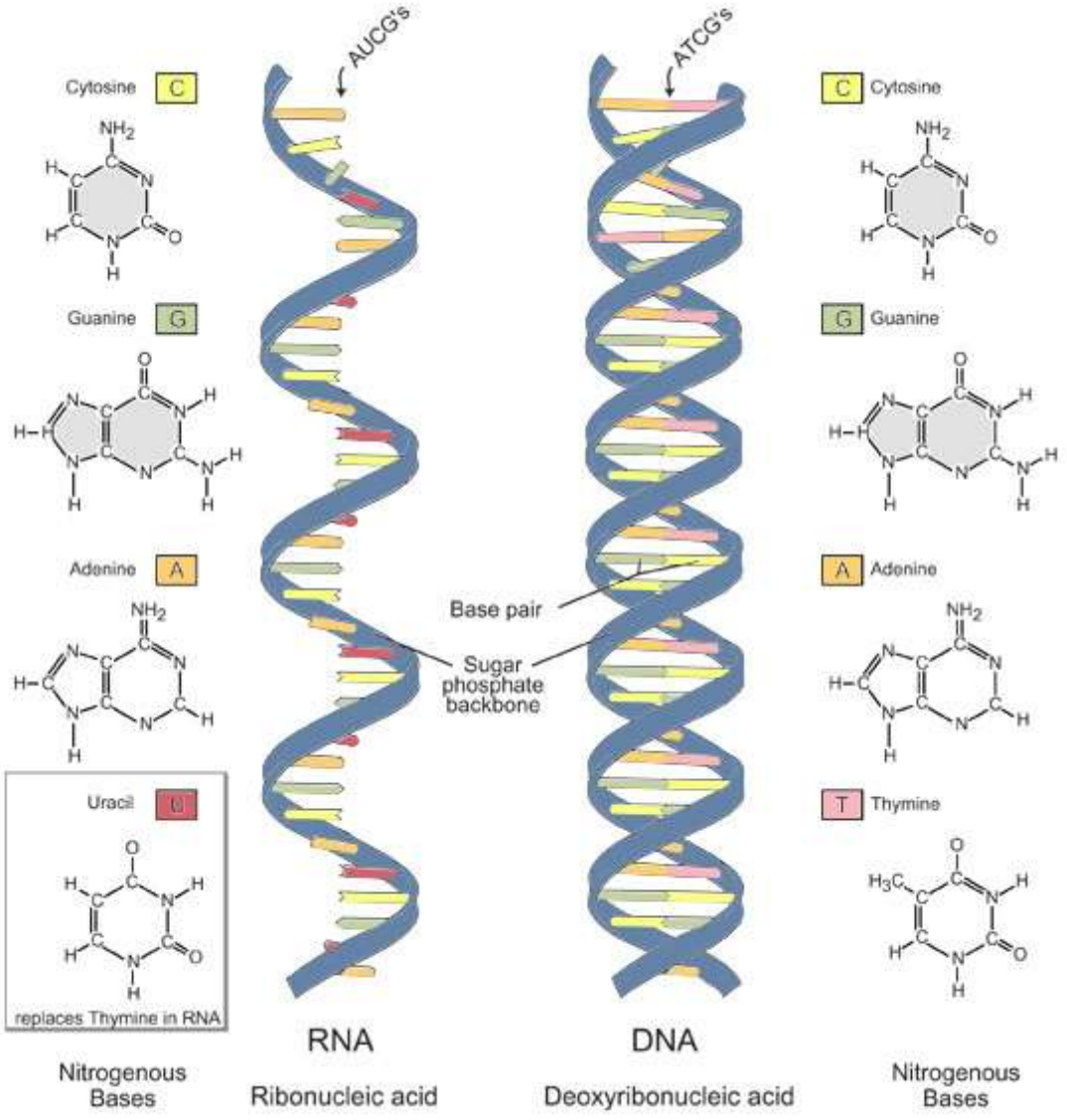
Nomenclature of the main nucleosides:

Base	Ribonucleoside	Deoxyribonucleoside
Adénine	Adenosine	Deoxyadénosine
Guanine	Guanosine	Deoxyguanosine
Uracile	Uridine	Deoxyuridine
Cytosine	Cytidine	Deoxycytidine
Thymine		Deoxythymidine

Nomenclature of the main monophosphate nucleotides

	ARN	ADN
Base	Ribonucleoside-5'-monophosphate	Deoxyribonucleoside-5'-mnophosphate
Adénine	Adénosine-5'-monophosphate = AMP	Deoxyadénosine-5'-monophosphate = dAMP
Guanine	Guanosine-5'-monophosphate = GMP	Deoxyguanosine-5'-monophosphate = dGMP
Uracile	Uridine-5'-monophosphate = UMP	Deoxyuridine-5'-monophosphate = dUMP
Cytosine	Cytidine-5'-monophosphate = CMP	Deoxycytidine-5'-monophosphate = dCMP
Thymine		Deoxythymidine-5'-monophosphate = dTMP

DNA vs RNA


AUCG's
ATCG's
Cytosine C
Guanine G
Adenine A
Uracil U
replaces Thymine in RNA
Base pair
Sugar phosphate backbone
C Cytosine
G Guanine
A Adenine
T Thymine
RNA
Ribonucleic acid
DNA
Deoxyribonucleic acid
Nitrogenous Bases
Nitrogenous Bases

DNA vs RNA

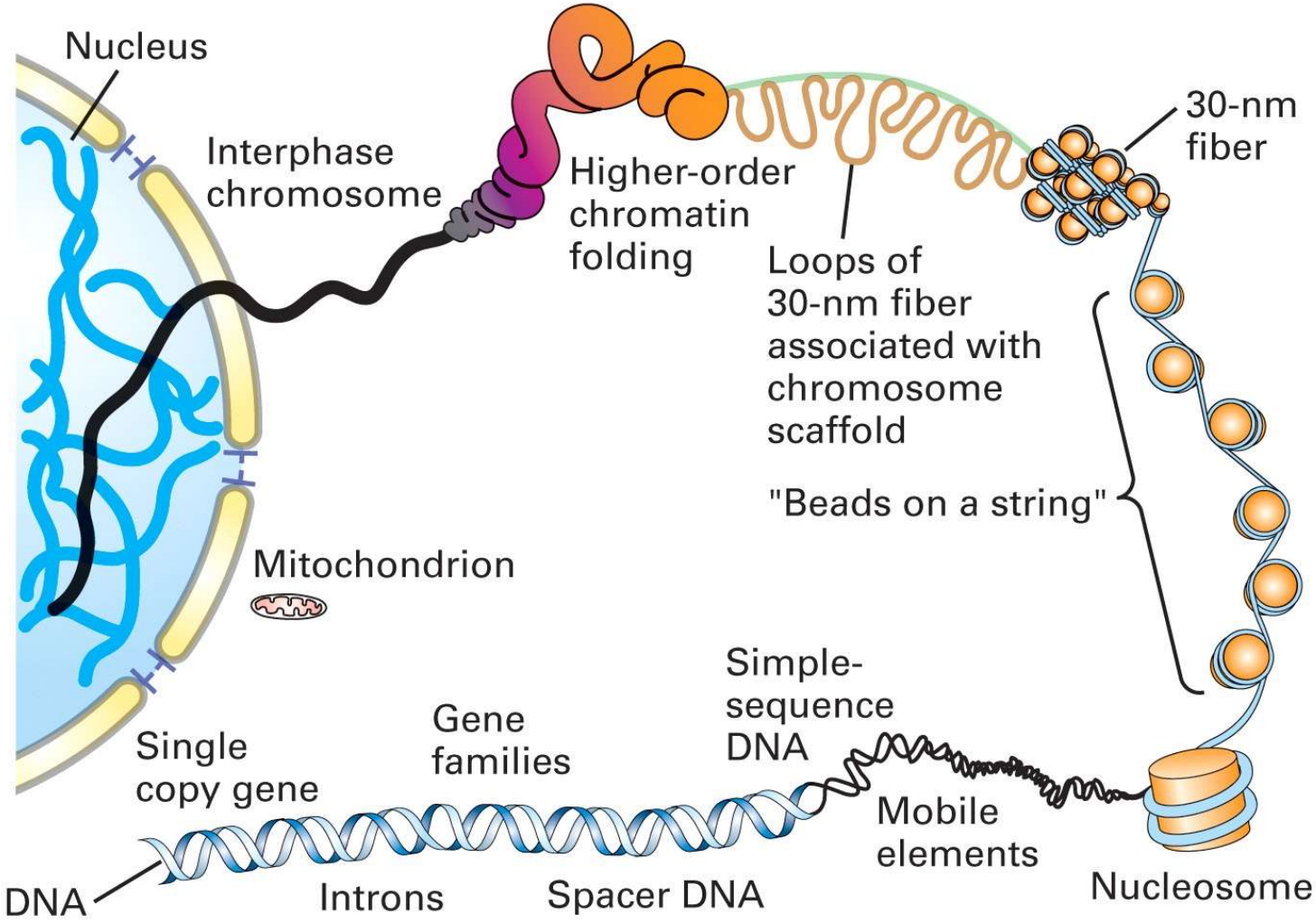
- Deoxyribonucleic Acid
- double helix
- Formes:
  - Nuclear
  - Mitochondrial
  - Chromosomic
- Don't leave cell nucleus
- Contient C, G, A, T

- Ribonucleic Acid
- Simple helix
- Formes:
  - Messenger
  - Transfer
  - Ribosomal, ….
- leave cell nucleus
- Content C, G, A, U

DNA organization in the nucleus

- **The DNA of eukaryotes is associated with proteins (histones) to form the chromatin**

DNA organization in the nucleus



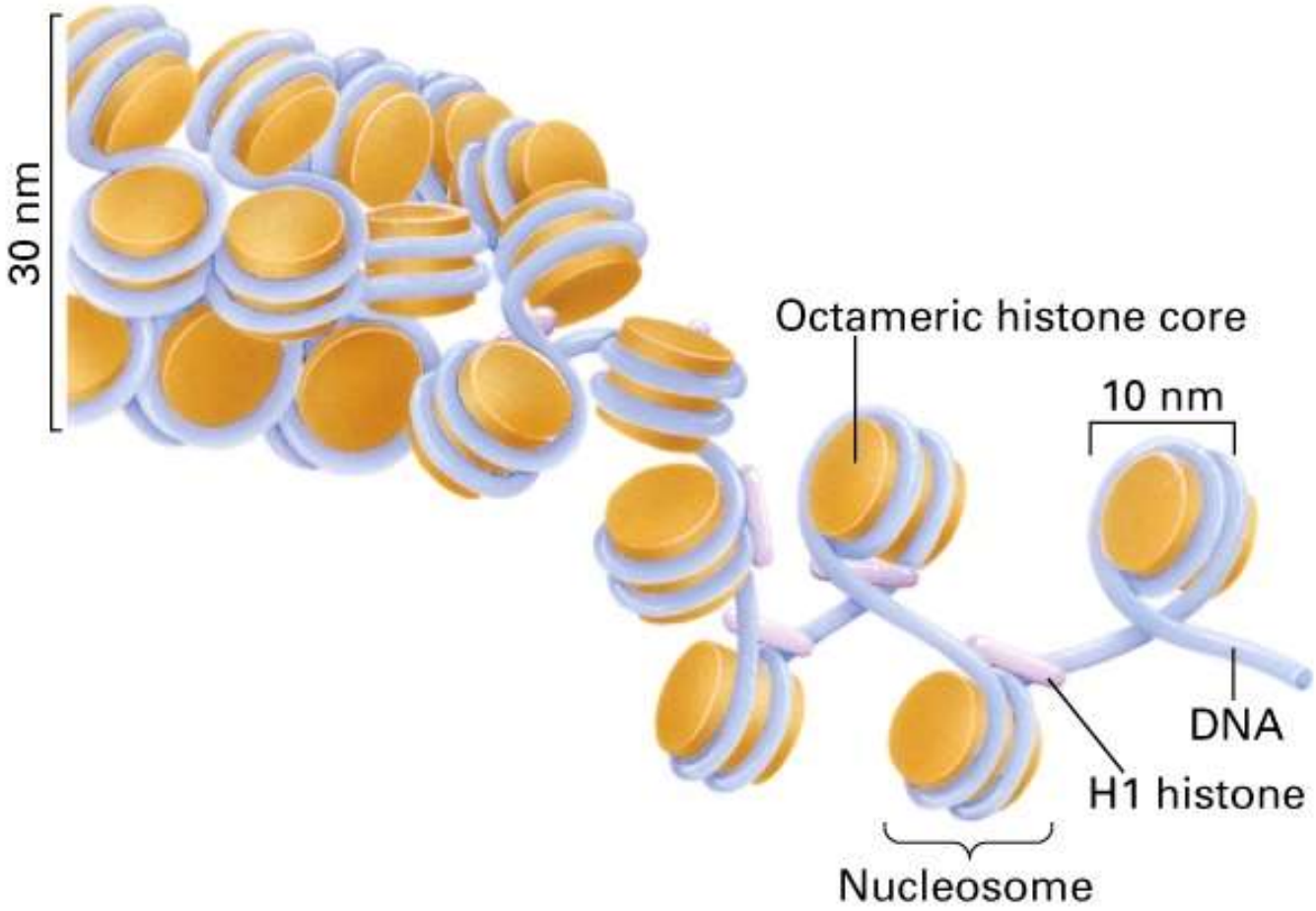
Chromatin structure

- Chromatin is found in an expanded (euchromatin) or condensed (heterochromatin) form.
- In the intact nucleus it is almost impossible to observe the structure of chromatin. The isolation of chromatin allowed us to establish its structure.

Condensed and decondensed form of chromatin

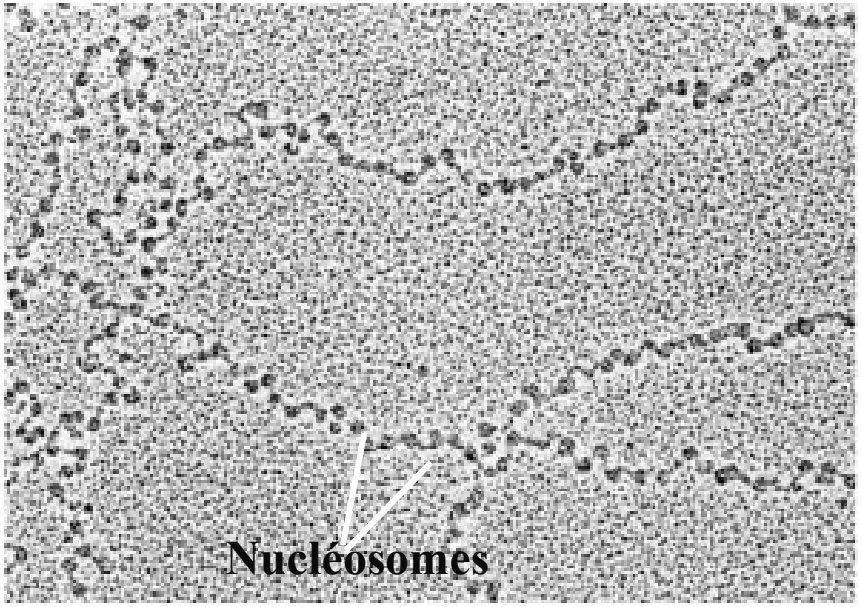
- Untranscribed chromatin (heterochromatin) would be mainly in condensed form (30nm)
  - Nucleosomes are compacted into spirals or solenoidal arrangements (30nm structure).
  - There are 6 nucleosomes per turn.
- The transcribed chromatin (Euchromatin) would be in a stretched form like a string of beads (10nm).

Chromatin condensation model



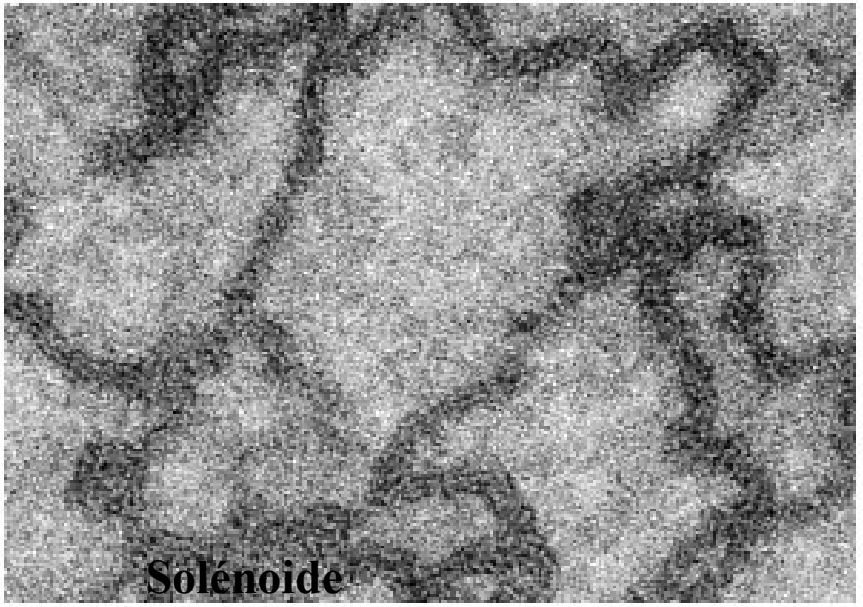
Chromatin is found in a dilated or condensed form

(a)



a) Low salt concentration isolation

(b)



b) Physiological saline concentration isolation

DNA association with histones: - The Chromatin

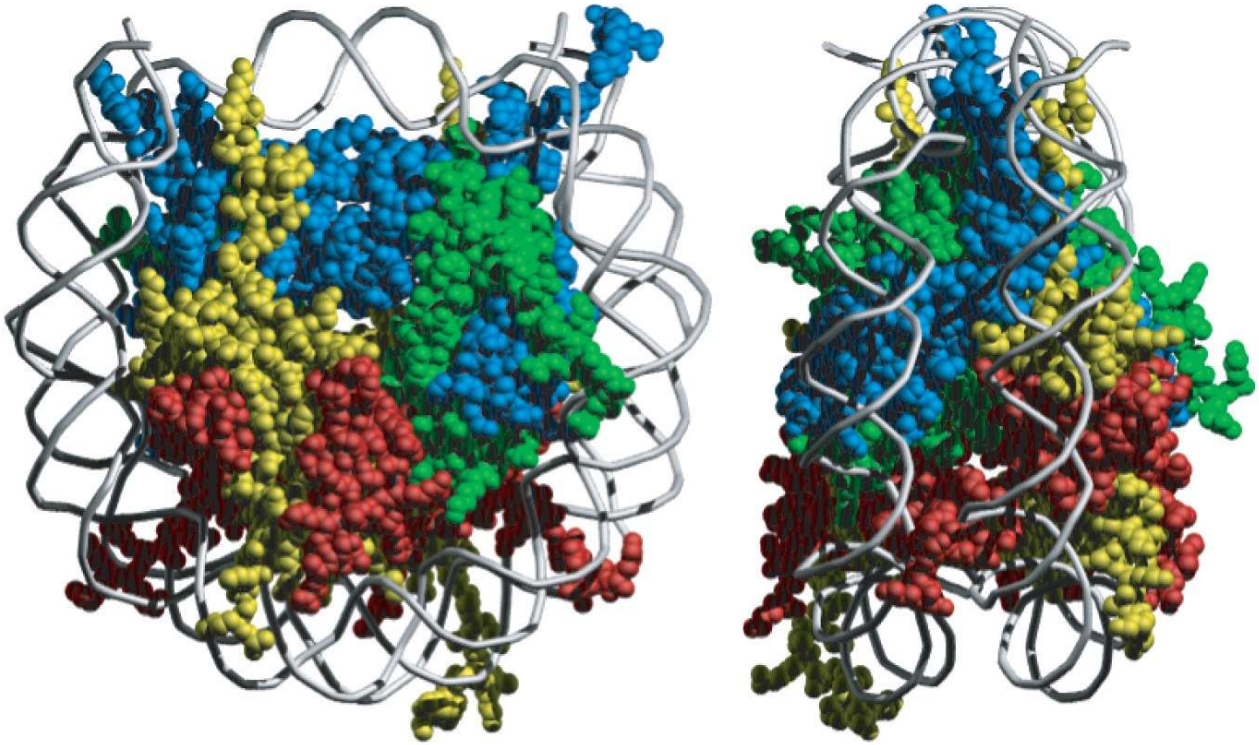
- Histones are the major proteins associated with eukaryotic DNA.
- Histones are basic proteins
- The main histones are H1, H2A, H2B, H3 and H4
- Histones are proteins rich in basic AAs (amino acids) that bind to the negative charges of phosphate groups on DNA.

Nucléosomes Structure

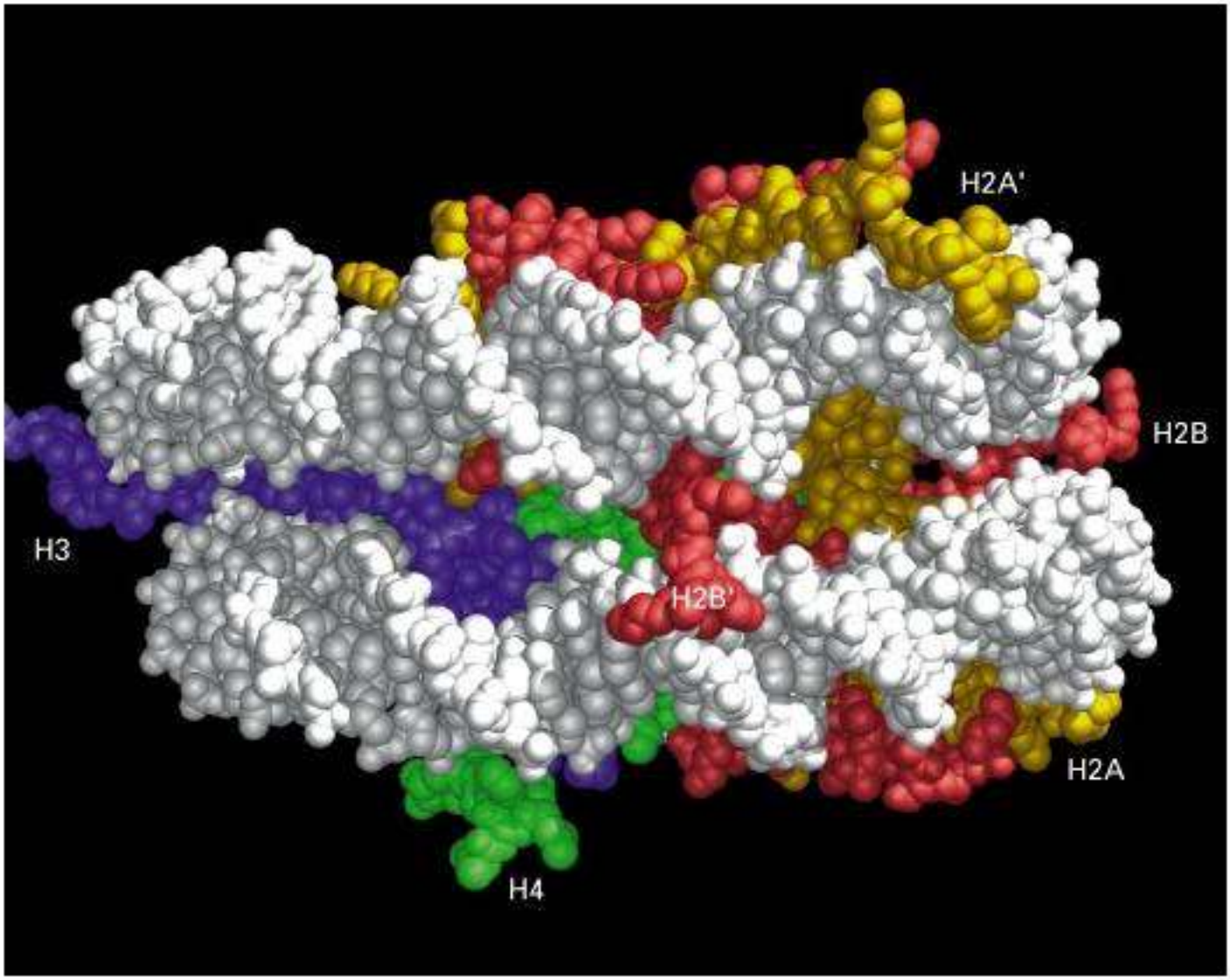
- **Nucleosome**
  - DNA, 147 base pairs, is wrapped around the structure formed by histones (histone octamer) (H2A, H2B, H3, H4).

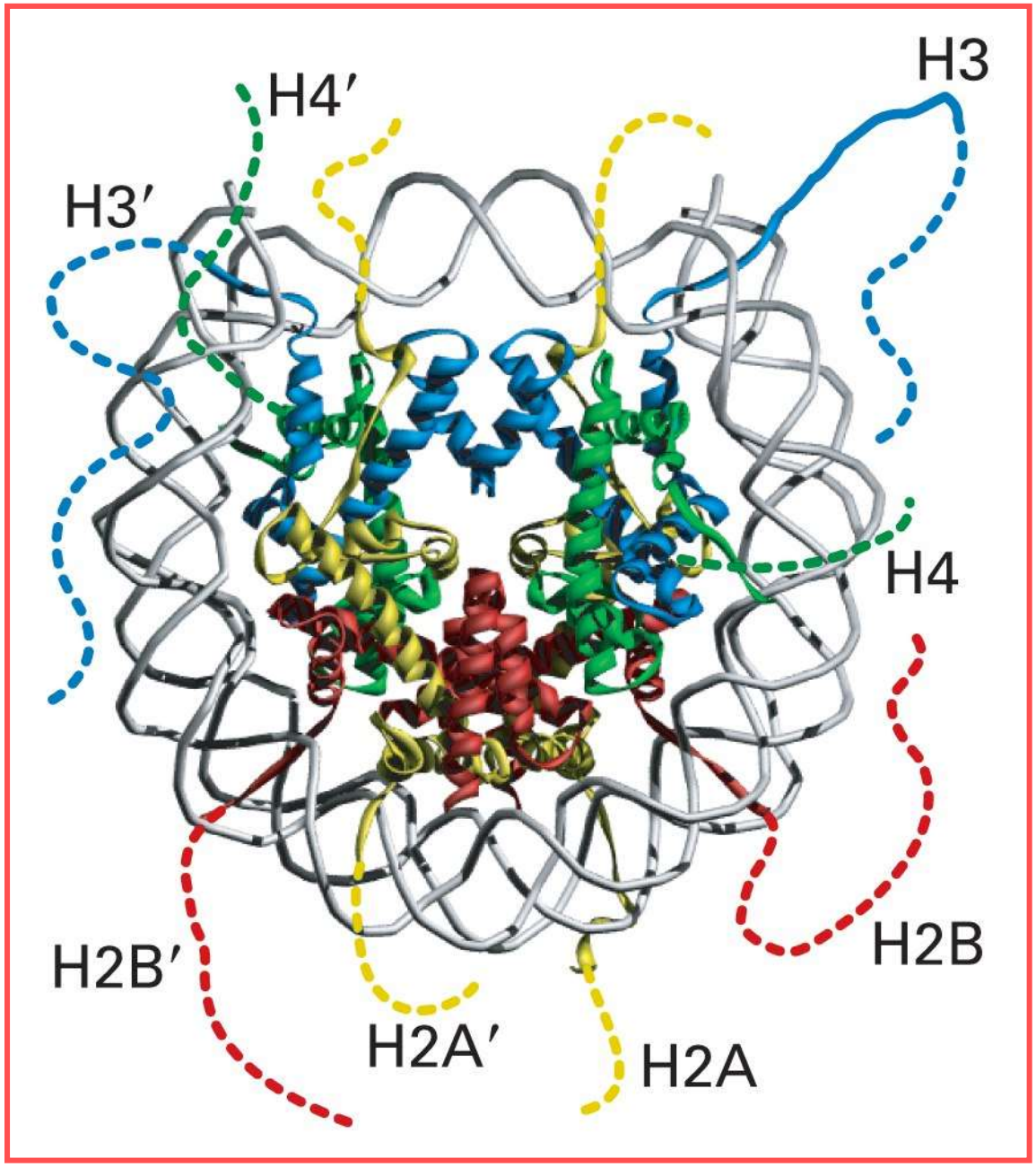
Nucleosomes are complexes of histones and DNA

(147 pairs of nucleotides)



H2A Yellow; H2B Red; H3 Blu; H4 Green


H2A'
H2B
H3
H2B'
H2A
H4

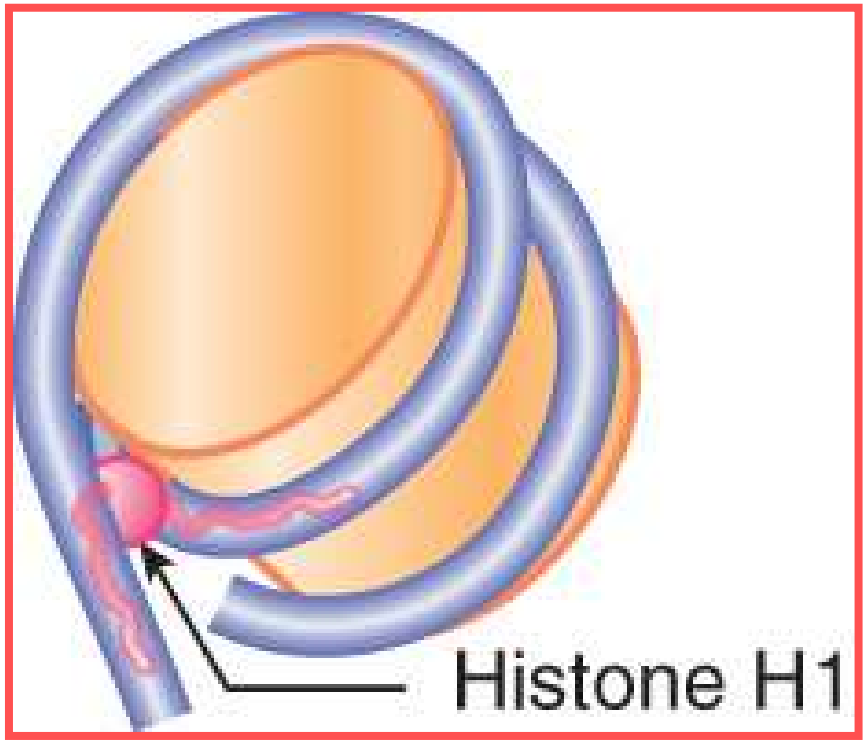


Queues d'histones

N- and C-termini of histones H2A, H2B, H3, and H4 emerge from the nucleosome. These regions are designated as histone tails

Interaction of histone 1 with the nucleosome

- **Histone H1 is attached to DNA at the exit of each nucleosome**



Histone 1 interacts with the central gyre of DNA at the axis, as well as with linker DNA either at the entrance or exit,

2. Organization of DNA into Chromosome

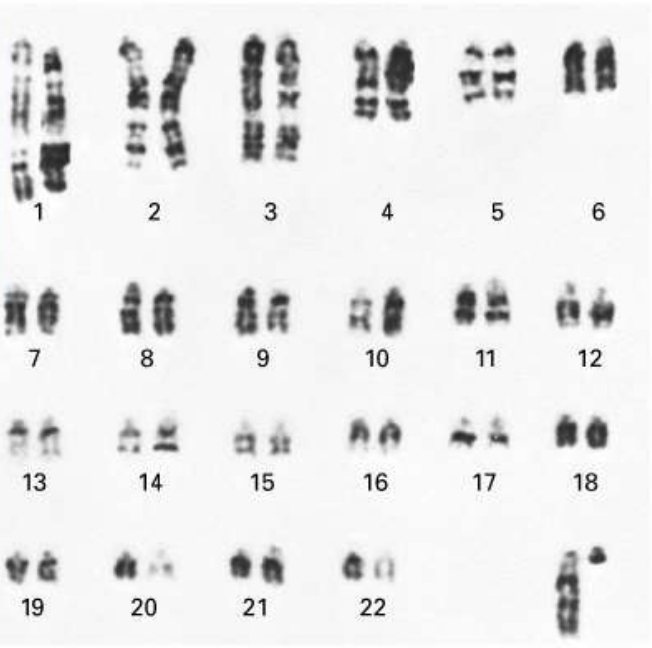
What a Chromosome?

- The chromosome is the highly condensed form of DNA
- It is wrapped in nucleosomes
- Wrapped in chromatin fiber
- Condensed during metaphase into the familiar form
- Humans have 22 autosomal pairs and a pair of sexual chromosome

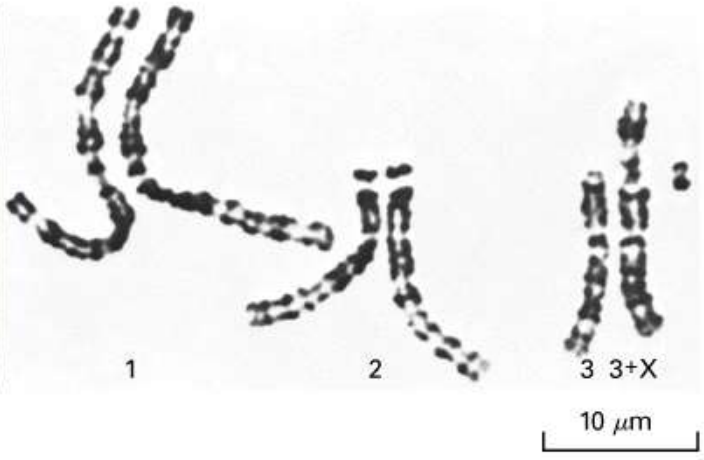
Chromosome number does not correlate with species complexity

Both species have about the same amount of DNA but the chromosomes are very different.

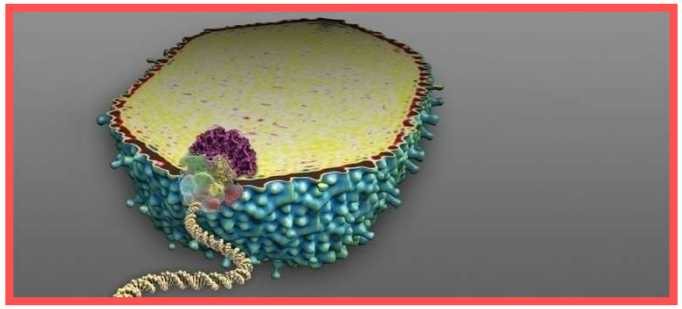
(b)

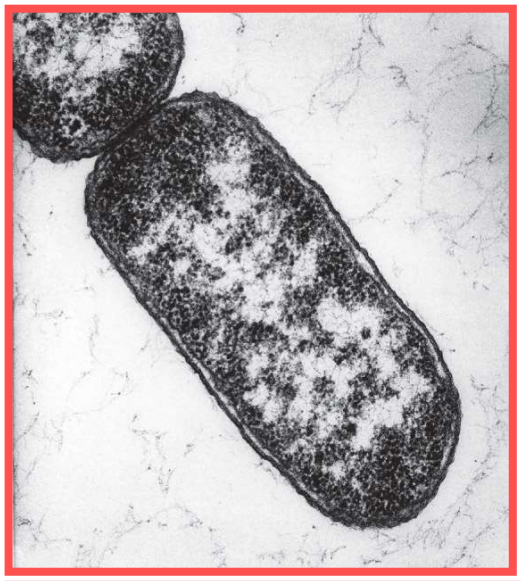


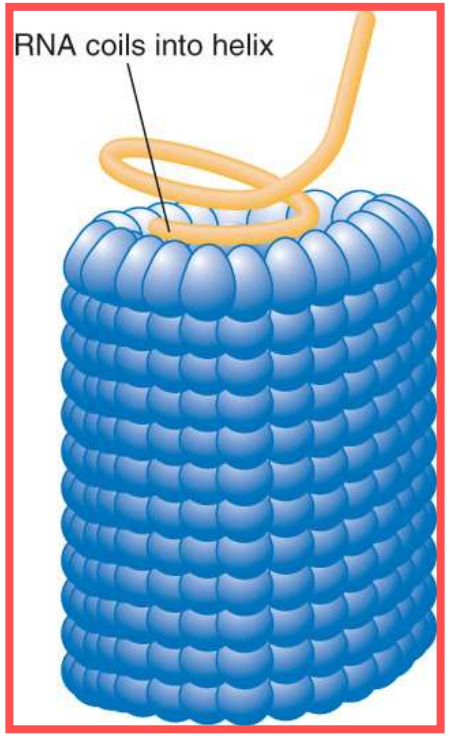
(a)

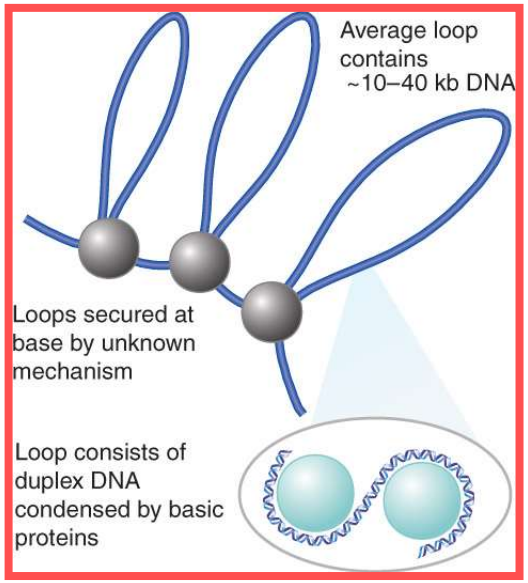


DNA compaction in microorganisms





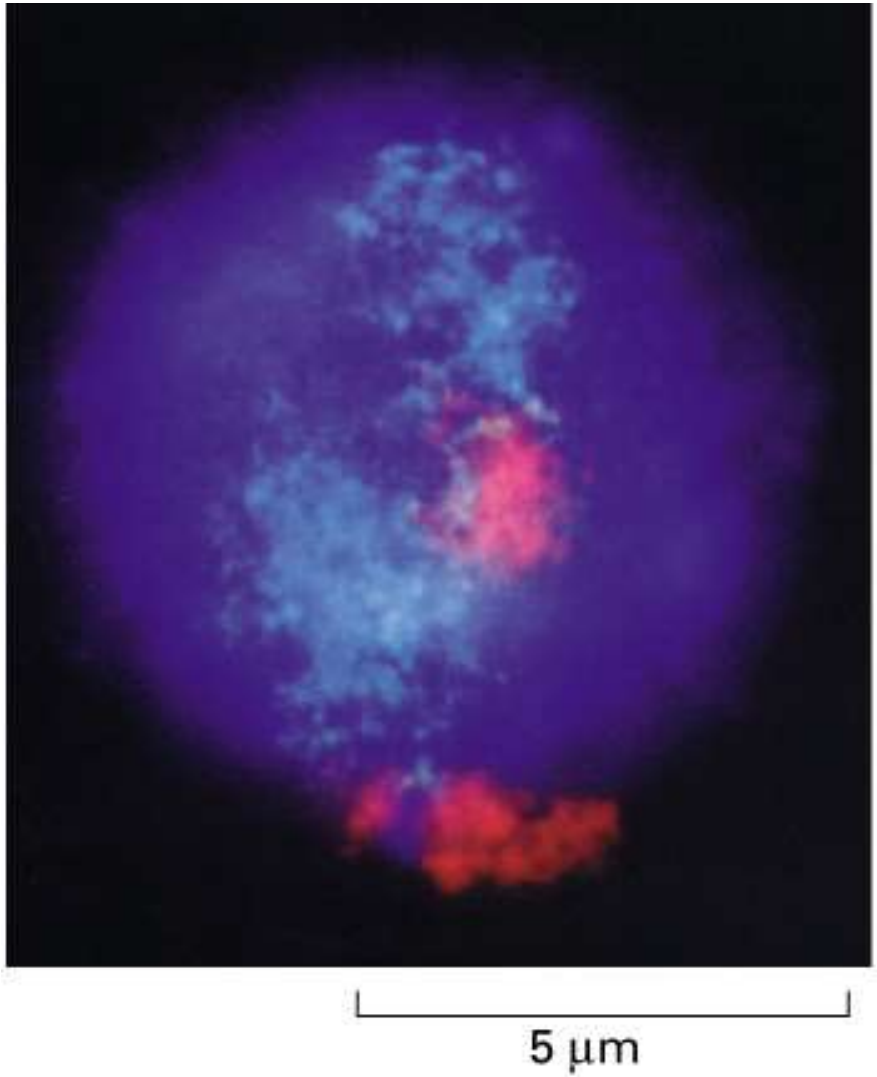




- In viruses, a genomic DNA molecule is associated with protein molecules and packaged into viral capsids.
- In bacteria, genomic DNA is associated with proteins and is packed as a compact mass inside the center of the cell. It is called as "nucleoid"

Organization of DNA into Chromosome in Eukaryotes

Selective localization of two interphase chromosomes
Chromosome 18 (red) and 19 (turquoise)



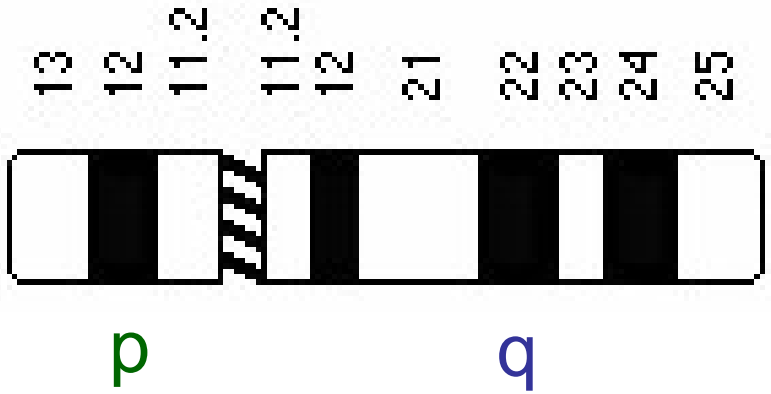
Organization of DNA into chromosomes

- DNA must be very compact to enter the nucleus.
  - DNA: 2 meters long while the nucleus is 5 to 10 μm in diameter.
  - Ex: Chromosome 22 represents 1.5% of the genome. The DNA of this stretched chromosome measures 1.5 cm
  - As an interphase chromosome it is compacted about 1,000X.
  - In mitotic chromosome form it measures 2 μm, compaction of approximately 10,000X.

<u>The organization of DNA must be well controlled to allow its use by the cell</u>

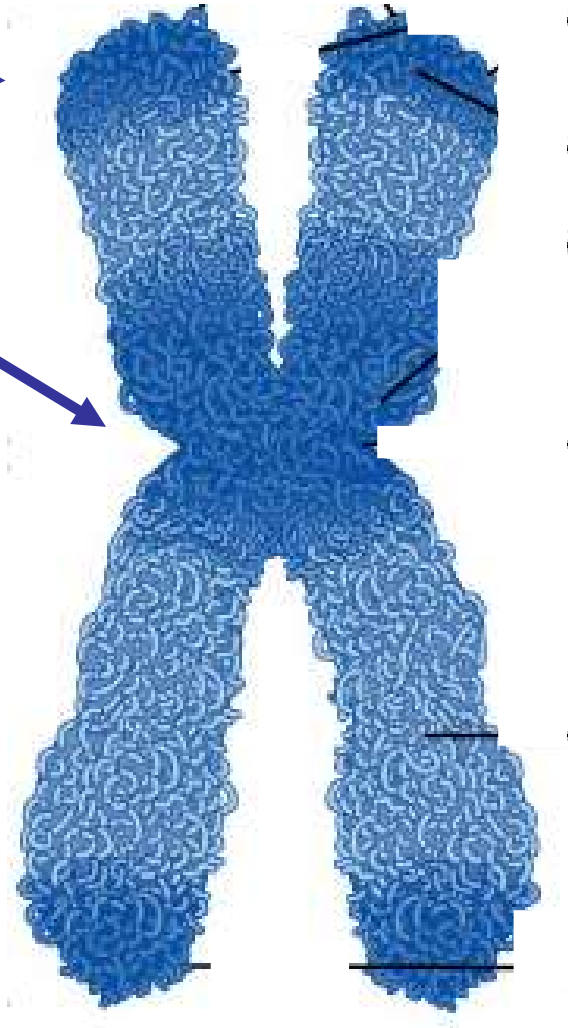
Chromosome Parts:

- p arm – the smaller of the two arms
  - p stands for petite
- q arm – the longer of the two arms
- Bands are numbered from centromere outward



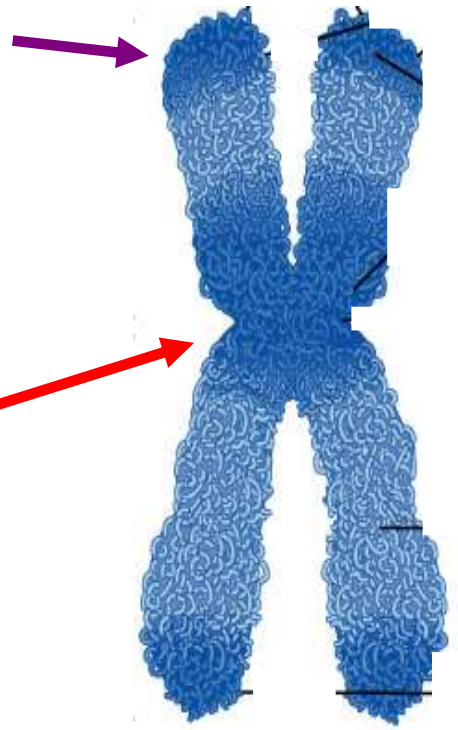
Chromosome Parts :

- Heterochromatin:
  - Highly condensed
  - Silent (methylated) genes
  - Poor in genes
  - Dark spots
- Euchromatin:
  - Less condensed
  - Expressing (Active) genes
  - Rich in genes
  - Lighter spots



Chromosome Parts :

- **Telomeres** - chromosome ends
  - Repetitive sequences
  - Known as a biological clock
  - Be reduced at each mitosis
- **Centromeres** - middle
  - Highly condensed
  - Repetitive sequences
  - Region where microtubules attach
  - Chromatid movement during mitosis



Types of Chromosomes

4 types of chromosomes:

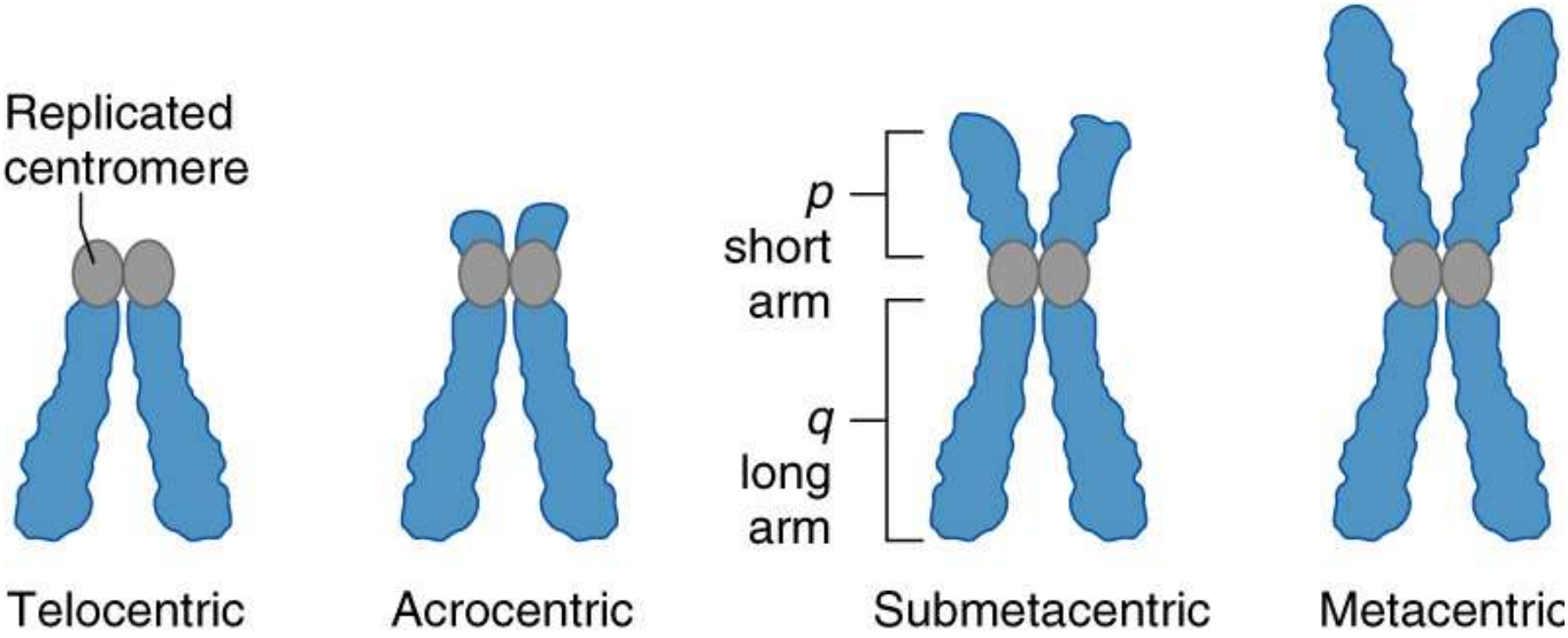
1. Telocentric
2. Acrocentric
3. Submetacentric
4. Metacentric

- Classification based on centromere position

Types de Chromosome :

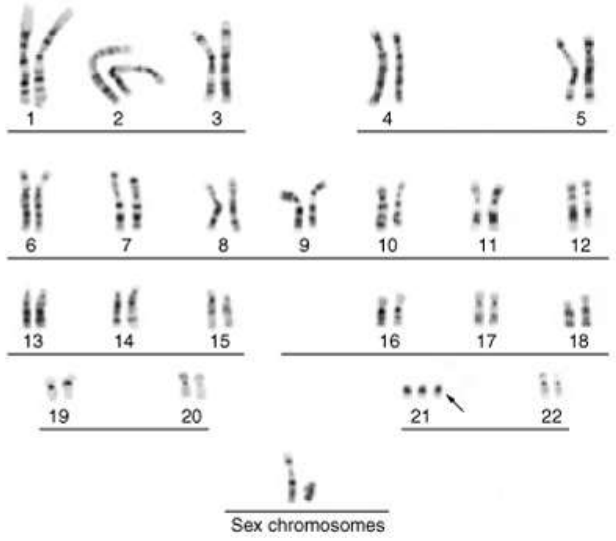
- Telocentric – no p arm; centromere is at the end
- Acrocentric - very small p arm; centromere is very close to the end
- Submétacentric - arm p a little smaller than q arm; centromere in the middle
- Metacentric - p et q les bras sont exactement la même longueur; centromere in the exact middle of the chromosome

Types of Chromosomes :



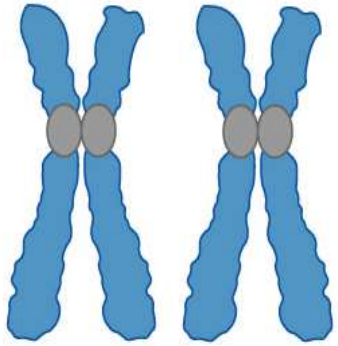
Karyotypes

- Individual's chromosomes in Metaphase, spread out on a slide
- Used to study chromosomes
- Identify chromosomal abnormalities
- Cytogenetics



Remember that…

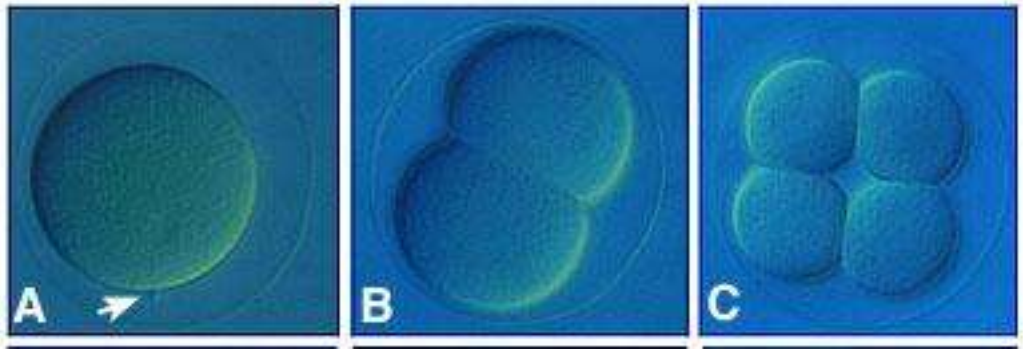
- Homologous chromosomes are not identical
  - May have different gene alleles
- Sister chromatids are identical



3. DNA Replication

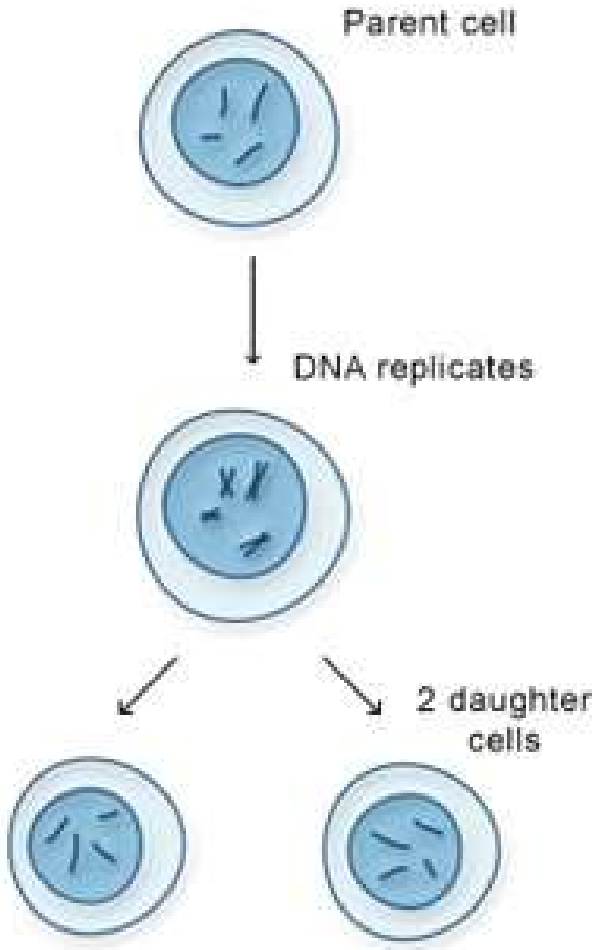
Cell Division and DNA Replication

- Cells divide for growth, repair and tissue replacement



Before cells divide they must duplicate the cell's structures, such as organelles and their genetic information.

DNA Replication

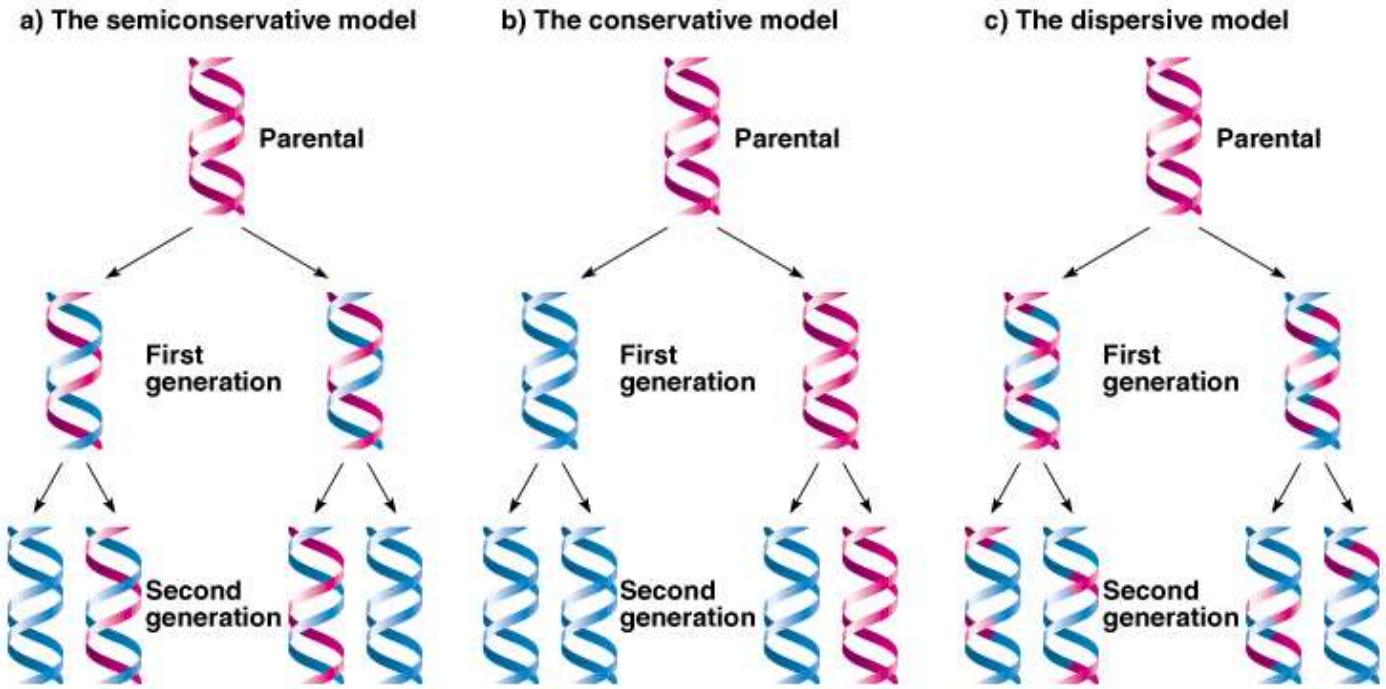


Proposed Models of DNA Replication

- In the 1950s, three different mechanisms were proposed for DNA replication.
- Conservative theory: Both parental strands remain intact after DNA replication
- Semi-conservative theory: Each daughter DNA molecule consists of a parent strand and a newly synthesized strand.
- Dispersive Theory: Parent and daughter DNA molecules are broken into fragments

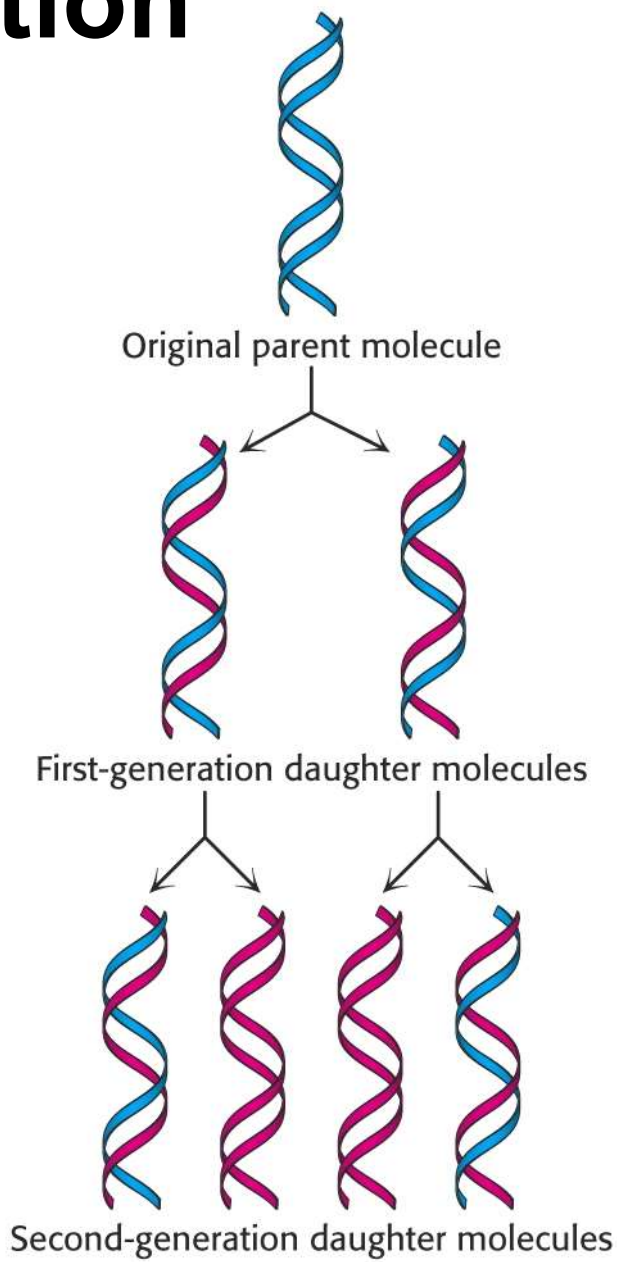
Semi-conservative Replication

Alternative models of DNA replication

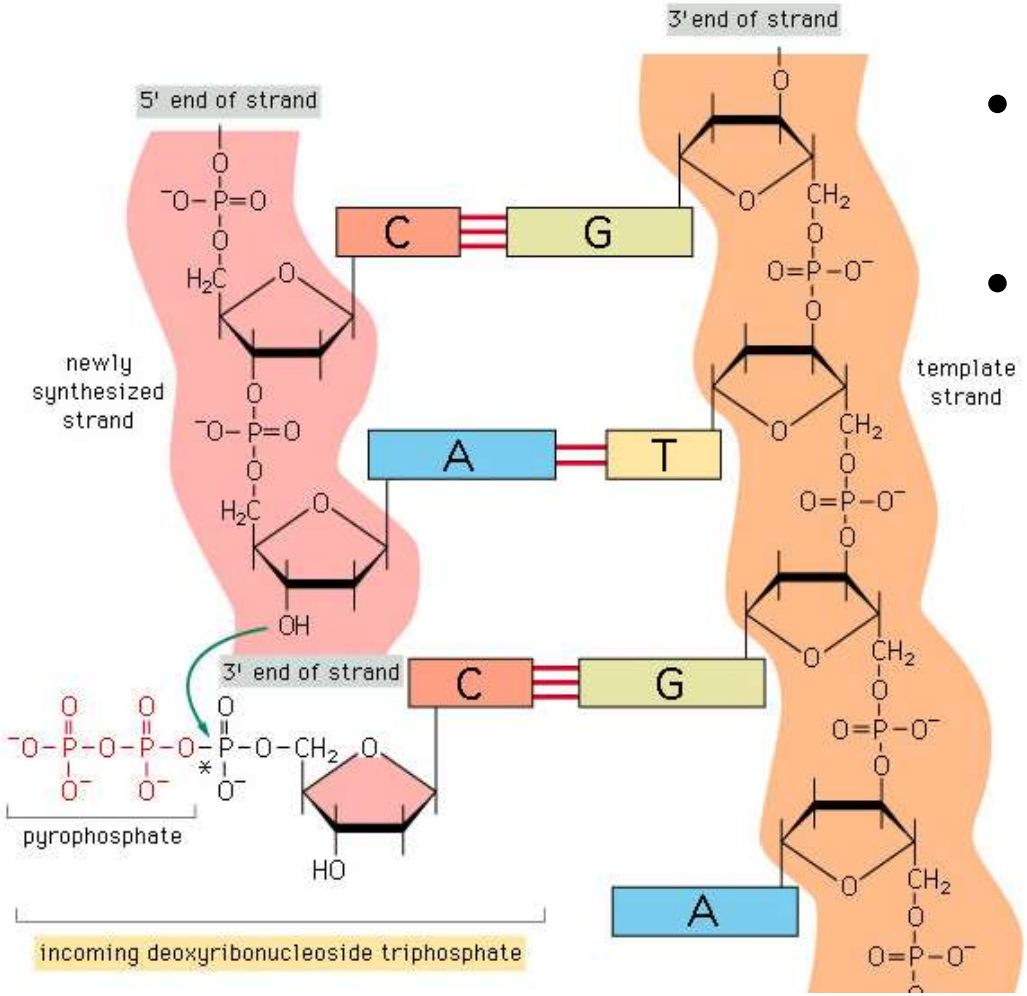


DNA Replication

- Semi-conservative theory!
- Watson and Crick theory

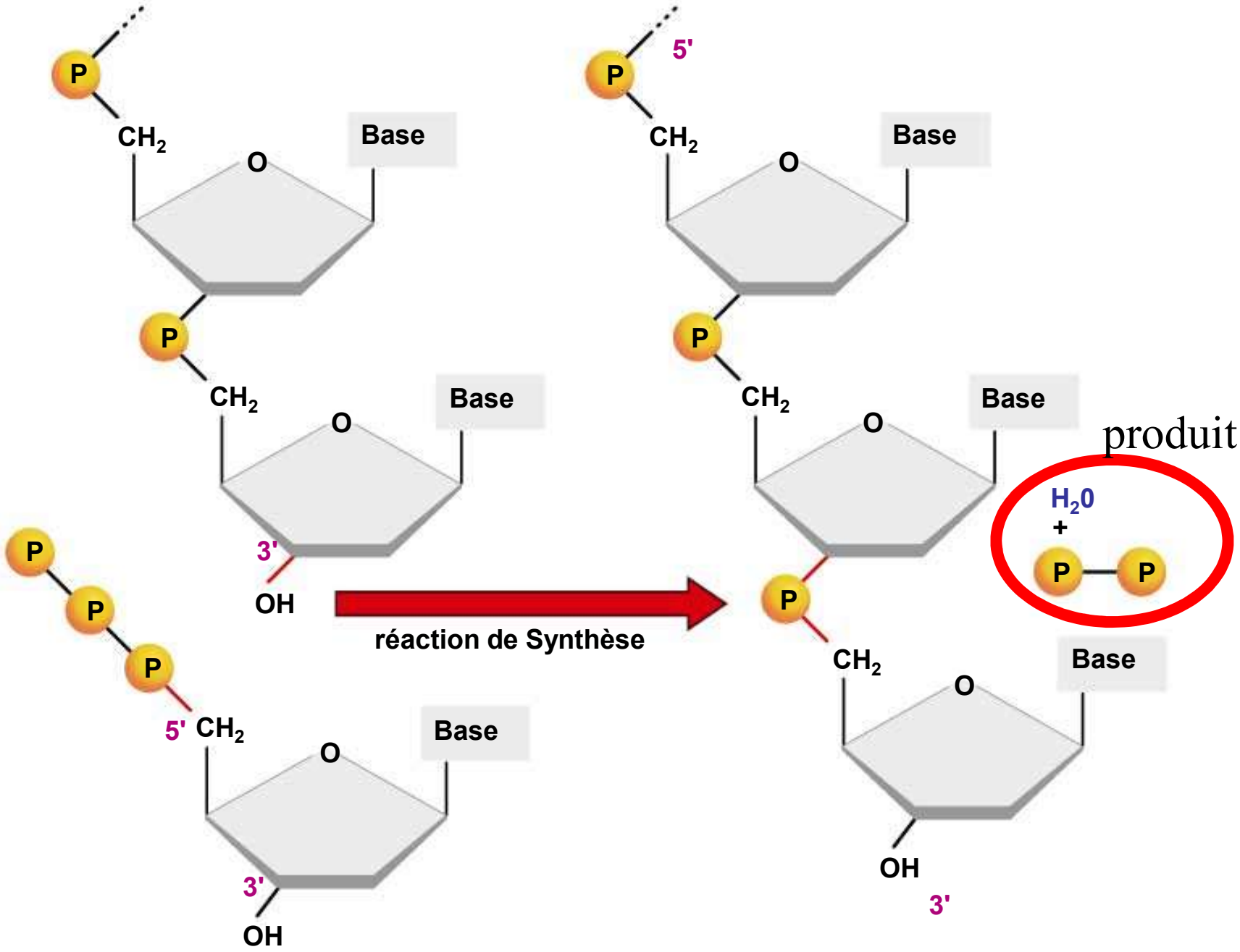


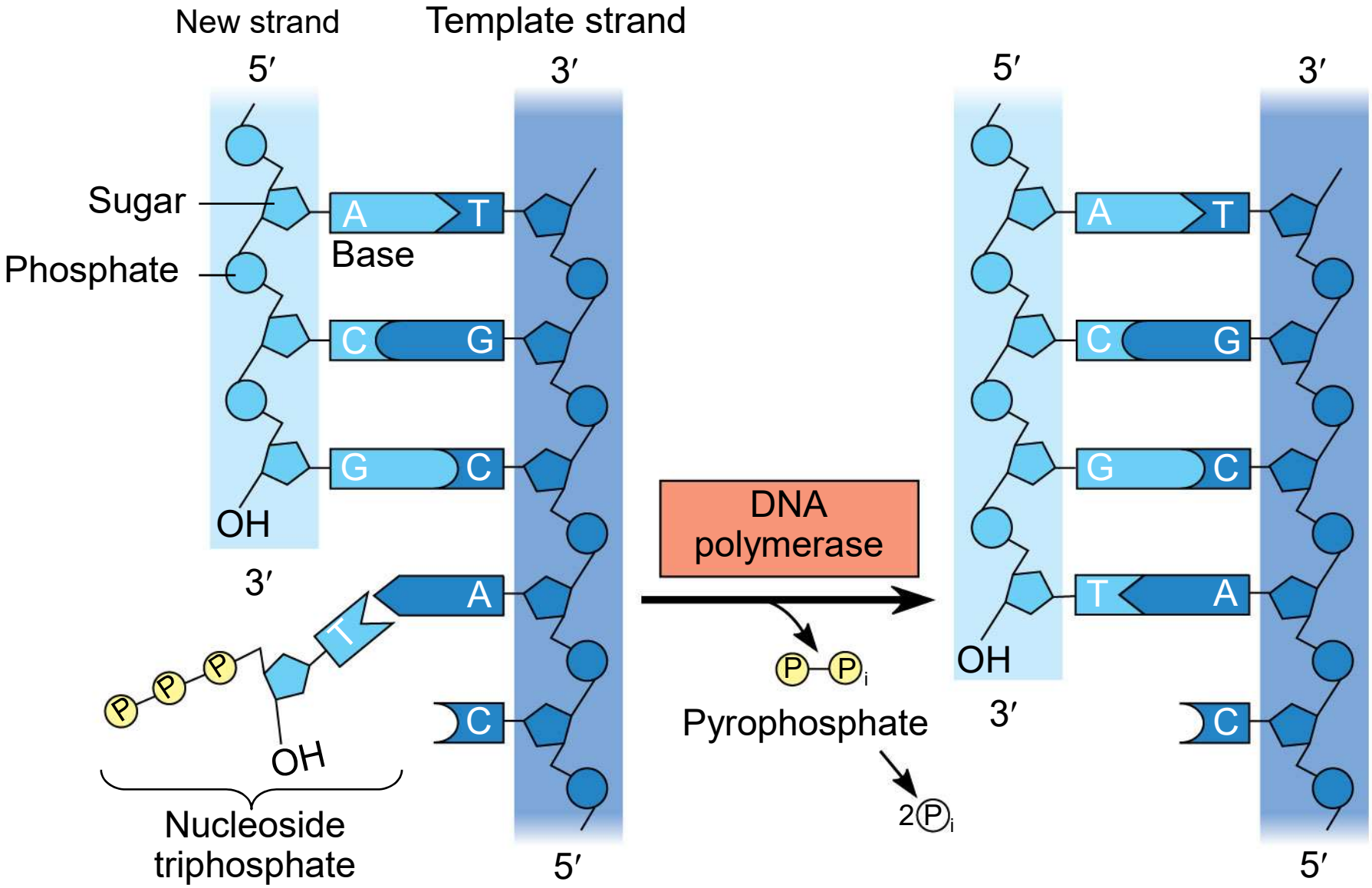
DNA Replication



- **Direction of the replication :**
- In all living organisms, DNA replication always occurs in the 5'→3' direction :
  - The daughter strand is elongated by the addition of new nucleotides at its 3' end;

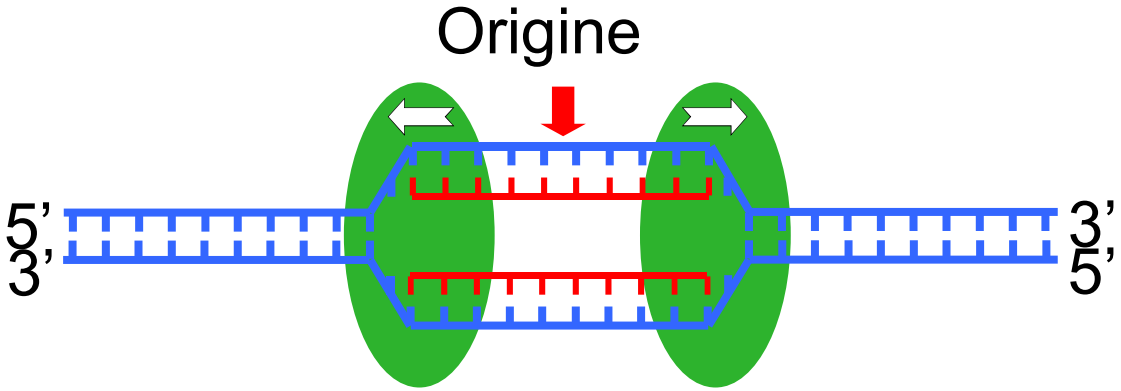
DNA Synthesis

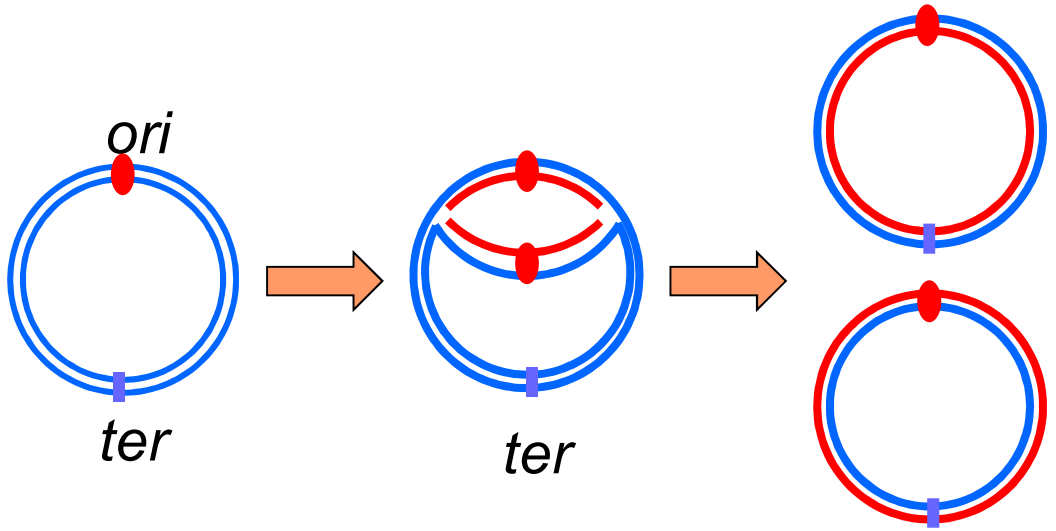




3. 1. DNA Replication in prokaryotes

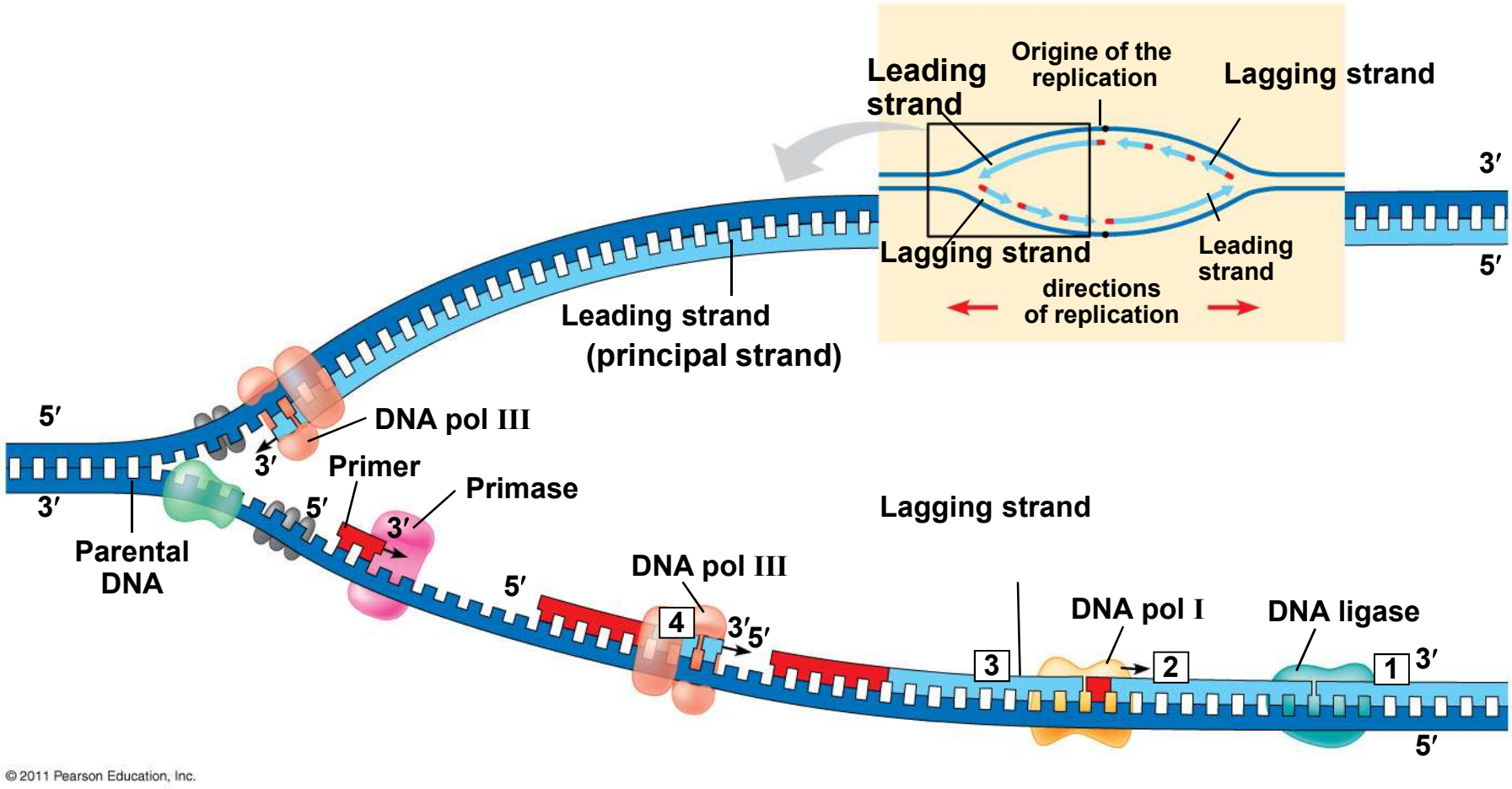
Replication of Prokaryote Chromosome (Bacteria)



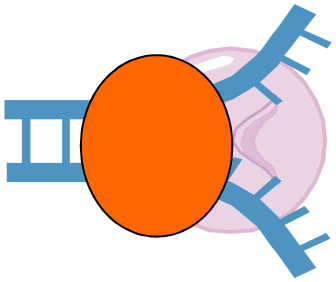


Bidirectional Replication

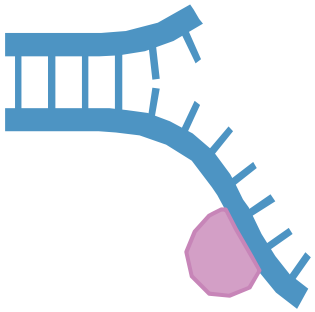
Two Replication Forks



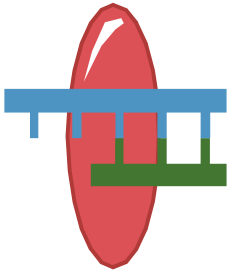
Enzymes in DNA replication



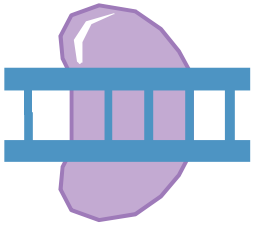
DnaA Helicase (Dna B) unwinds parental double helix



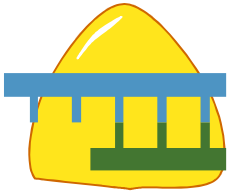
Single Strand Binding (SSB) proteins stabilise separate strands



Primase adds short primer to template strand



DNA polymerase binds nucleotides to form new strands

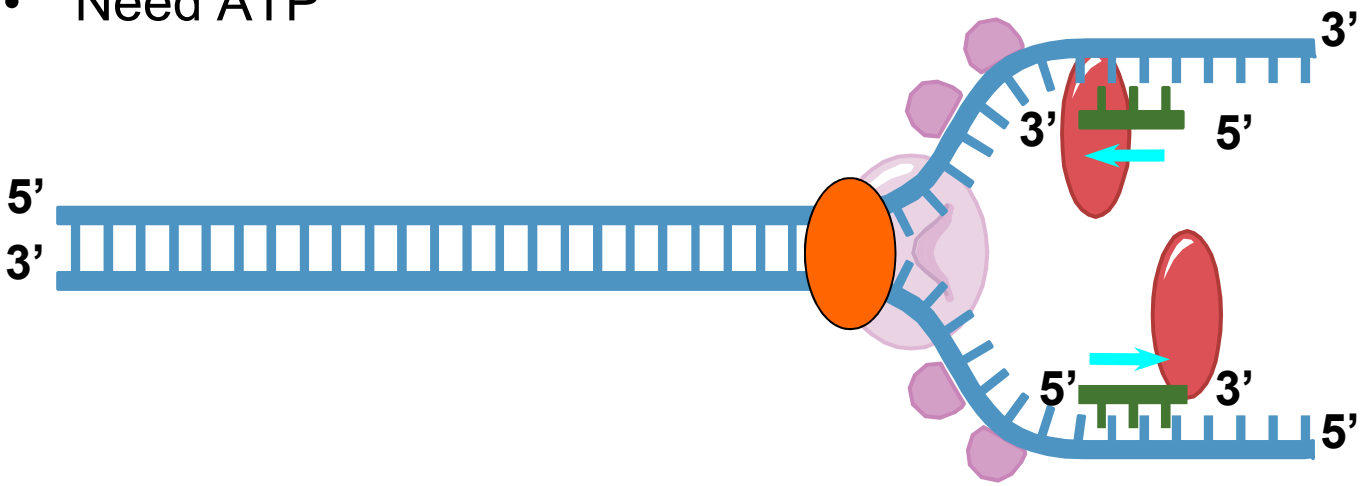


DNA polymerase I (Exonuclease) removes RNA primer and inserts the correct bases

DNA Ligase joins Okazaki fragments and seals other nicks in sugar-phosphate backbone

DNA Replication Process

- **1. Initiation**: Part of the DNA double helix is unwound to expose the nitrogenous bases :
  - The enzyme Dna A opens the double helix at the C origin (origin of replication)
  - The helicase enzyme (Dna B) cuts and unwinds short segments of DNA before the replication fork (they cut the hydrogen bonds between the bases).
    - Need ATP

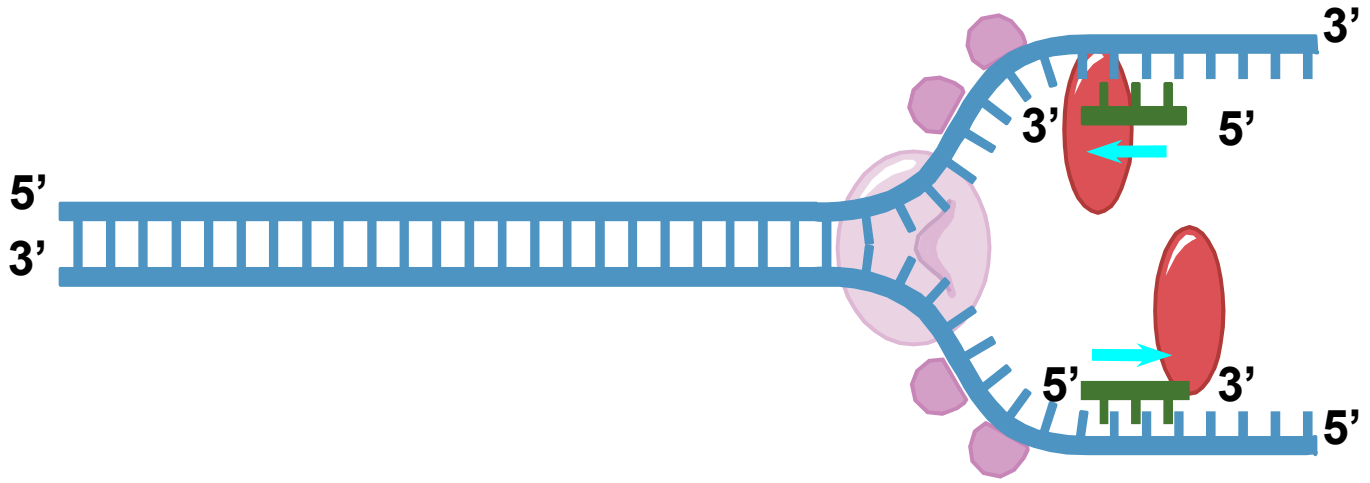


Assistants

- Single-stranded DNA-binding proteins (SSB)
  - Bind to single-stranded DNA to stabilize the structure
  - Called RPA (replication protein A) in eukaryotes
- Topoisomerases
  - Topoisomerase nicks DNA to relieve tension from unwinding
  - Two types
    - Type I - Break only one strand then ligate it
    - Type II – Break the two strands then ligate them

DNA Replication

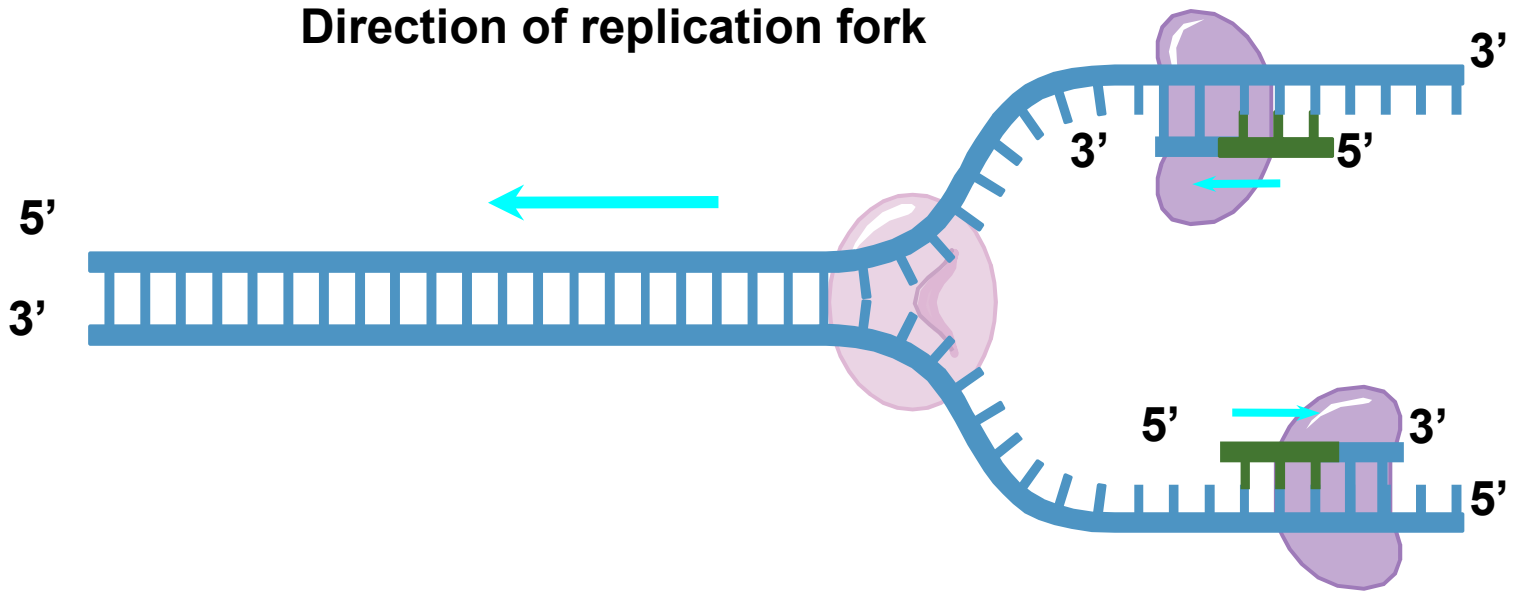
- 2. **Elongation**: two new strands of DNA are copied using the parental DNA as a template.
  - RNA Primase enzyme: Synthesizes an **RNA primer** to begin the elongation process



DNA Replication

2. Continued Elongation:

– **DNA Polymerase III**: Begin to add one nucleotide at a time to create a new complementary strand (in the 5’ to 3’ direction)



- DNA polymerase reads back the added bases and replaces incorrect nucleotides.

DNA Replication

- Leading strand:
  - Replicated without interruption (5’ to 3’)
  - It follows the direction of the replication fork
  - Faster

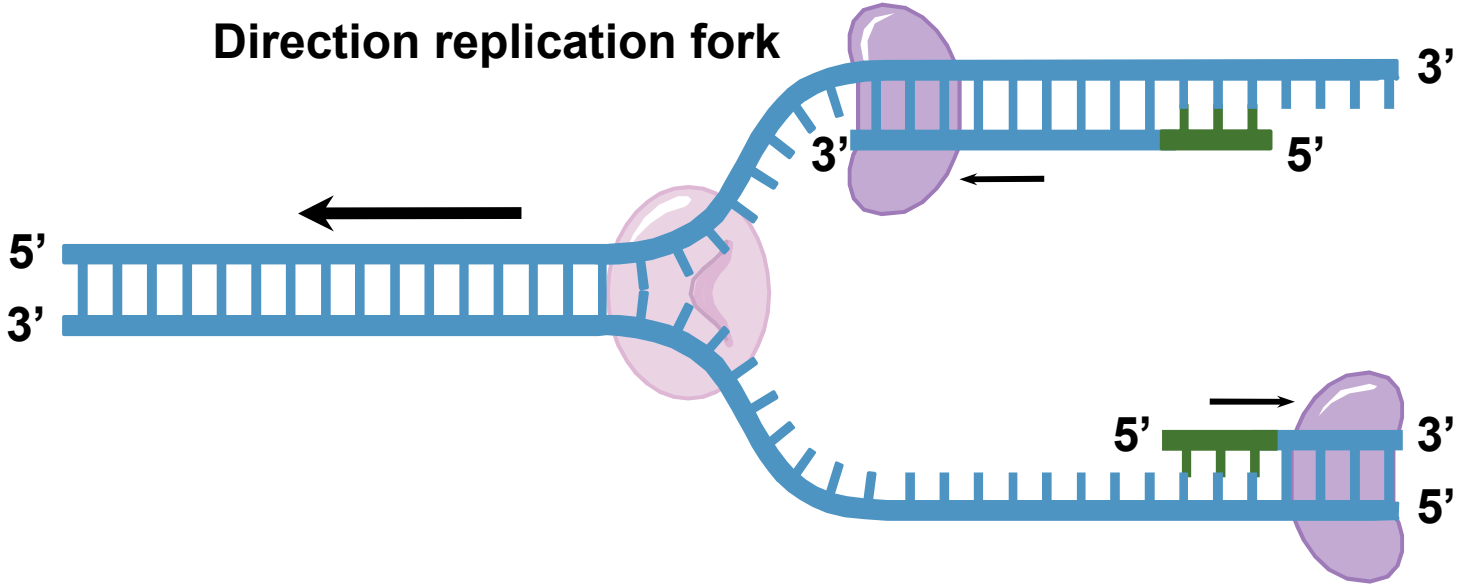
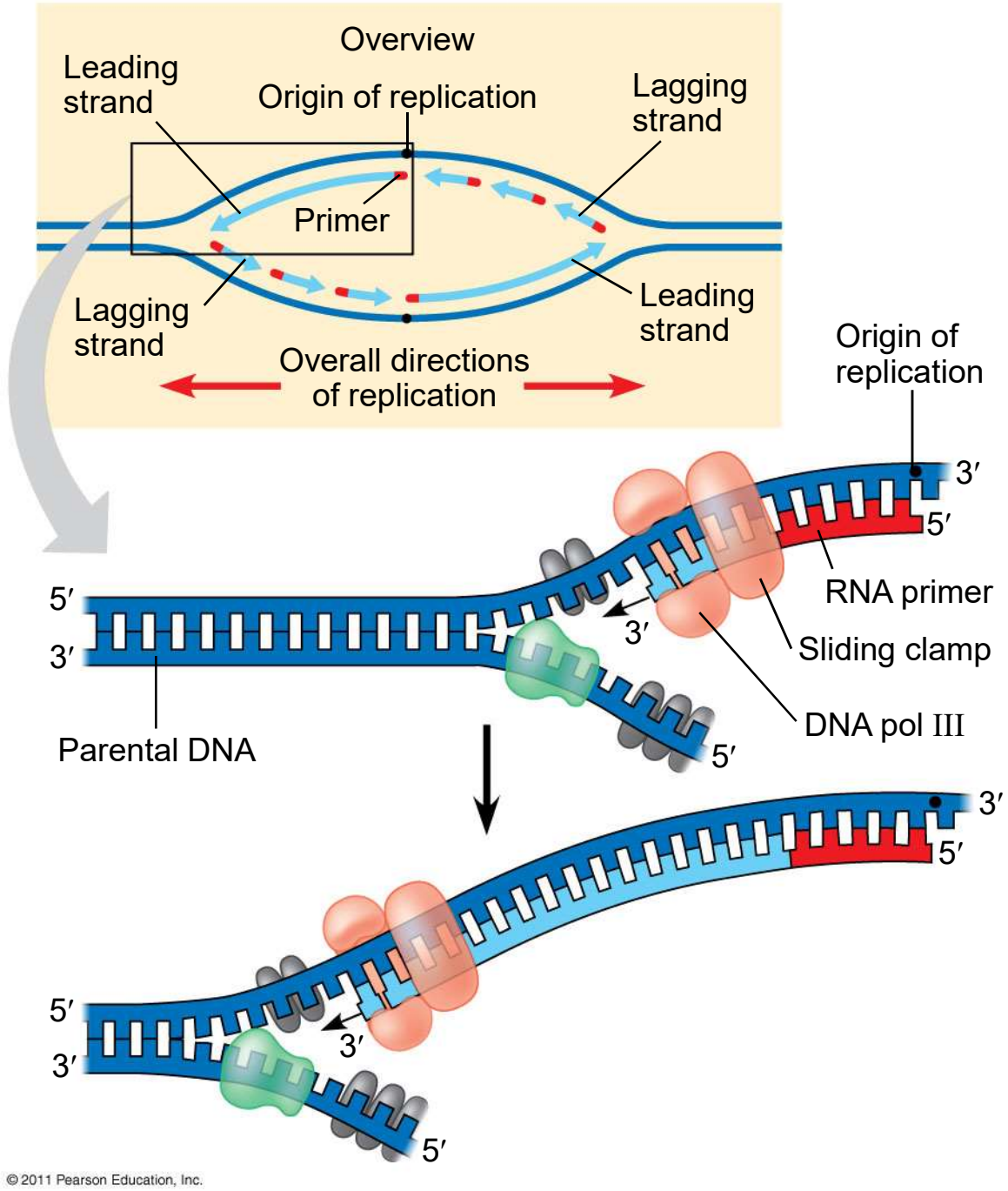
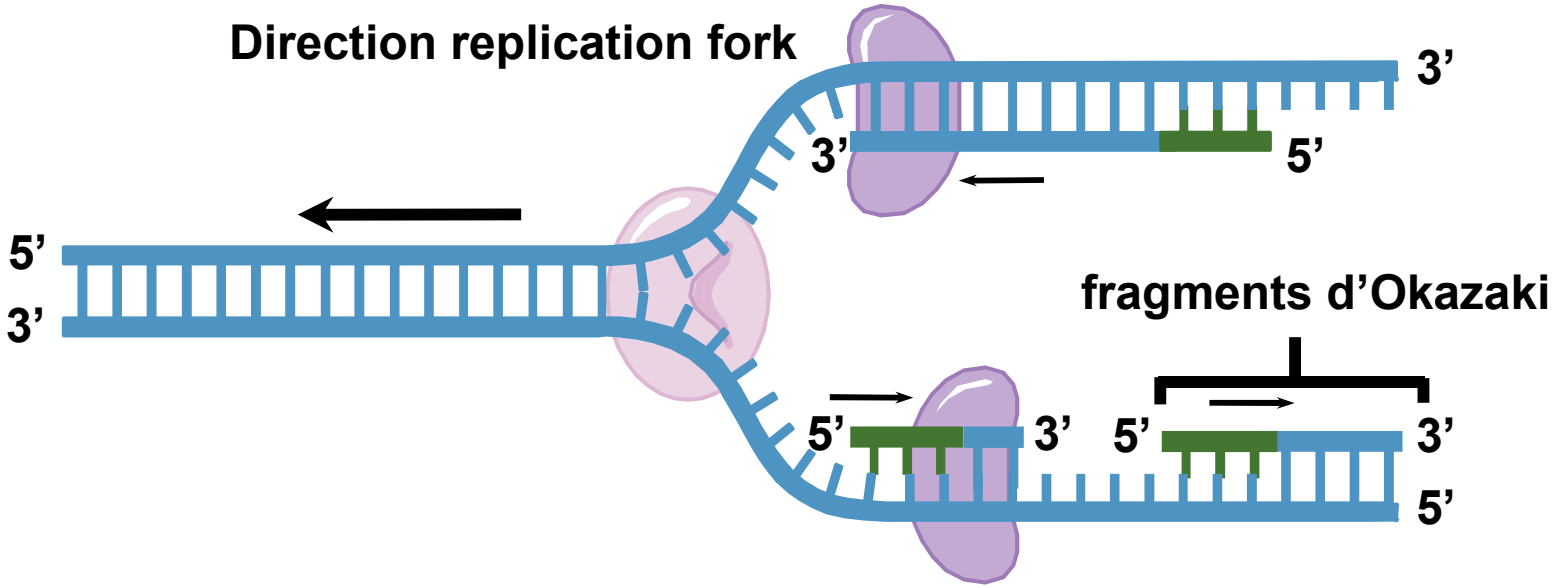


Figure 16.1



DNA Replication

- **Lagging strand:**
  - Replicated in the direction (3’ to 5’)
  - It does not follow the direction of the replication fork
  - Slower
  - Makes fragments called **Okazaki Fragments**



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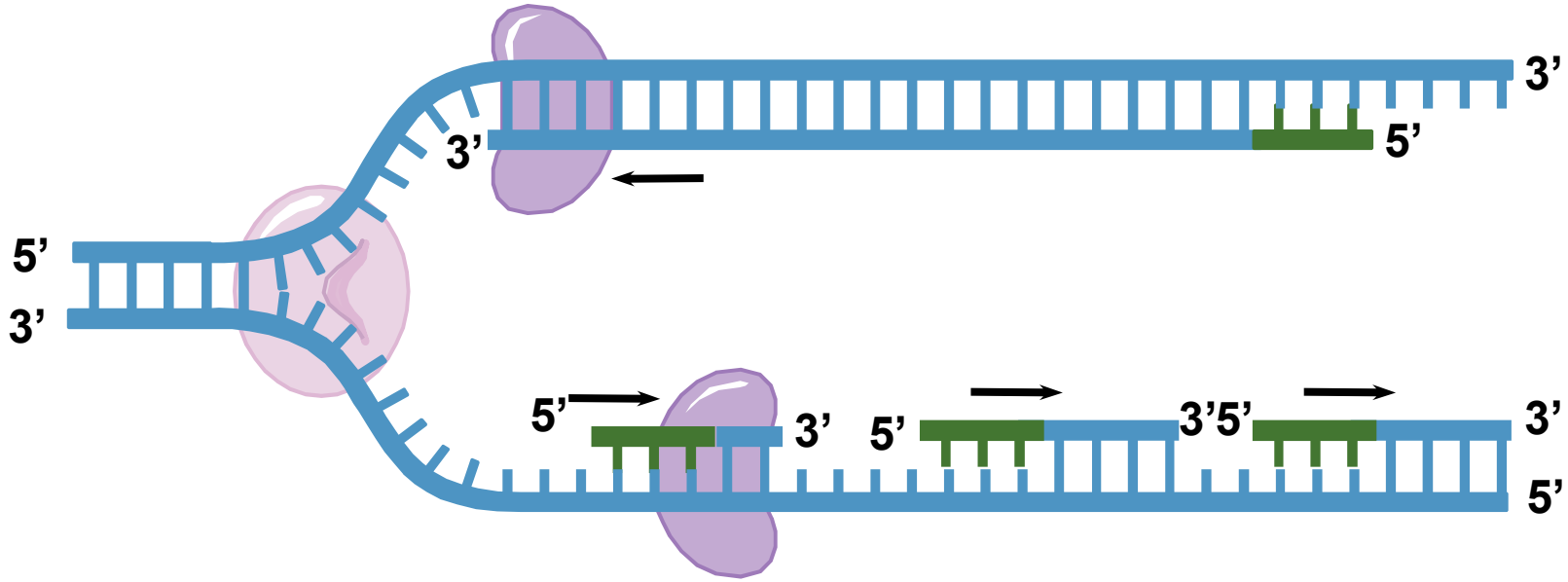
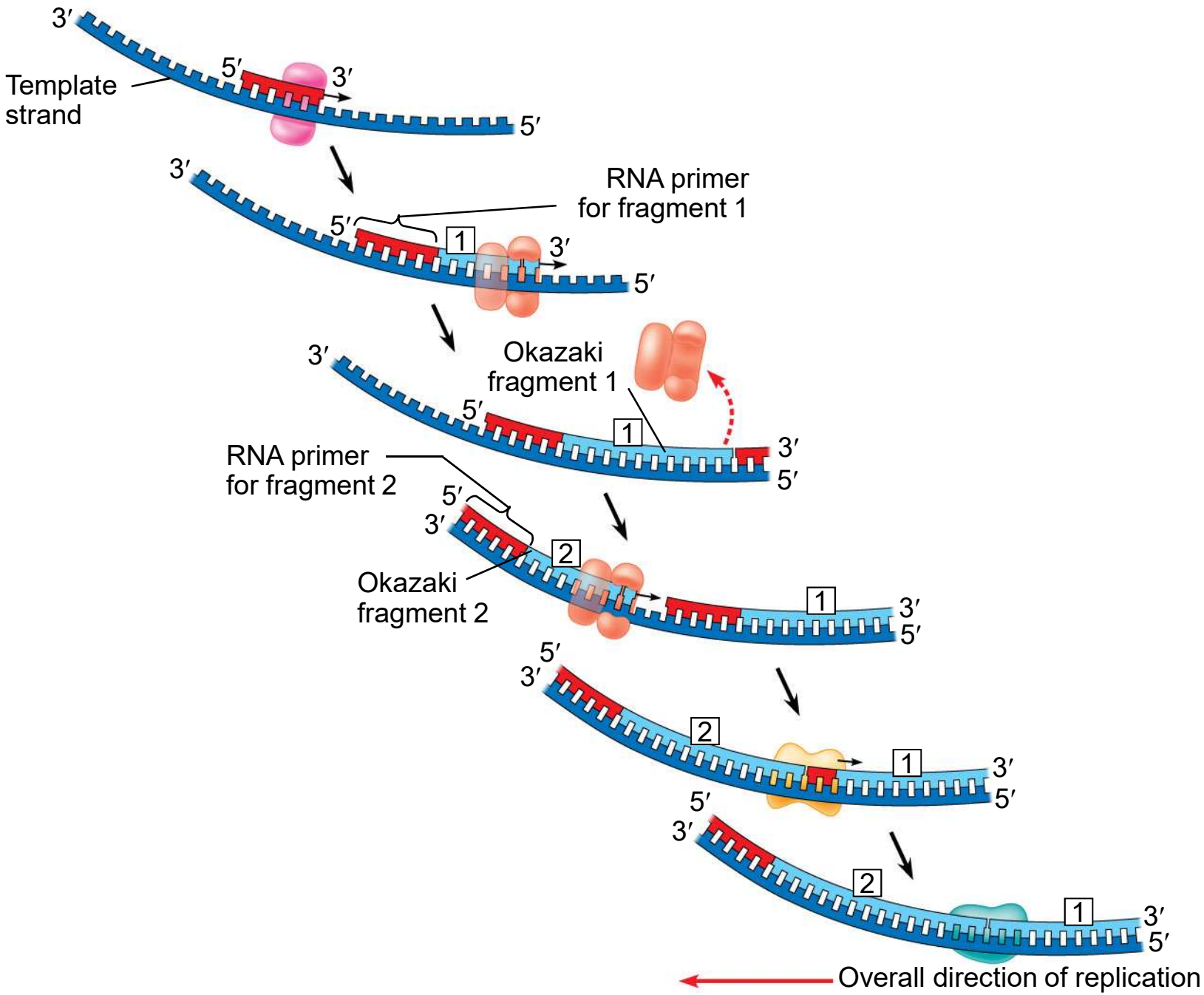
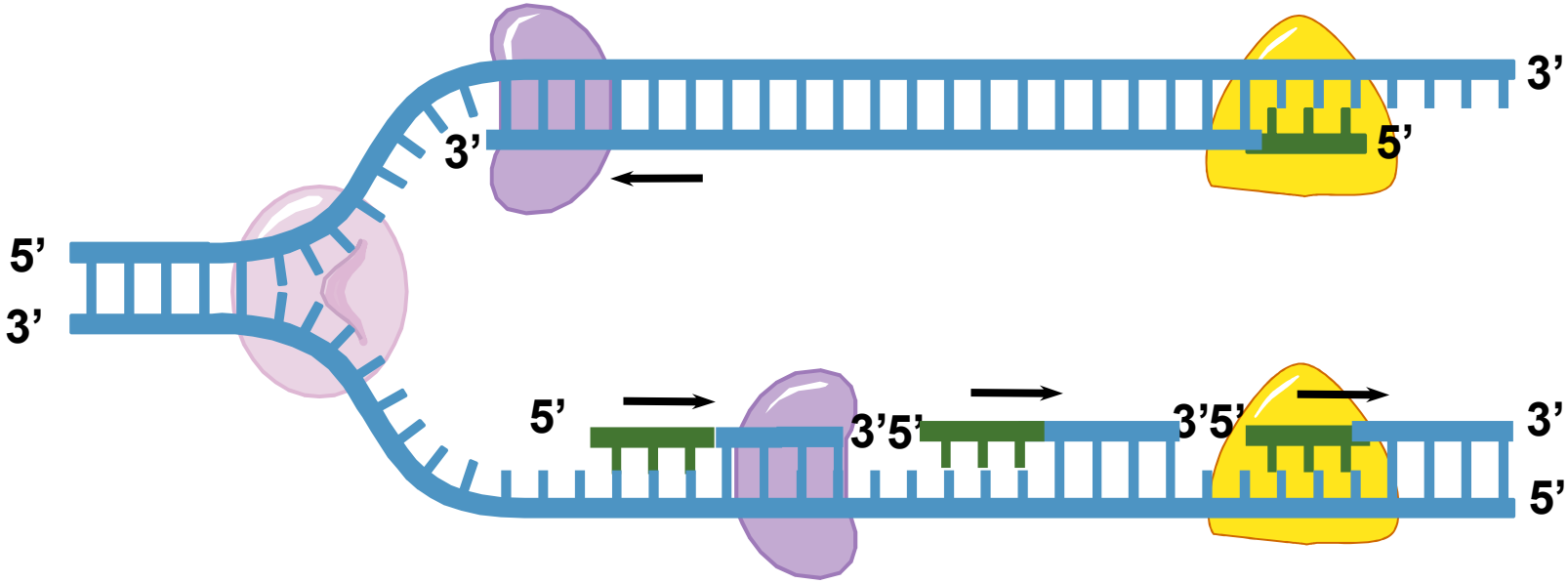




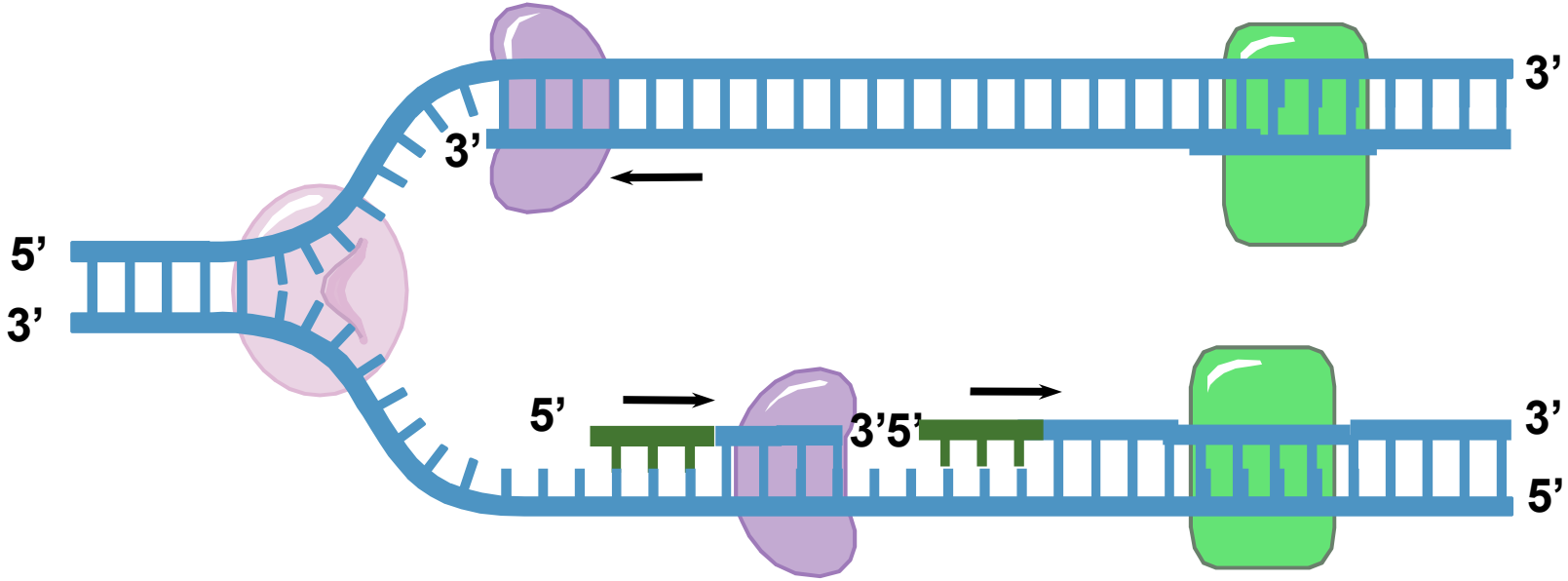
DNA Replication

DNA polymerase I (exonuclease activity) removes the RNA primer on both strands and inserts the correct DNA bases

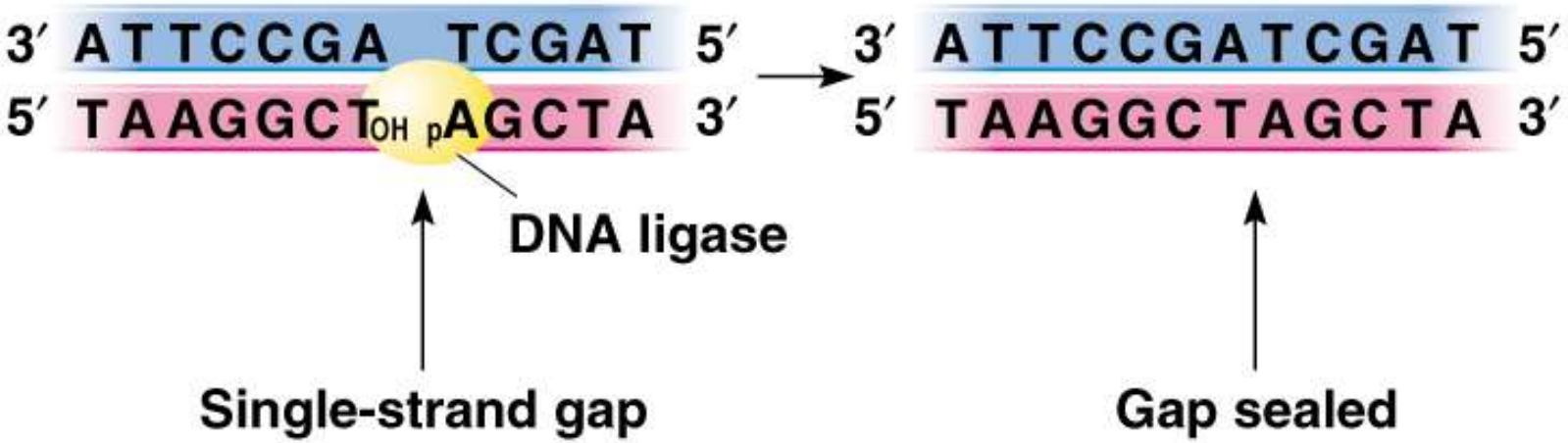


DNA Replication

– **DNA Ligase**: Ligates the Okazaki fragments together (catalyzes the formation of phosphodiester bonds between nucleotides)

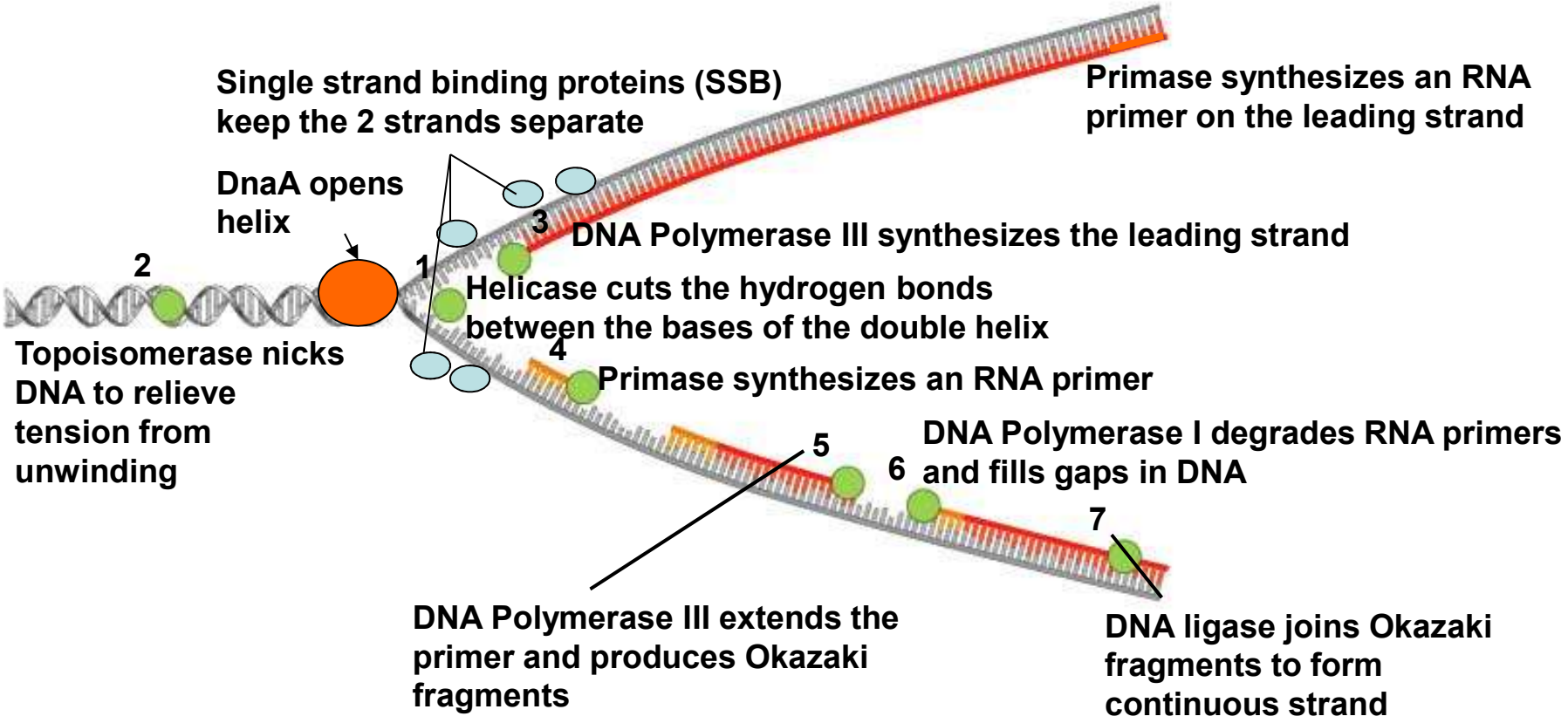


DNA Replication - DNA ligase



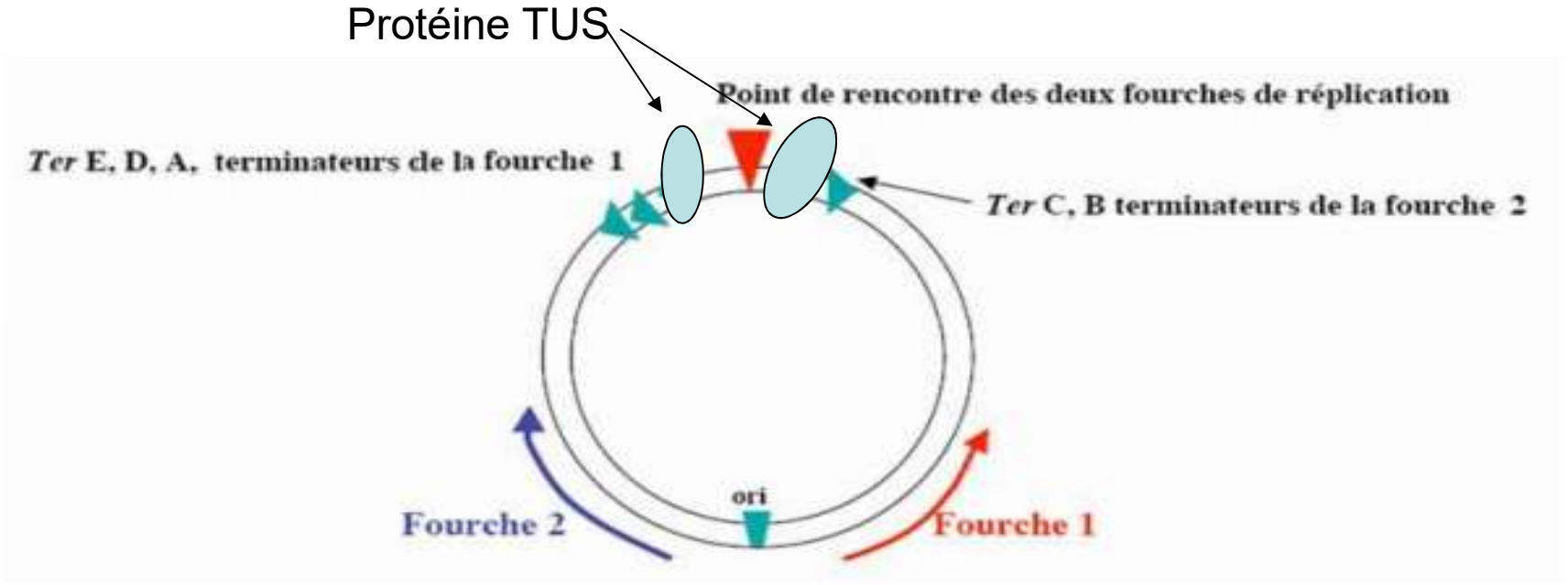
DNA Replication

Enzymes involved in DNA replication (Replisome)



Terminaison de la réplication

- Replication stops when two replication forks meet or when a fork encounters a replication termination signal (ter). There are many "ter": terA terD terB terC which slow down the replication forks.
- A protein called TUS (terminator utilisation substance) which binds to the terminal sequence of DNA ‘Ter’.
- Bacterial DNA being circular, two replisomes copy the double strands of DNA in opposite directions until they encounter TUS : the replisome can in fact dislodge TUS as it advances if it approaches from one side but is blocked when he gets to the other.



Intervention of a topoisomerase IV to catalyze the separation of the 2 chromosomes

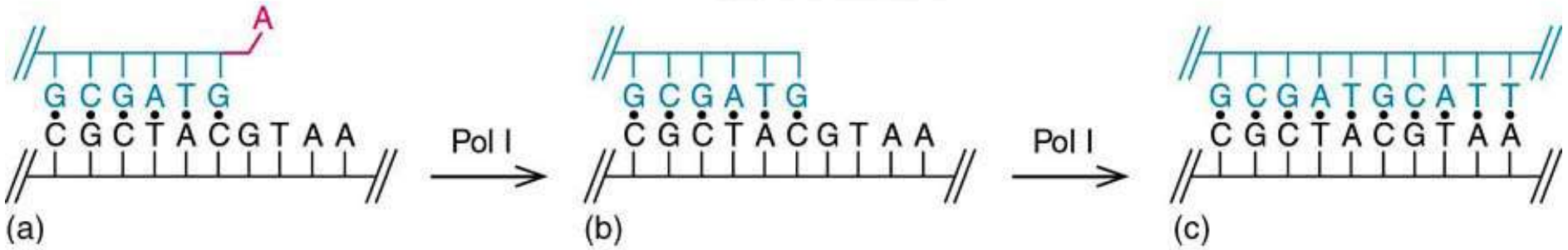
Proteins Involved in DNA Replication in *E. coli*

Protein Name	Function
DNA Gyrase	Unwinding DNA
SSB	Single-stranded DNA binding
DnaA	Initiation factor
HU	Histone-like (DNA binding and bending)
PriA	Primosome assembly
PriB	Primosome assembly
PriC	Primosome assembly
DnaB	DNA unwinding (helicase)
DnaC	DnaB chaperone
DnaT	Assists DnaC in delivery of DnaB
Primase	Synthesis of an RNA primer
DNAP III holoenzyme	Elongation (DNA synthesis)
DNAP I	Excises RNA primer, fills in with DNA
Ligase	Covalently links Okazaki fragments
Tus	Termination

Proofreading and Repairing DNA

- The error rate of DNA polymerase is around 10^6
- Error repair mechanisms :
  - The activity of repairing errors or "Proofreading":
    - DNA polymerase has a 3' →5' exonuclease activity which removes the mismatched nucleotide.
  - Mismatch Repair :
    - In **mismatch repair** of DNA, repair enzymes correct errors in base pairing.
    - Fixing errors after replication is complete

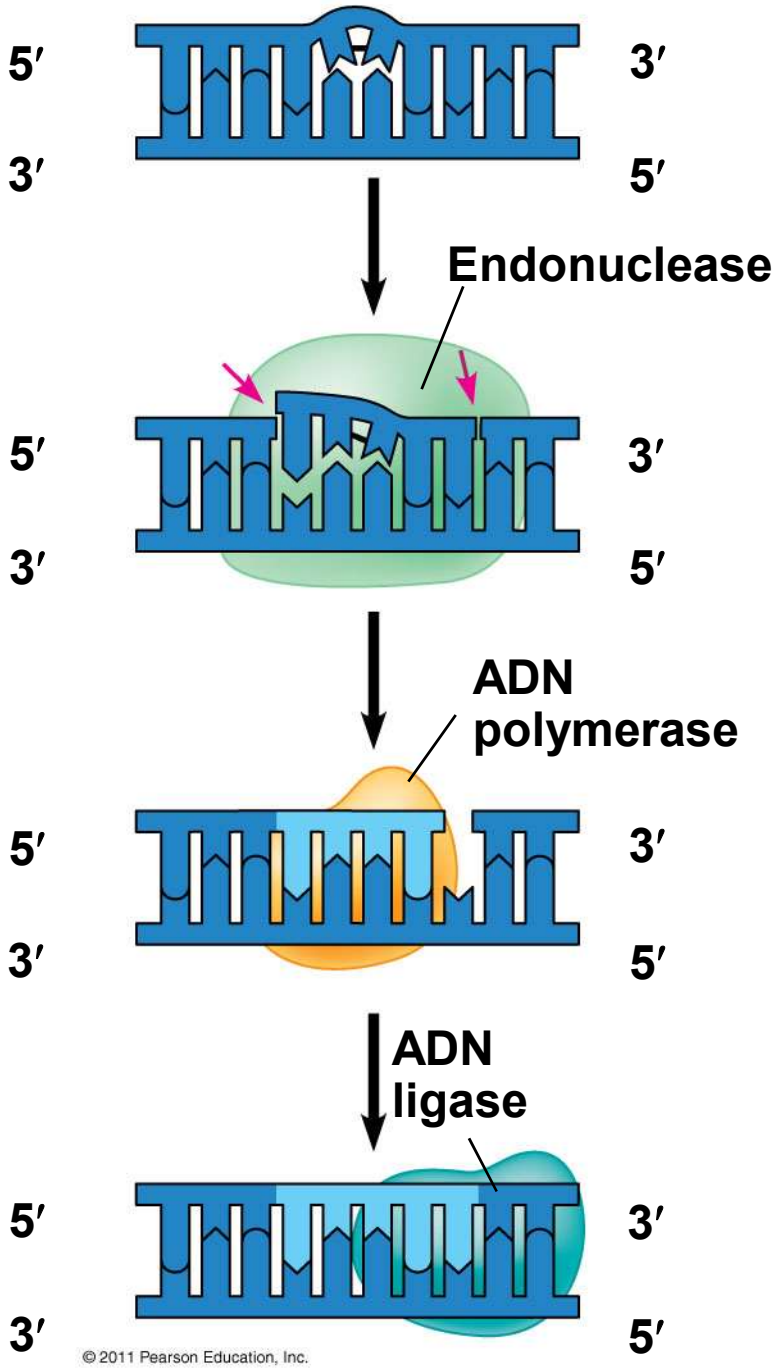
Proofreading and Repairing DNA



Inserting the wrong nucleotide causes DNA polymerase to stop, and then the 3'-5' exonuclease activity removes the mismatched nucleotide. The polymerase then continues by adding the correct nucleotides.

Proofreading and Repairing DNA

•In **nucleotide excision repair**, a **nuclease** cuts out and replaces damaged stretches of DNA then a DNA polymerase fills the gap, and finally a DNA ligase ligates the DNA segments.



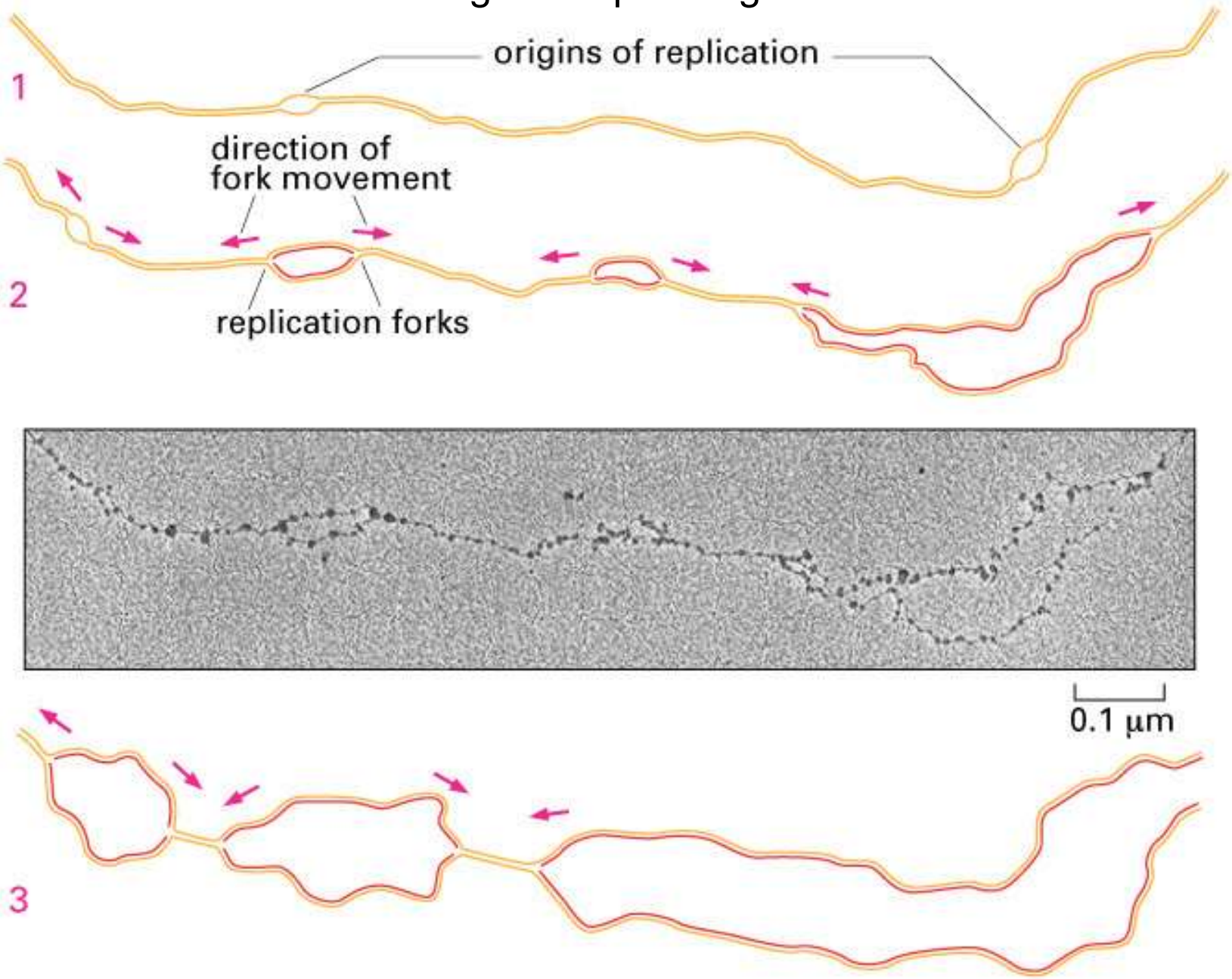
Evolutionary Significance of Altered DNA Nucleotides

- Error rate after proofreading repair is low but not zero
- Sequence changes may become permanent and can be passed on to the next generation
- These changes (mutations) are the source of the genetic variation upon which natural selection operates

Replication in Eukaryotes

- In general, replication in eukaryotes is nearly identical to replication in prokaryotes.

Different regions of a chromosome are replicated at different times
Arrows indicate regions replicating at different times



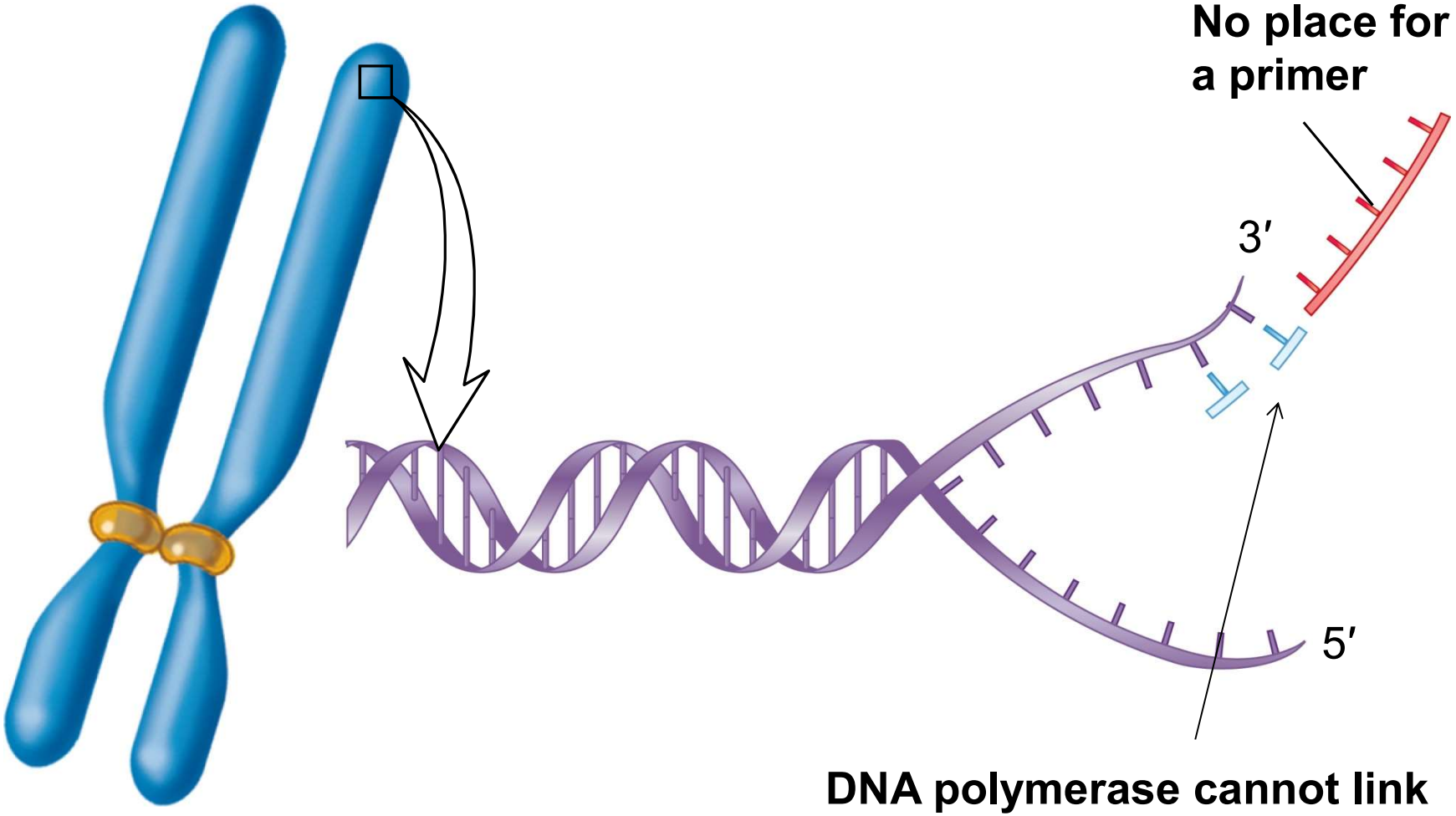
DNA Replication in Eukaryote

- DNA polymerases in Eukaryotes
  - Polymerase α– acts as a Primase in initiation
  - ADN polymerase δ- replies the lagging strand
  - ADN polymerase ε- replicates the leading strand

Differences between eucaryotes vs procaryotes

	Eukaryotes	**Prokaryotes**
Origin	multiple	single
Single-stranded DNA stabilizing protein	RP-A or RF-A	SSB
RNA Primers	oui synthesized by Polymerase alpha followed by alpha or beta or epsilon degraded by RNAse H gap synthesized by alpha or béta	oui synthesized by primase followed by DNA pol III degraded by DNA pol I gap synthesized by DNA pol I
DNA polymerases	**alpha**: polymerase activity primase activity no exonuclease activity 3'->5' **delta**: exonuclease activity 3'->5‘ no primase activity **epsilon**: exonuclease activity 3'->5' pas de primase **béta**: repairs short DNA fragments **gamma**: mitochondrial DNA replication exonuclease activity 3'->5'	**ADN pol III**: polymerase activity exonuclease activity 3'->5' **ADN pol I**: exonuclease activity 5'->3' polymerase activity 5'->3' exonuclease activity 3'->5'
Okazaki Fragments	200 nucleotides	2000 nucleotides

Linear chromosomes (eukaryotic) cannot easily replicate the ends of chromosomes



Brooker, fig 13.21

DNA polymerase cannot link these two nucleotides together without a primer.

Telomerase

Two components of the human telomerase:

- **the human RNA subunit(hTR)**
  5’-CUAACCCUAAC-3’

- **The human telomerase reverse transcriptase(hTERT)**

Telomerase

- Function:
  - Specialized **reverse transcriptase**
  - Prevents “shortening ends problem” problem by adding telomeres to the end
  - Copies only a small segment of **RNA** that it carries by itself
  - Requires a 3’ end as a primer
  - Synthesis proceeds in 5’ – 3’ direction
  - Synthesizes one repeat then repositions itself
  - When active provides **cell immortality**

Telomerase is composed of both RNA and protein

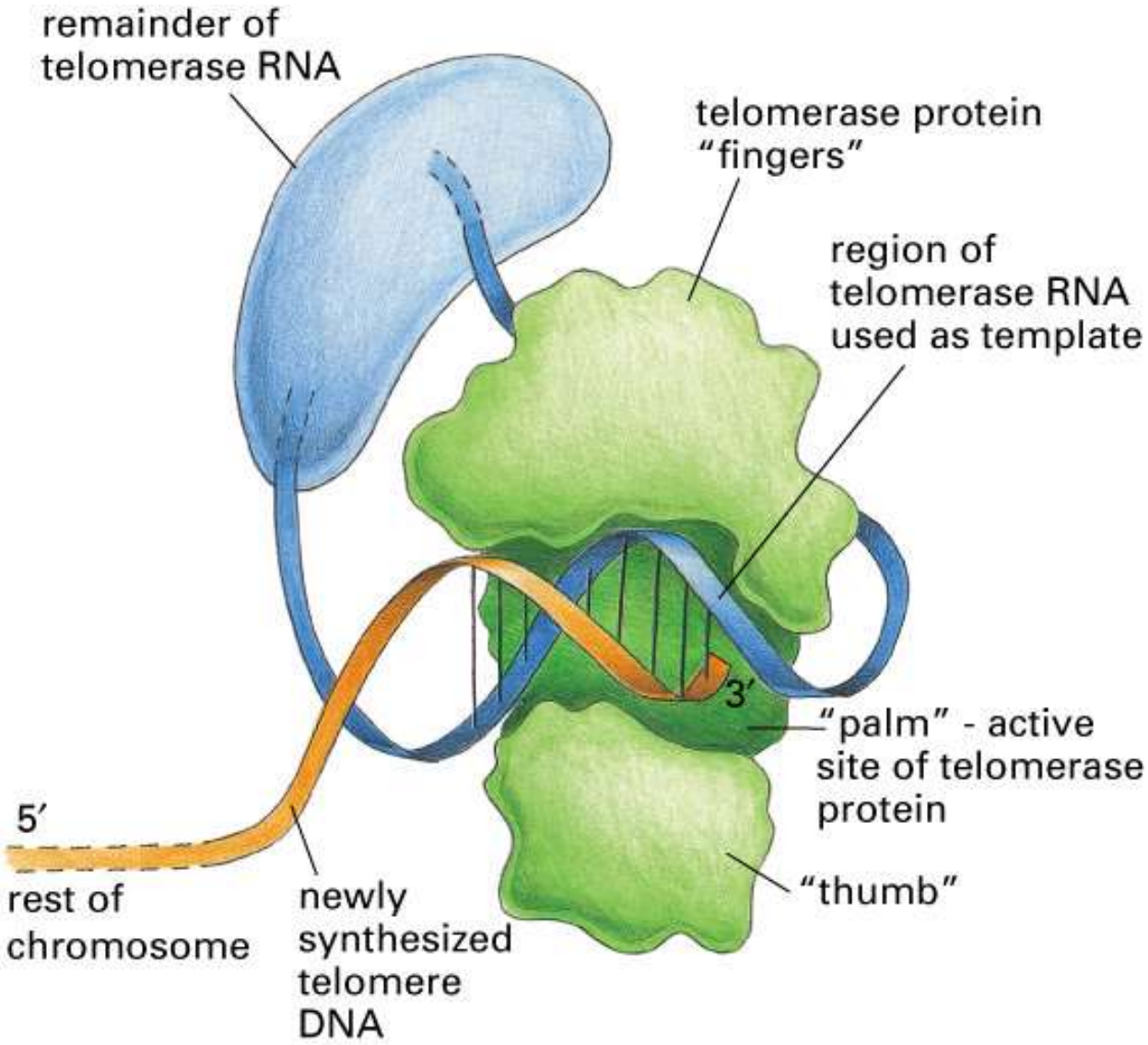
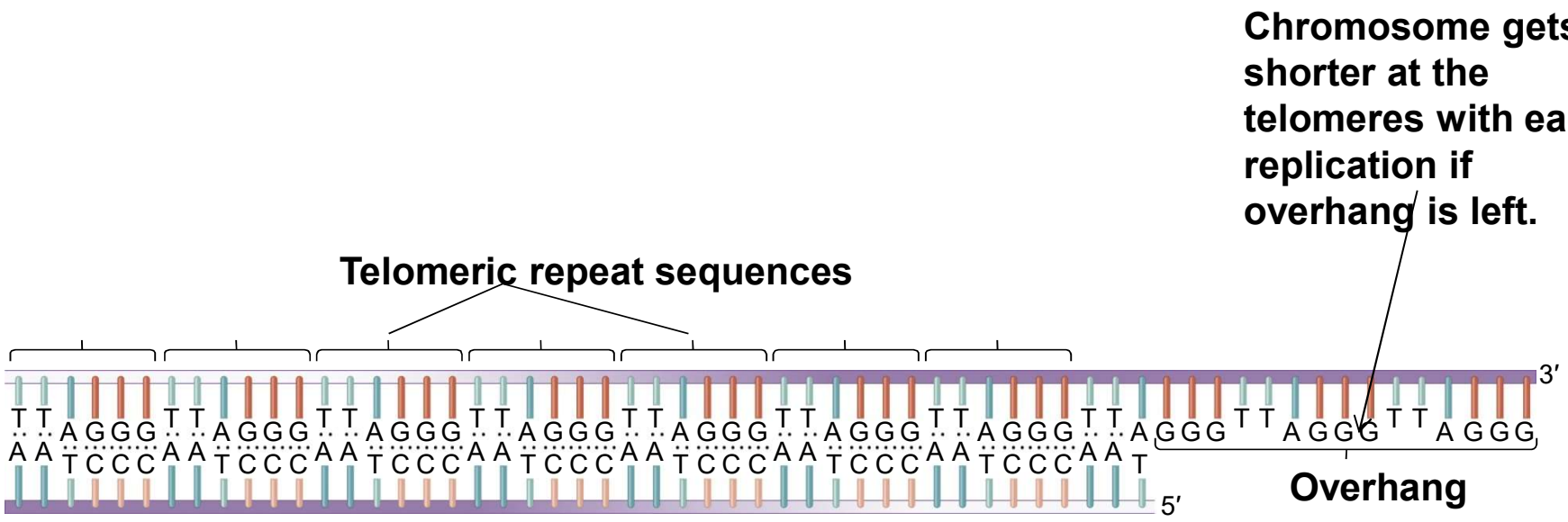


Figure 5–42. Molecular Biology of the Cell, 4th Edition.

What are telomeres?

- Telomeres are…
  - Repetitive DNA sequences at the ends of all human chromosomes
  - They contain thousands of repeats of the six-nucleotide sequence, TTAGGG
  - In humans there are 46 chromosomes and thus 92 telomeres (one at each end)

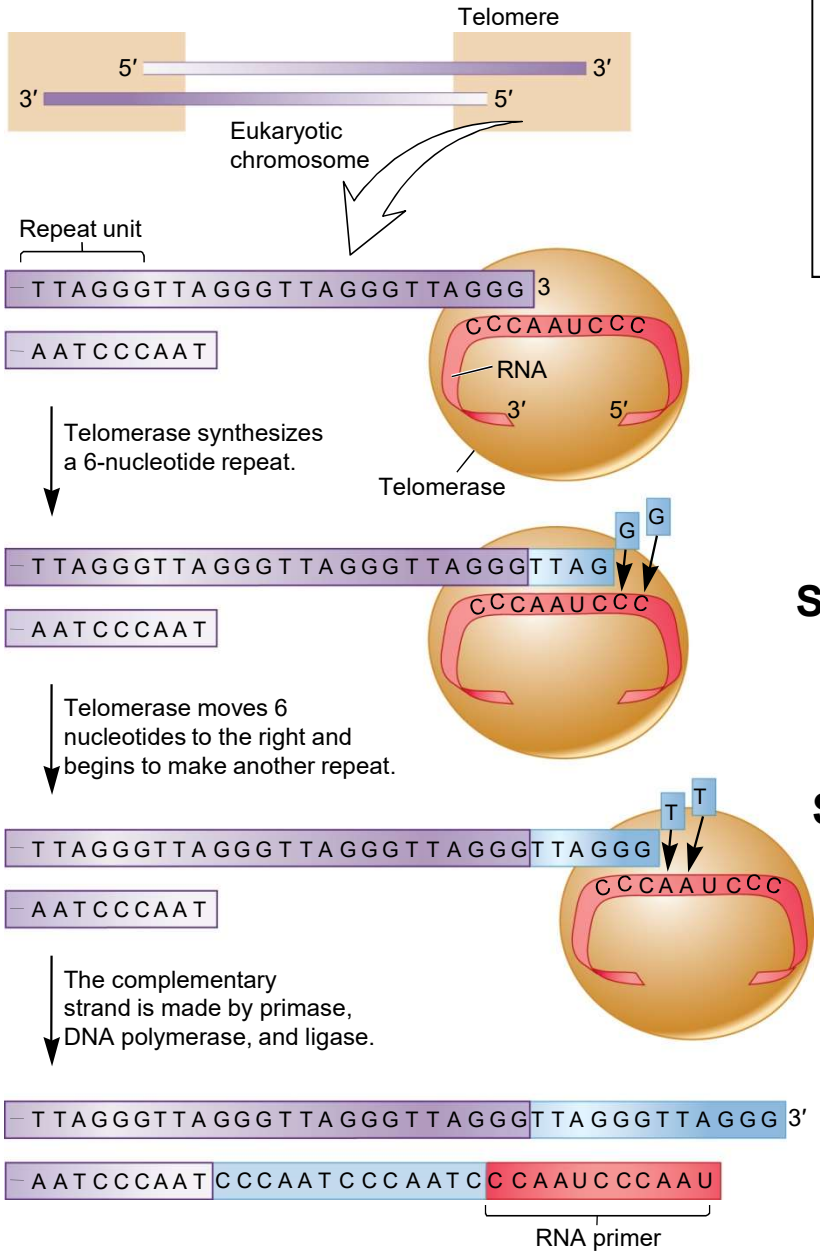
Linear chromosomes (eukaryotic) must fill in gap left by RNA primer



In humans and most complex organisms, telomerase is only used in continuously dividing stem cells (e.g. spermatogonia stem cells) → most cells get shorter telomeres over time (age). What happened to Dolly, the cloned sheep? (she was generated from a skin cell with shorter telomeres, and she aged early)

Brooker, fig 13.20

How telomerase "finishes" the replication of linear chromosomes



The binding-polymerization-translocation cycle occurs many times

This greatly lengthens one of the strands

Step 1 Binding

Step 2 Polymerization

Step 3 Translocation

The end is now lengthened

Brooker, figure 13.22