SAPIENZA
UNIVERSITÀ DI ROMA

MOLECULAR BIOLOGY

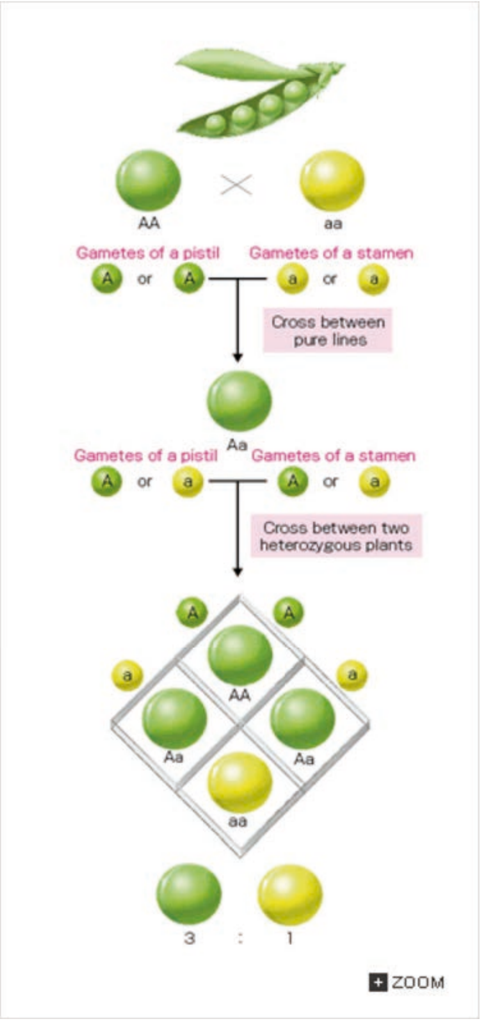
A.A. 2018-19

DNA Structure and Replication

Dr. Stefano Cacchione

Milestones in Genetics and Molecular Biology

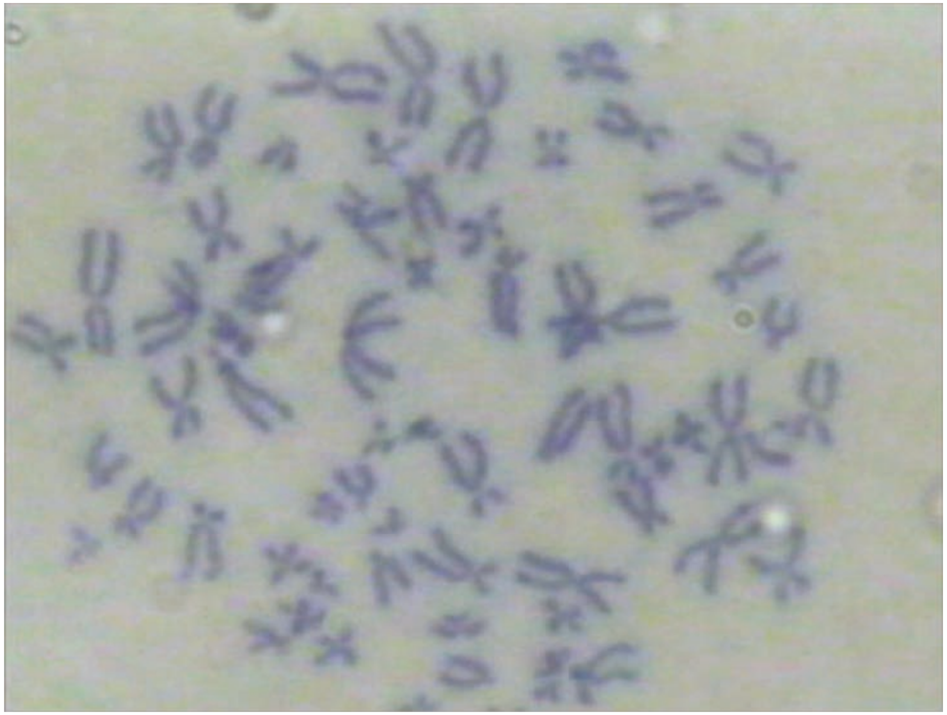
1865 Characters (genes) are particulate factors (Mendel)



Milestones in Genetics and Molecular Biology

1865 Genes are particulate factors (Mendel)

1910 Genes lie on chromosomes (Morgan)



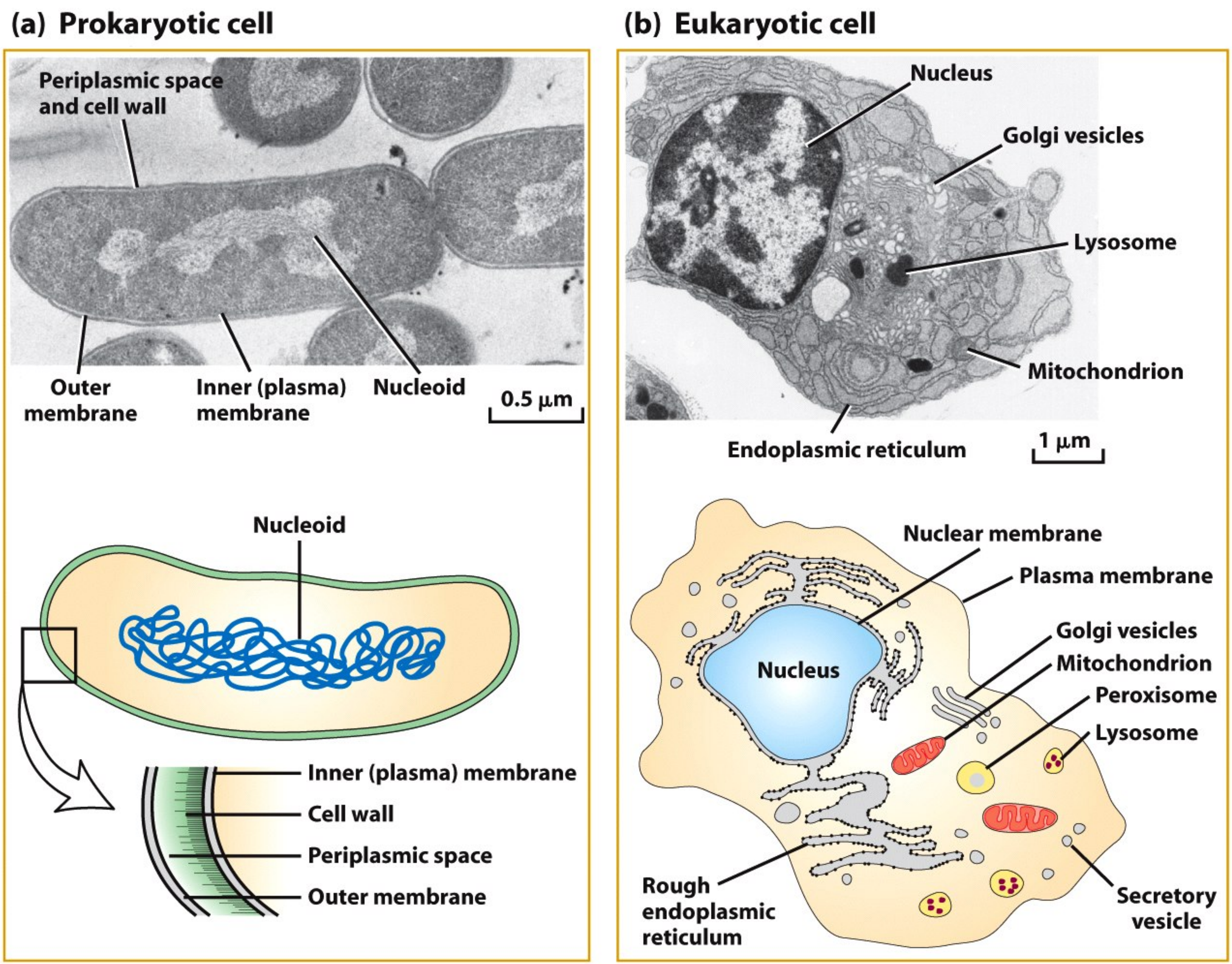


Figure 1-2
Molecular Cell Biology, Sixth Edition
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What genes are made of?

Chromosomes contain two main components:
DNA and **Proteins**

Four requirements for the genetic material

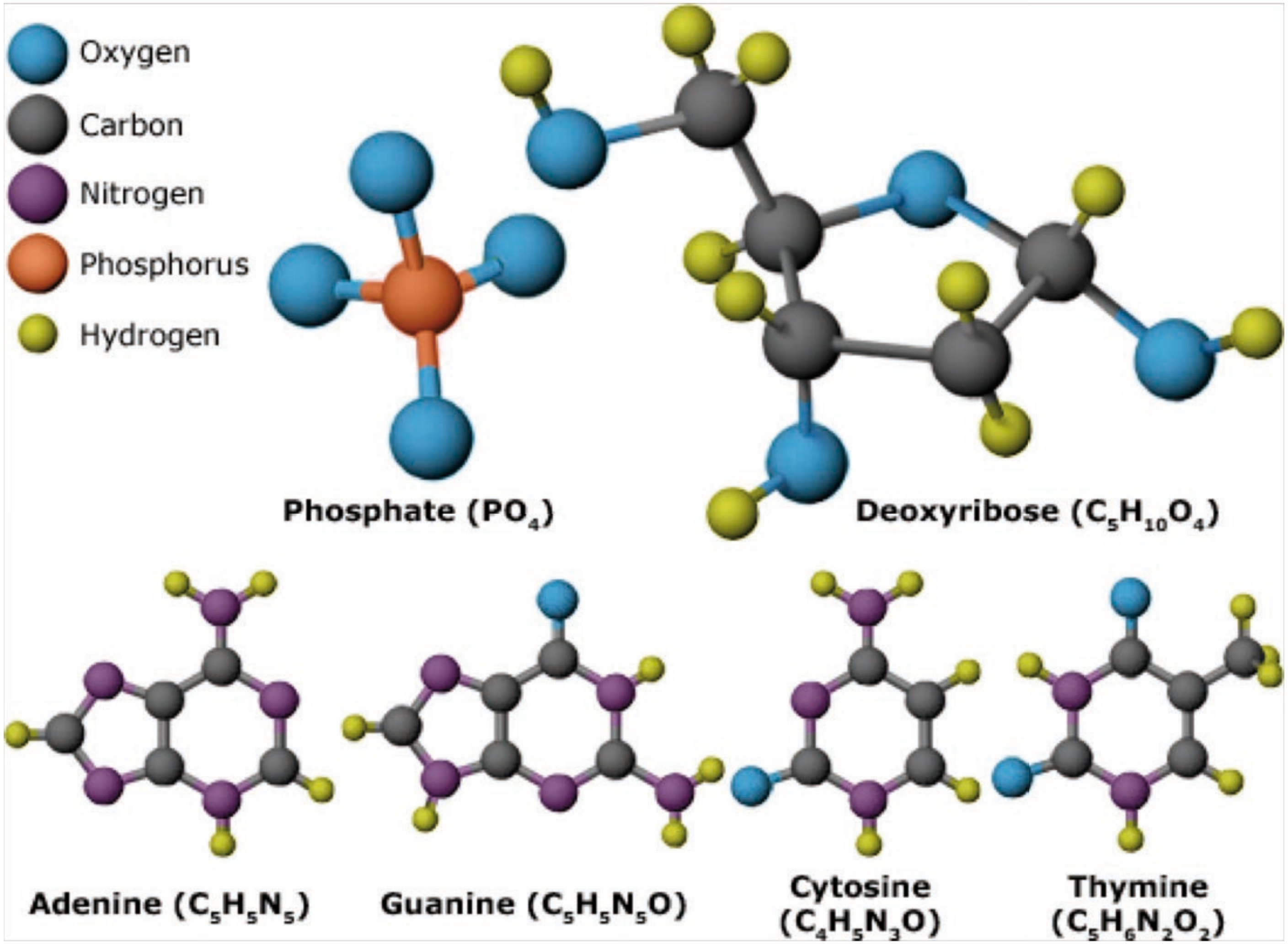
Must carry information

Must replicate

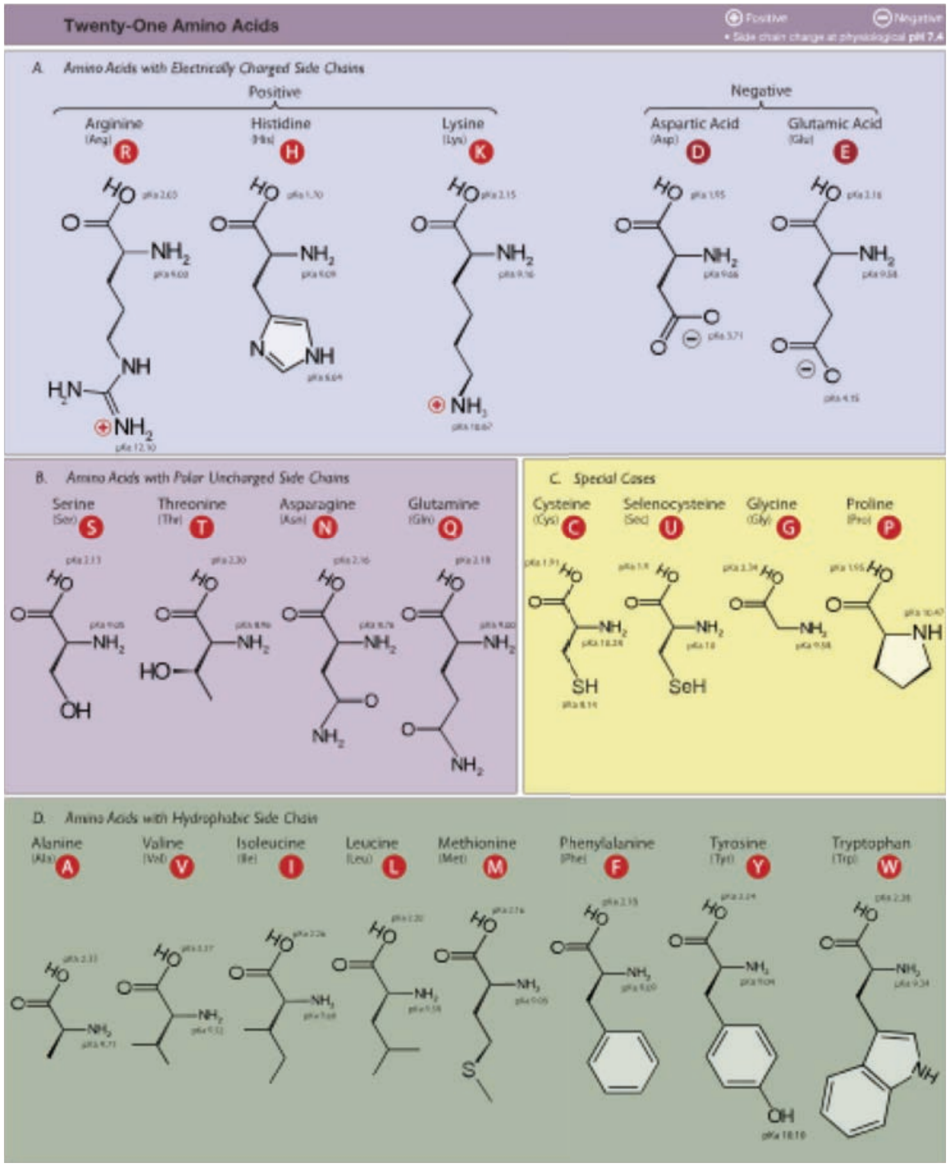
Must allow for information to change

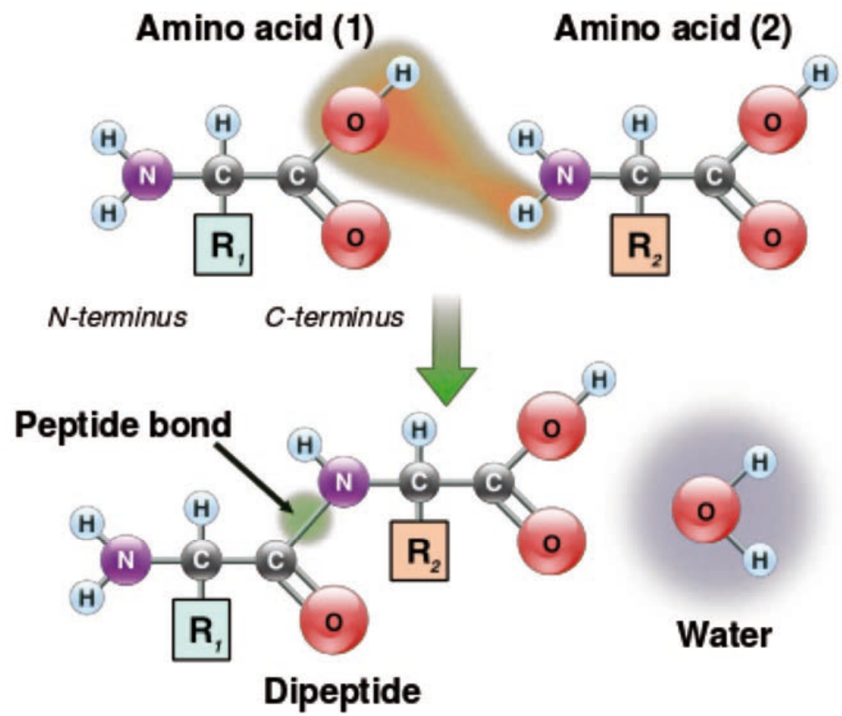
Must regulate the expression of the phenotipe

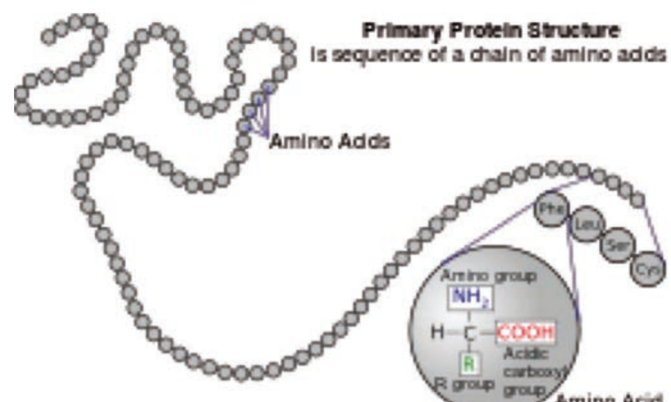
What DNA is made of

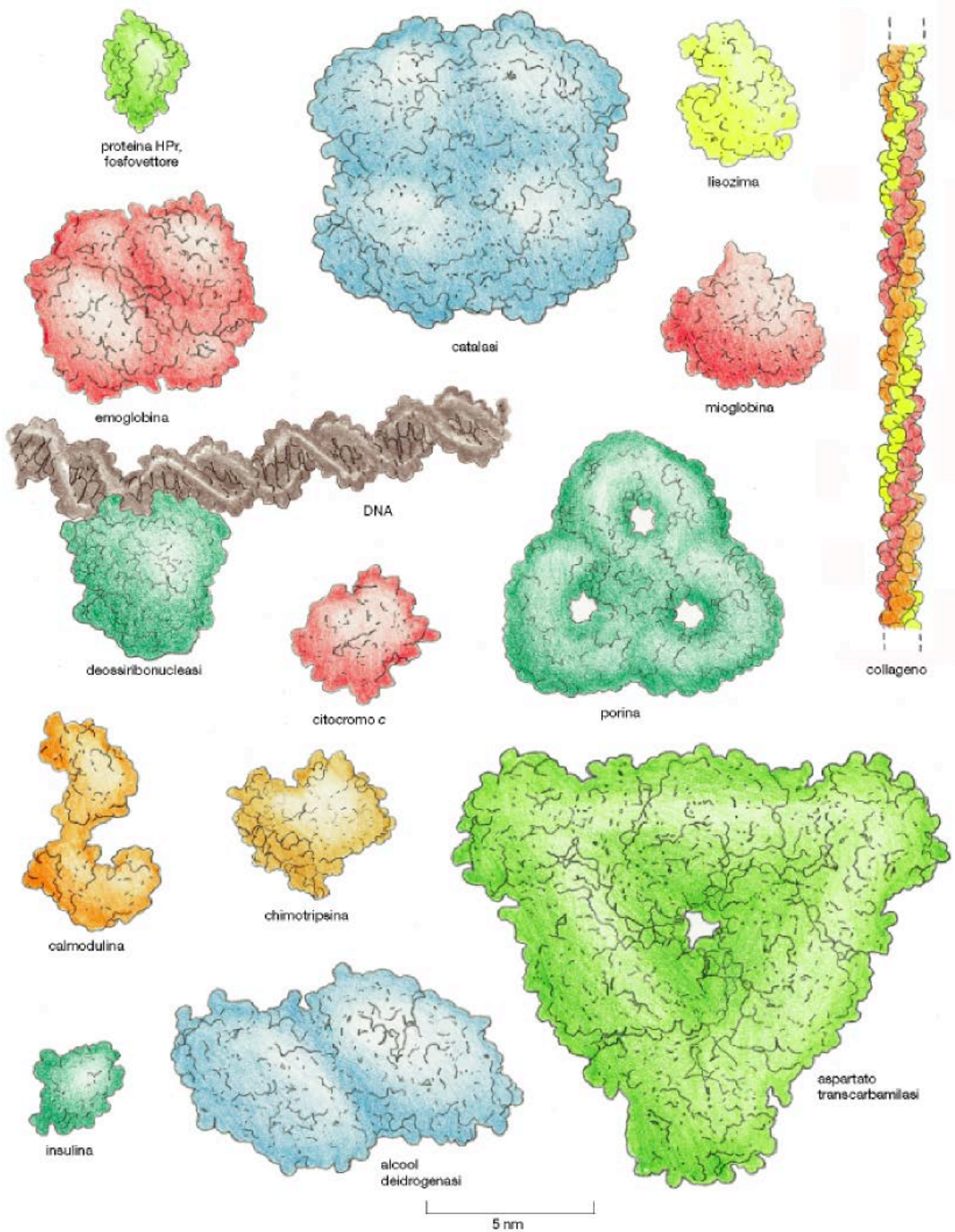


What Proteins are made of








proteina HPr,
fosfovettore
catalasi
lisozima
collageno
emoglobina
mioglobina
DNA
deossiribonucleasi
citocromo c
porina
calmodulina
chimotripsina
aspartato
transcarbamilasi
insulina
alcool
deidrogenasi
5 nm

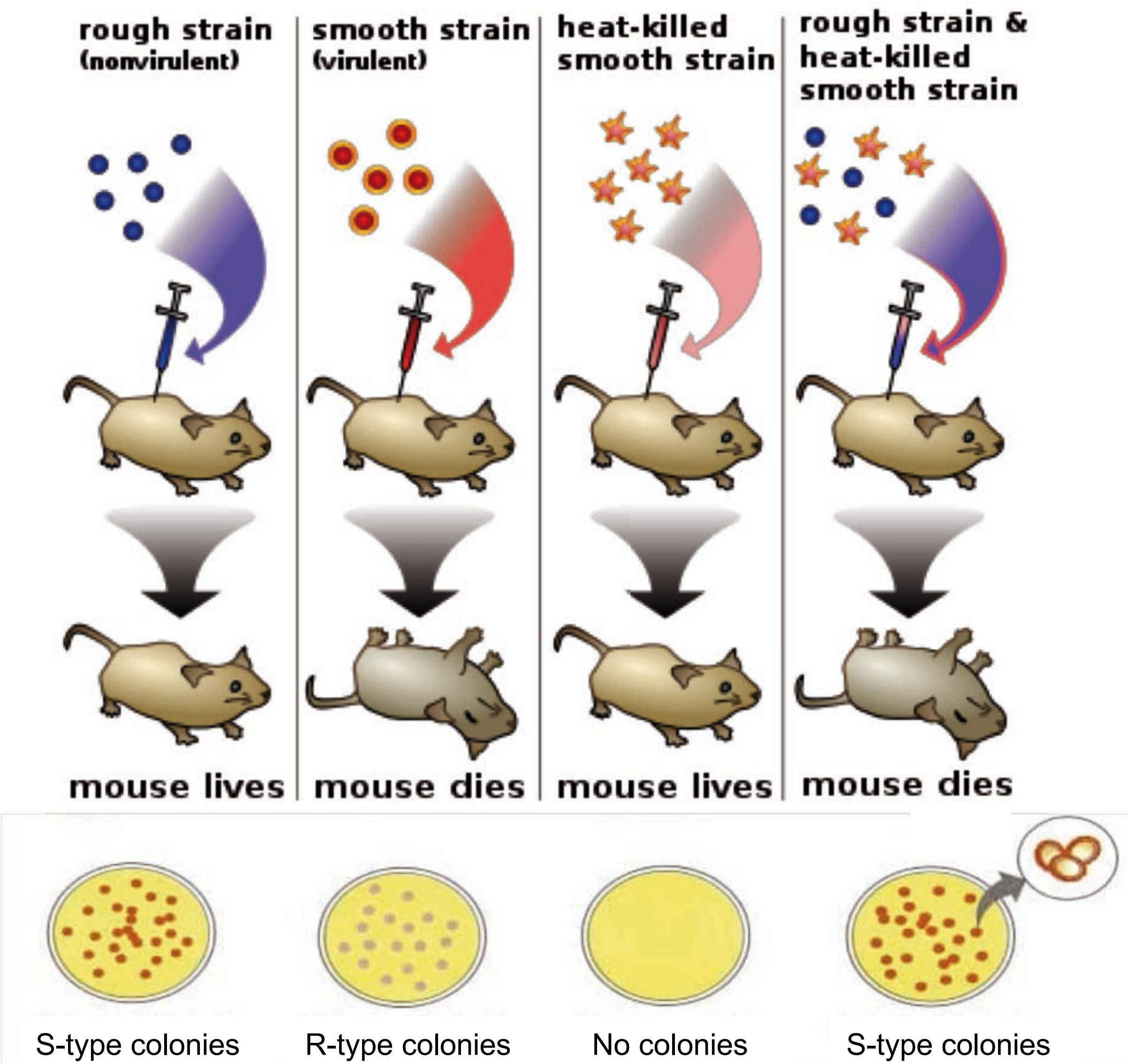
Milestones in Genetics and Molecular Biology

1865 Genes are particulate factors (Mendel)

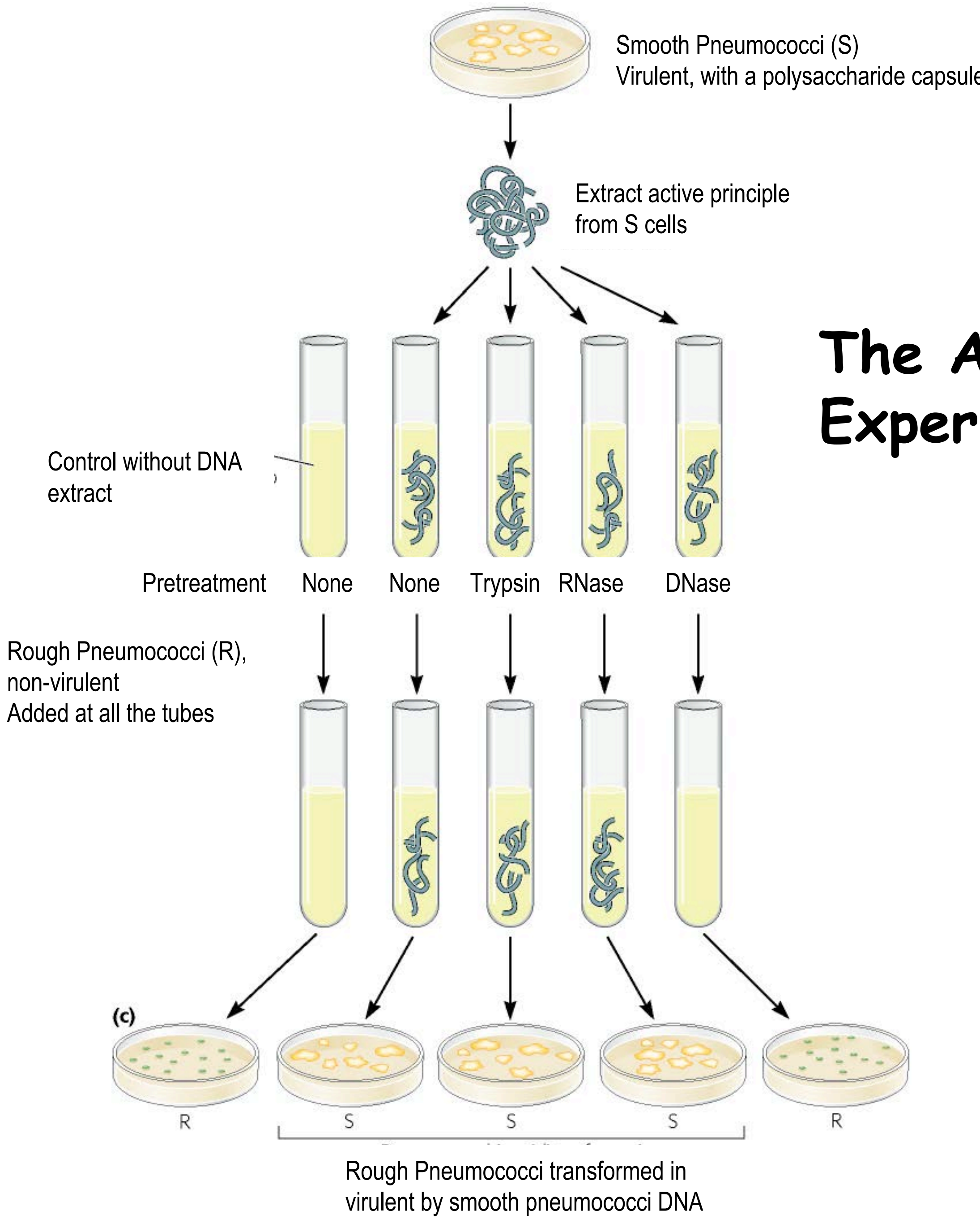
1910 Genes lie on chromosomes (Morgan)

1944 DNA is the genetic material (Avery)

The Griffith Experiment (1928)



The Avery–MacLeod–McCarty Experiment (1944)



Four requirements for DNA to be genetic material

Must carry information

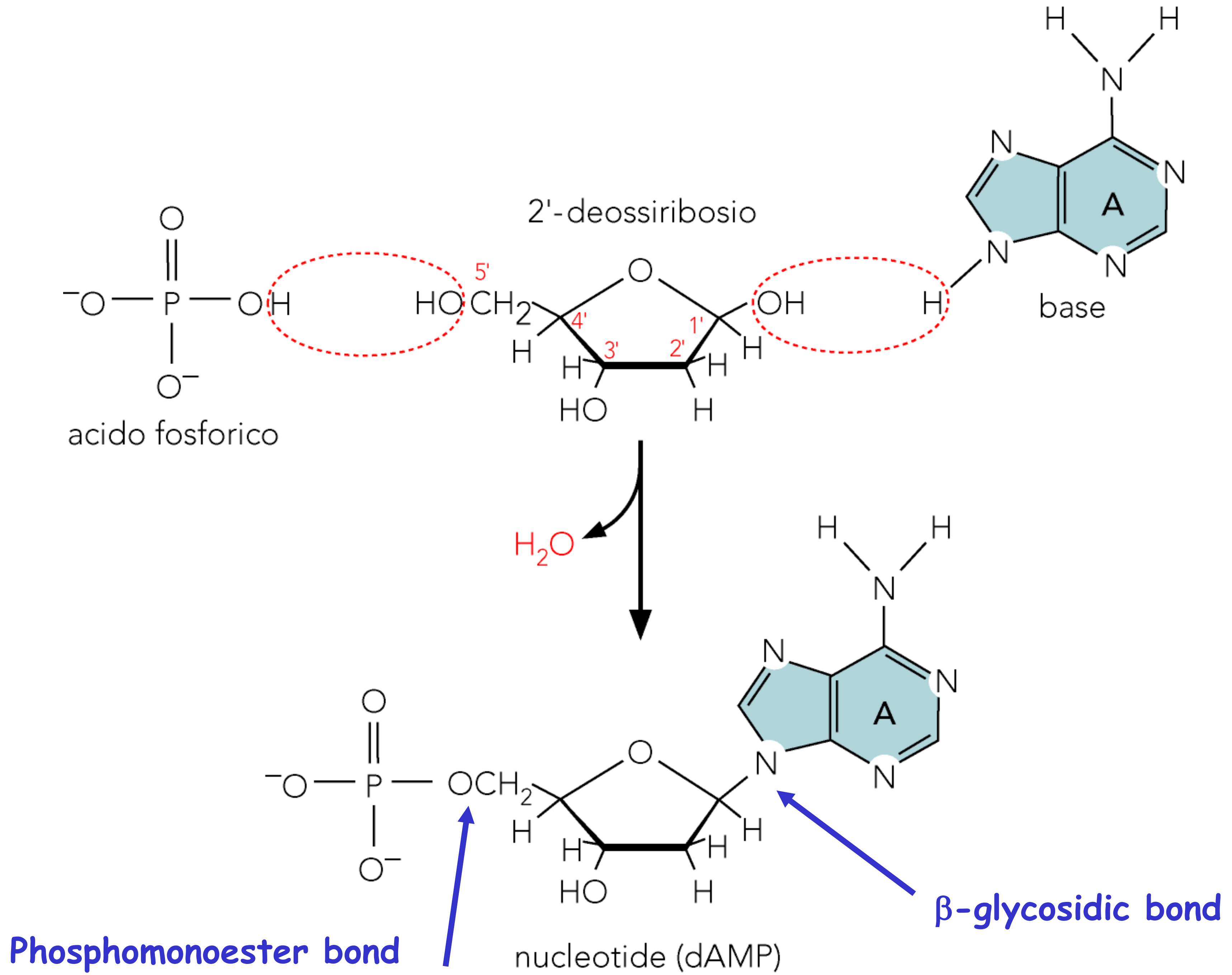
Must replicate

Must allow for information to change

Must regulate the expression of the phenotipe

The DNA race


Wilkins
University of London
Franklin
Watson
Cambridge University
Caltech
Pauling
Crick


2'-deossiribosio
acido fosforico
base
A
5'
4'
3'
2'
1'
H_2O
nucleotide (dAMP)
Phosphomonoester bond
β-glycosidic bond

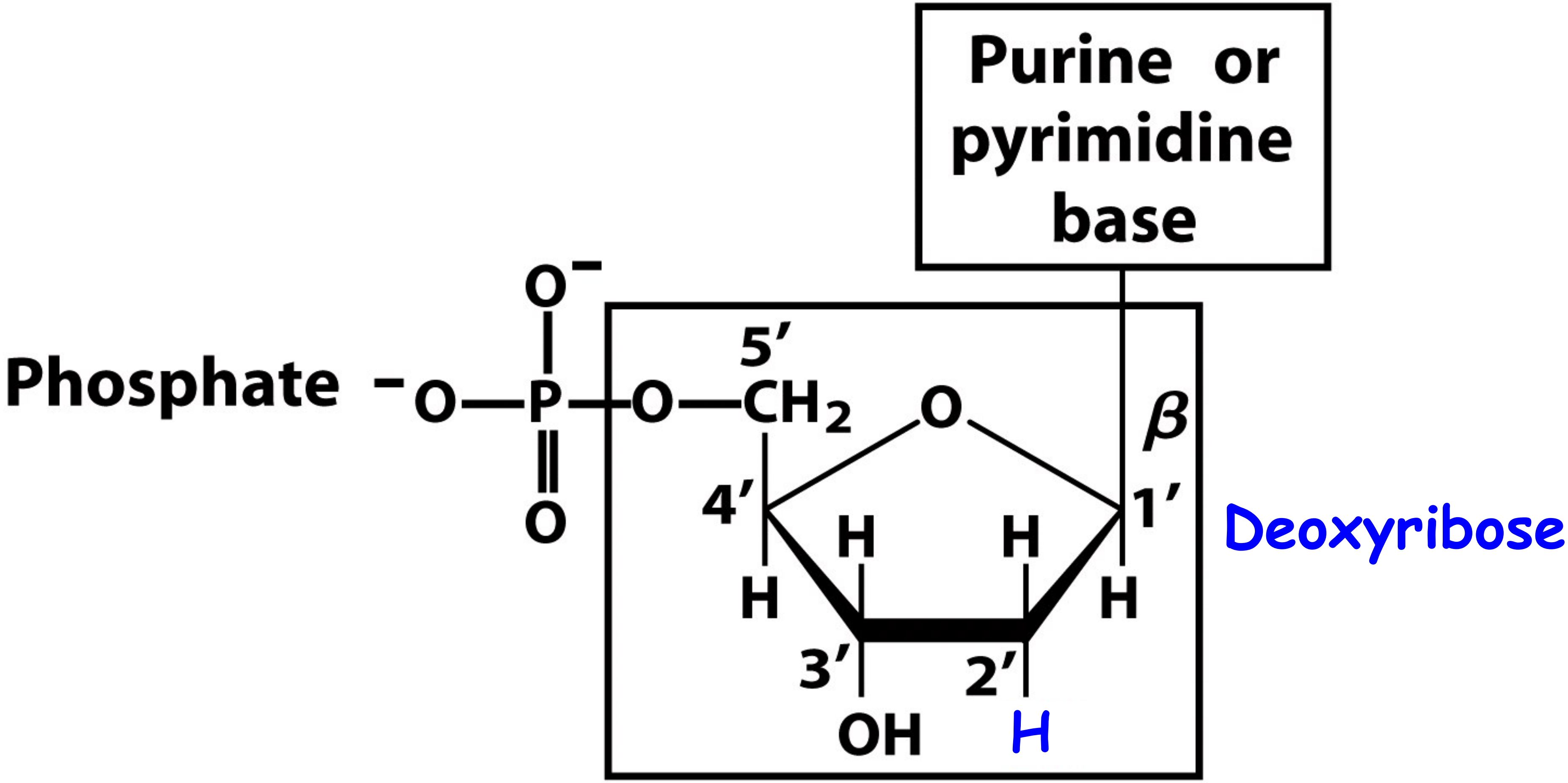


Figure 8-1a
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DNA

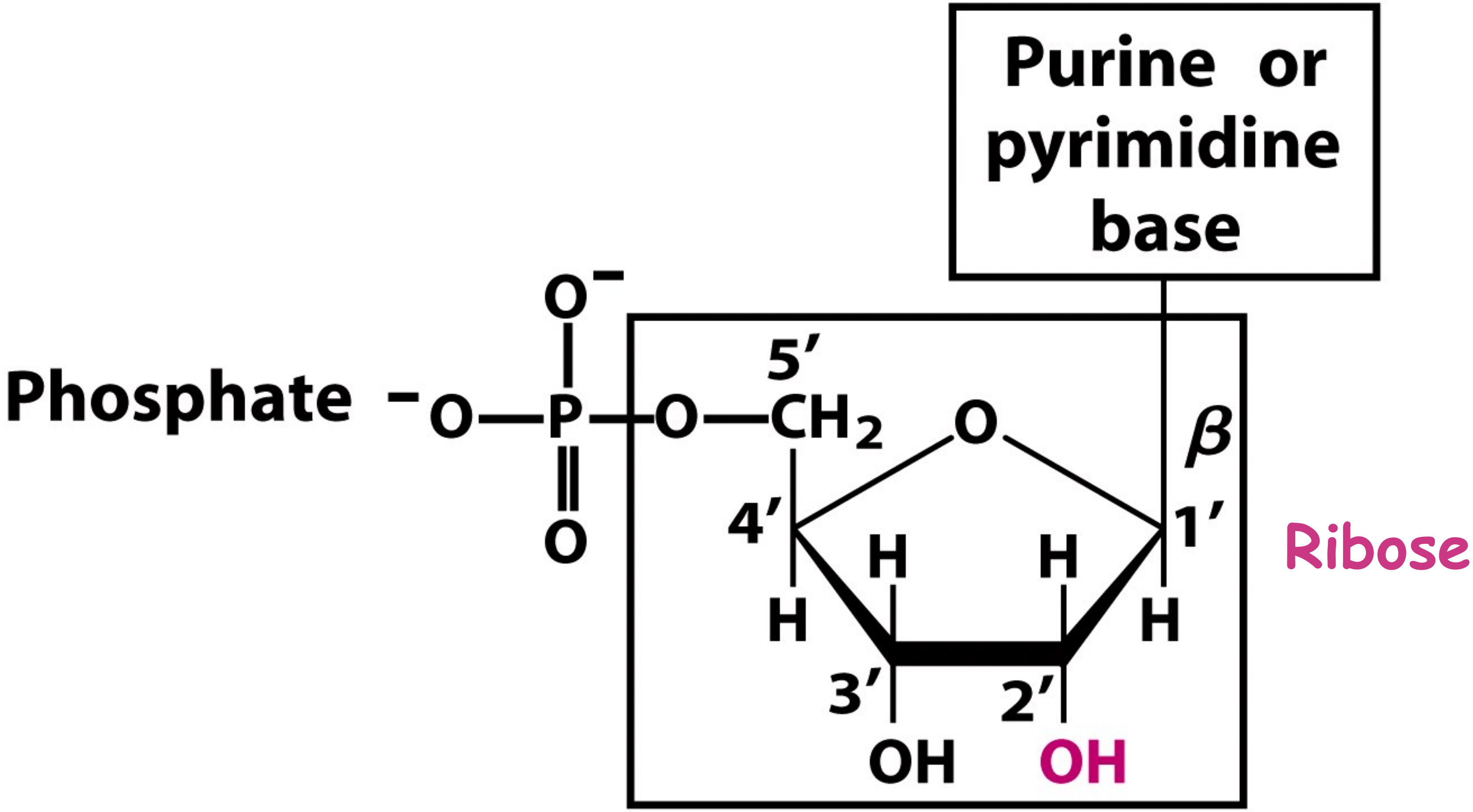

Figure 8-1a
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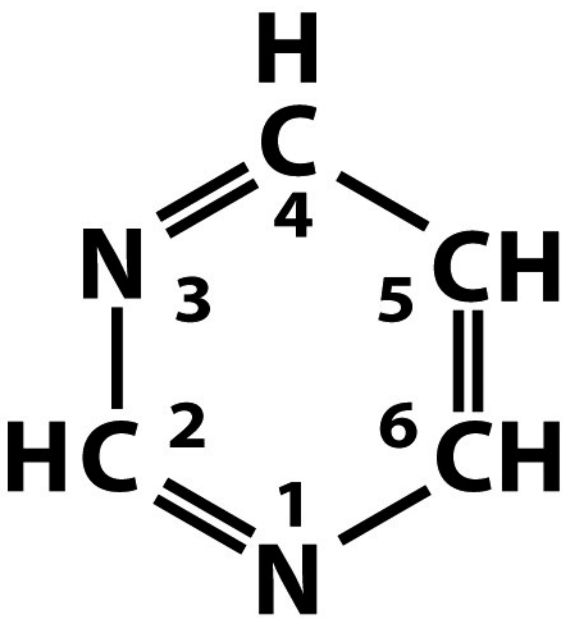
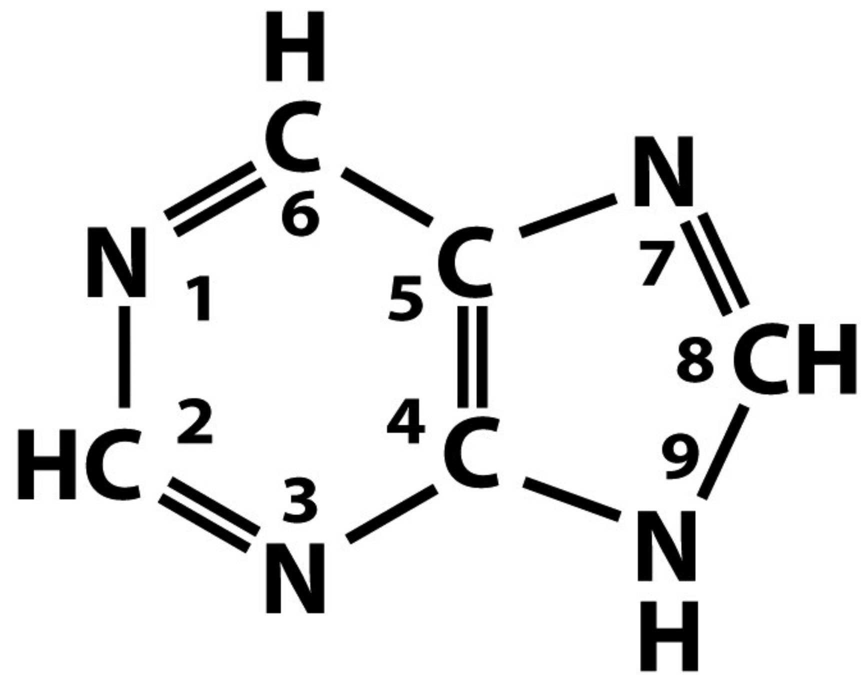
RNA



Pyrimidine



Purine

Figure 8-1b

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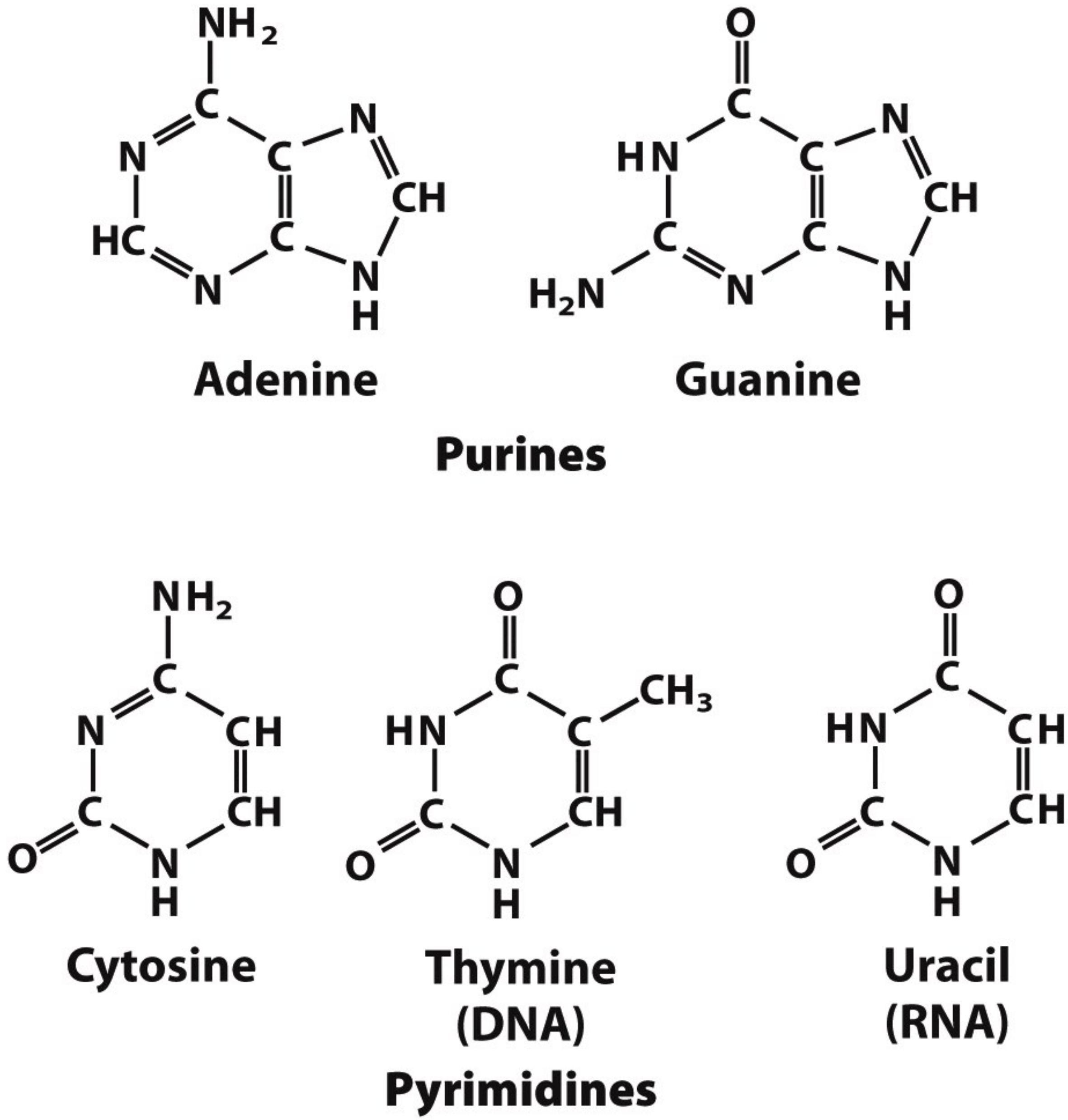

Figure 8-2
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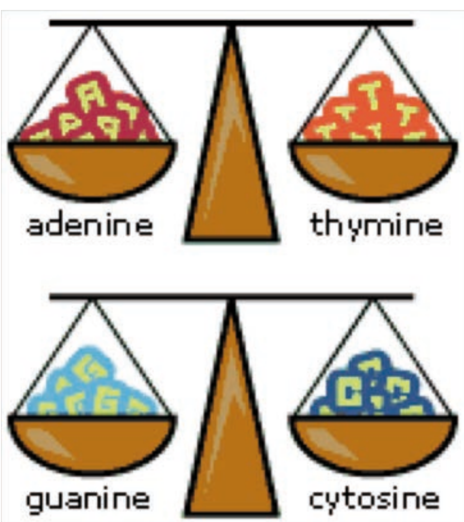
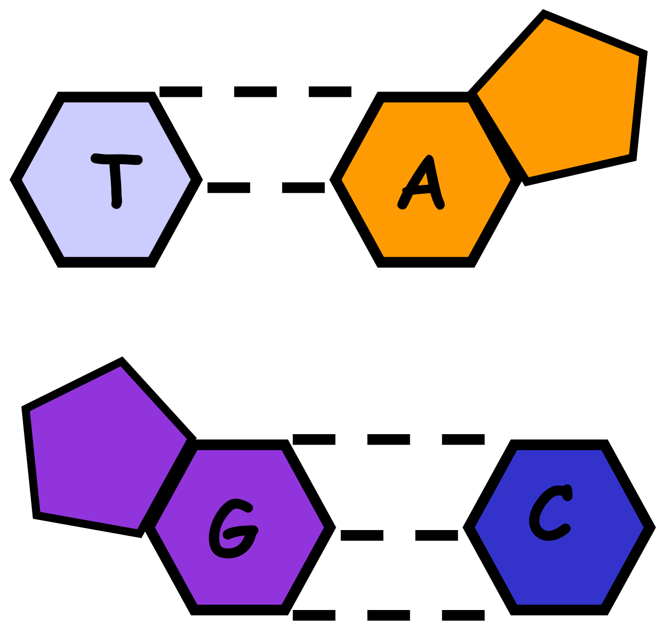
SOURCE	ADENINE TO GUANINE	THYMINE TO CYTOSINE	ADENINE TO THYMINE	GUANINE TO CYTOSINE	PURINES TO PYRIMIDINES	AMINO GROUPS TO ENOLIC HYDROXYLS
Ox	1.29	1.43	1.04	1.00	1.1	1.4
Man	1.56	1.75	1.00	1.00	1.0	1.3
Hen	1.45	1.29	1.06	0.91	0.99	1.5
Salmon	1.43	1.43	1.02	1.02	1.02	1.4
Wheat	1.22	1.18[1]	1.00	0.97[1]	0.99	1.4
Yeast	1.67	1.92	1.03	1.20	1.0	1.3
Hemophilus influenzae, type C	1.74	1.54	1.07	0.91	1.0	1.5
B. coli K-12	1.05	0.95	1.09	0.99	1.0	1.6
Avian tubercle bacillus	0.4	0.4	1.09	1.08	1.1	1.7
Serratia marcescens	0.7	0.7	0.95	0.86	0.9	1.6
Hydrogen organism Bacillus Schatz	0.7	0.6	1.12	0.89	1.0	1.7

[1] In these computations the sum of cytosine and methylcytosine was used. If cytosine alone is considered, the thymine to cytosine ratio is 1.62 and that of guanine to cytosine 1.33.

In 1949 ***Erwin Chargaff*** shows that in DNA (from every source) the amount of Adenine is approximately equal to the amount of Thymine, and the amount of Guanine is approximately equal to the amount of Cytosine.





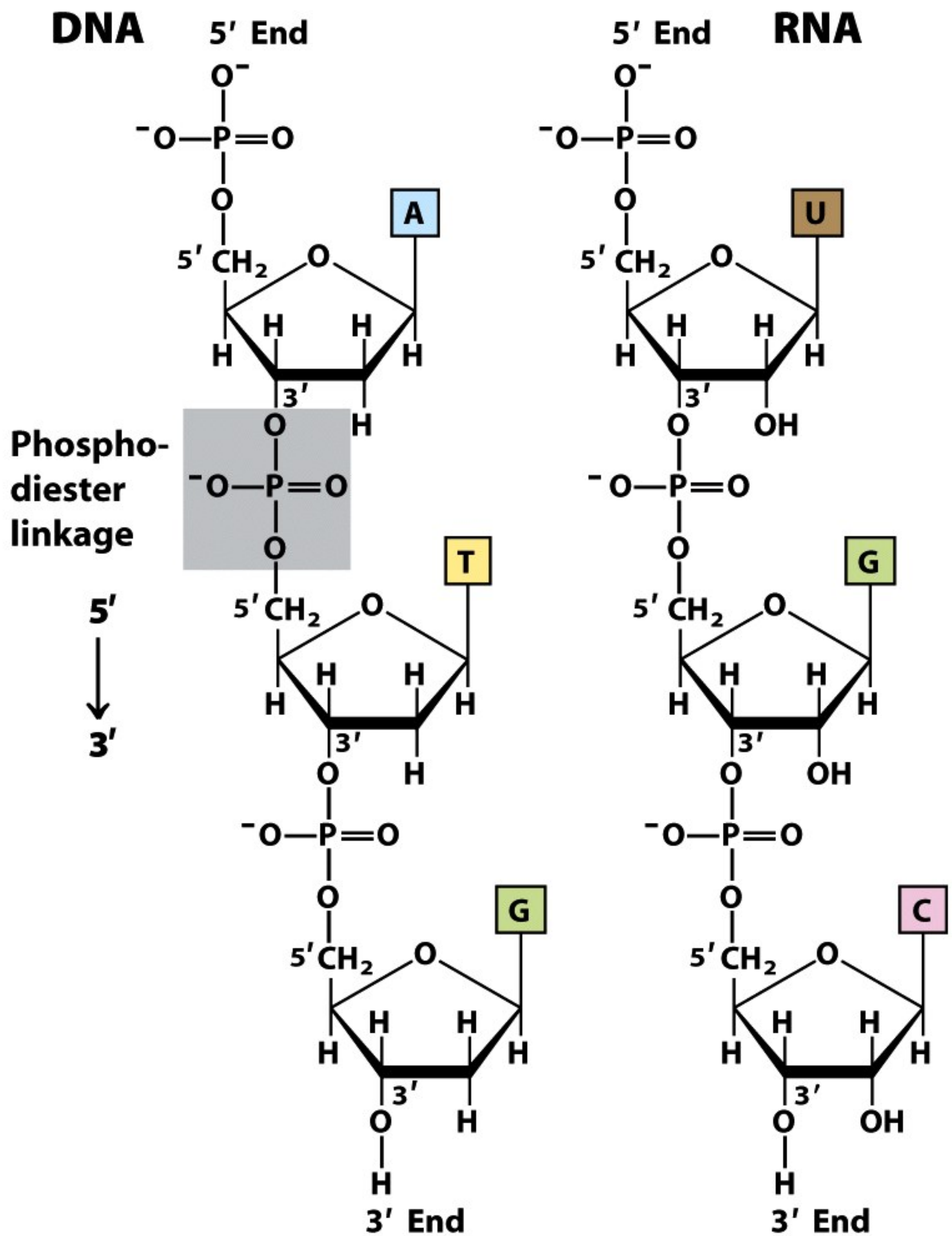


Figure 8-7

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In 1952 ***Alexander Todd*** showed that nucleotides are linked by phosphodiester bonds

Milestones in Genetics and Molecular Biology

1865 Genes are particulate factors (Mendel)

1910 Genes lie on chromosomes (Morgan)

1944 DNA is the genetic material (Avery)

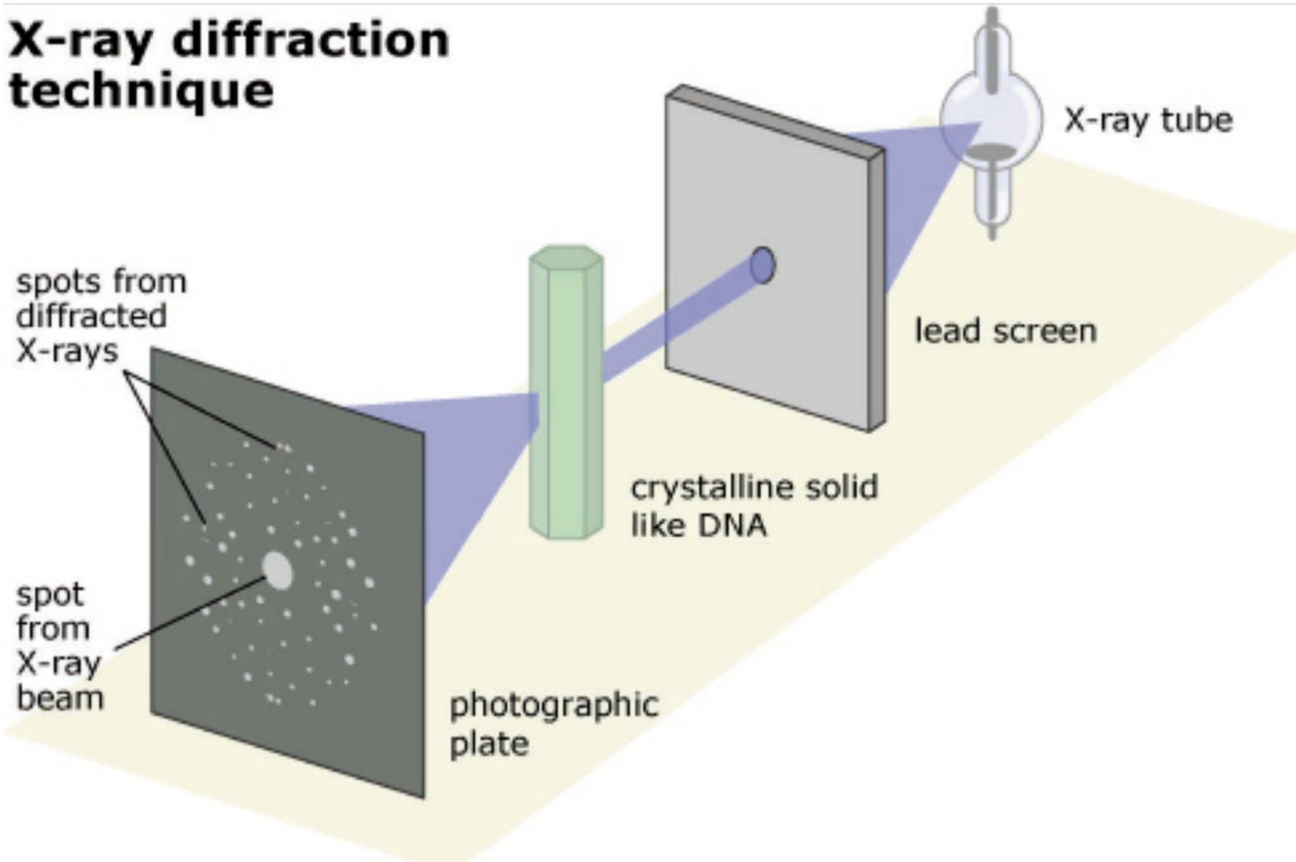
1953 DNA is a double helix (Watson, Crick, Franklin)

Rosalind Franklin, 1920–1958

Formerly: Expert on the structure of coal

Maurice Wilkins, 1916–2004

Formerly: Nuclear physicist

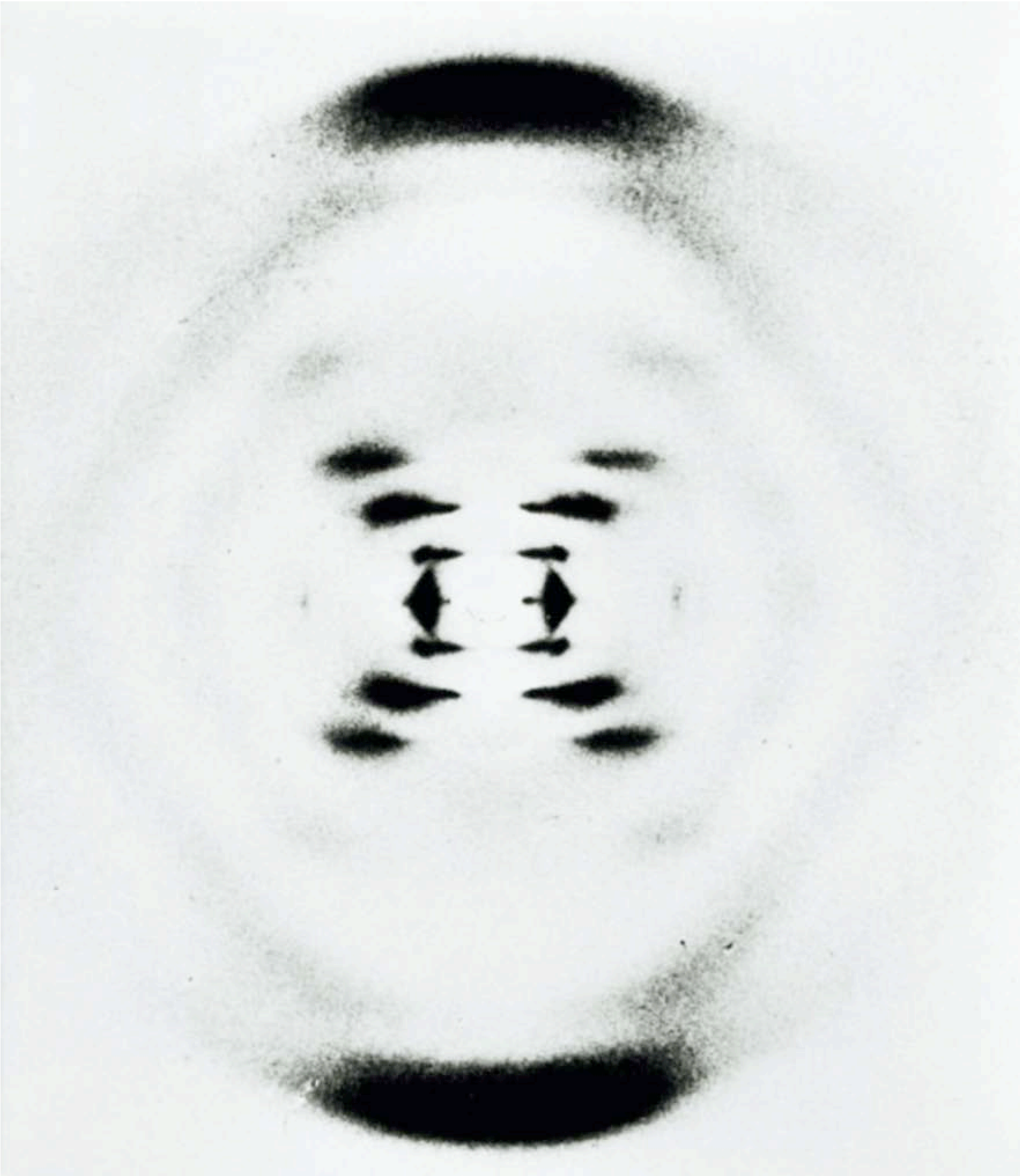

X-ray diffraction technique
X-ray tube
lead screen
crystalline solid like DNA
spots from diffracted X-rays
spot from X-ray beam
photographic plate

James D. Watson

Formerly: Bird Biologist

Francis Crick, 1916–2004

Formerly: Designer of underwater mines



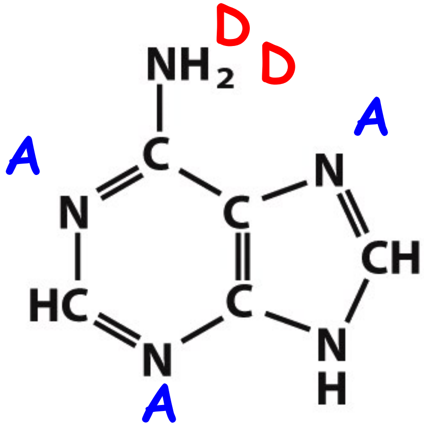
*The **B-51** Image*

From this image (1952) ***Rosalind Franklyn*** (and independently Watson & Crick) deduced that DNA has a helical structure. Other information from her work:

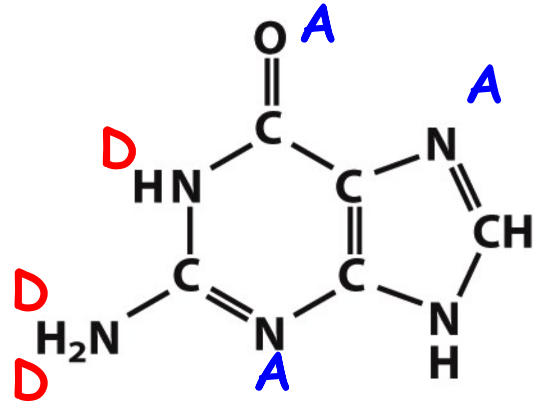
- The diameter of the helix is 2 nm
- Bases are stacked on the inside of the helix, 0.34 nm apart
- DNA molecules are symmetrical

A=Acceptor

D=Donor

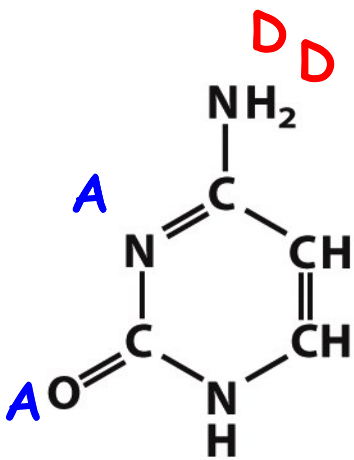


Adenine

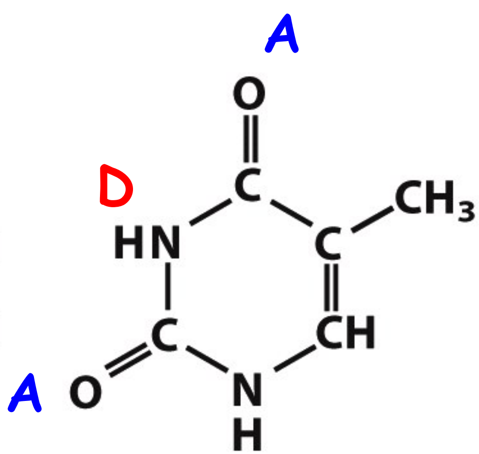


Guanine

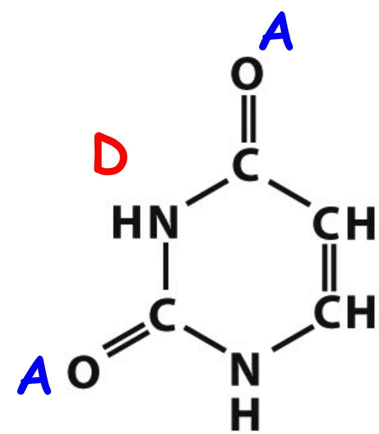
Purines



Cytosine



Thymine (DNA)



Uracil (RNA)

Pyrimidines

Figure 8-2

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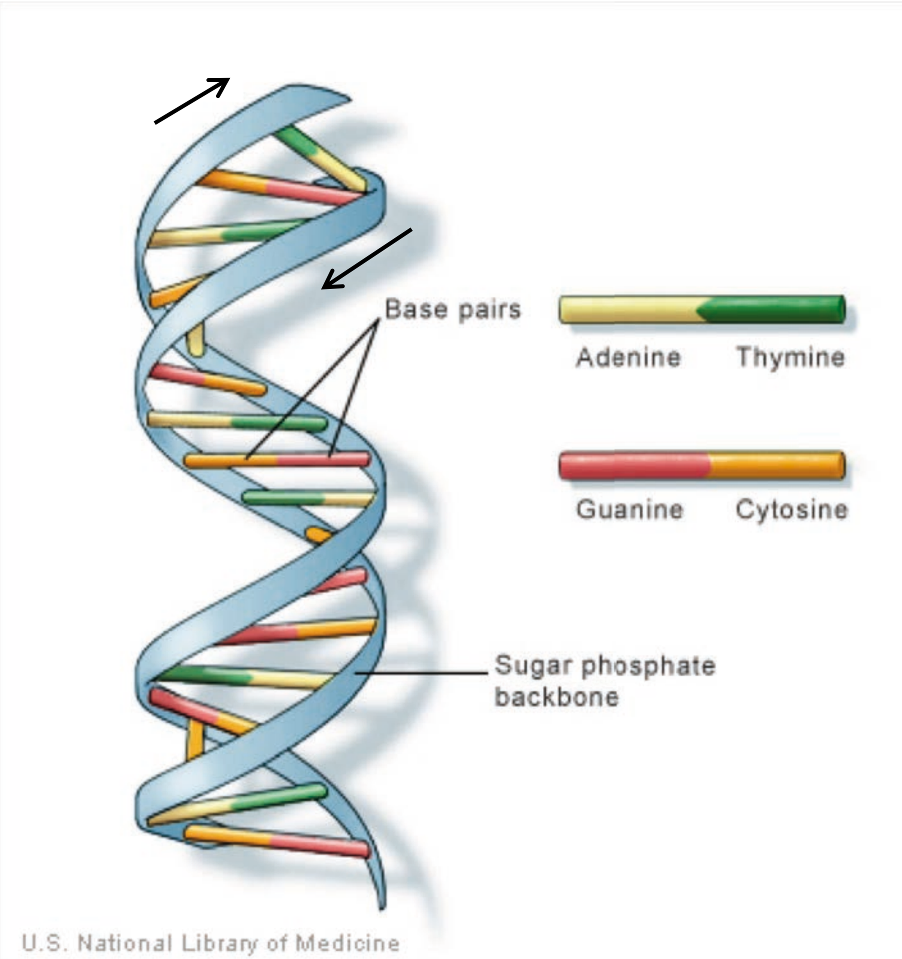
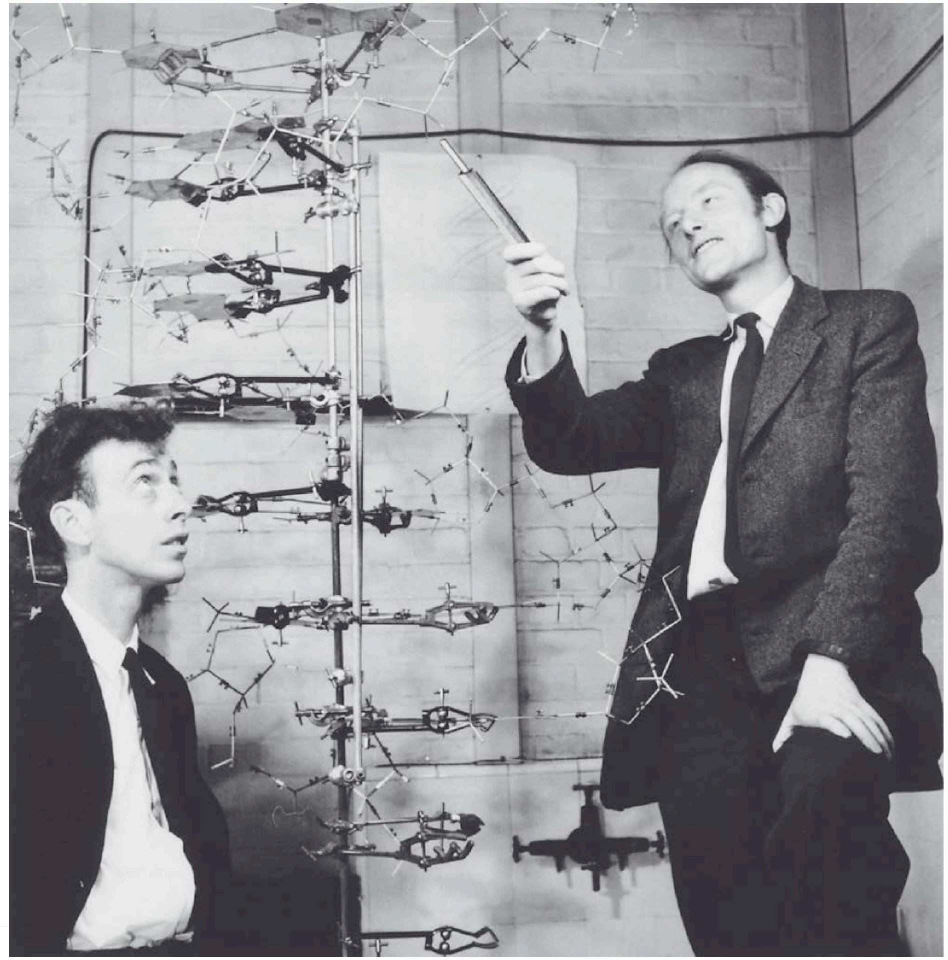

Base pairs
Adenine
Thymine
Guanine
Cytosine
Sugar phosphate backbone
U.S. National Library of Medicine



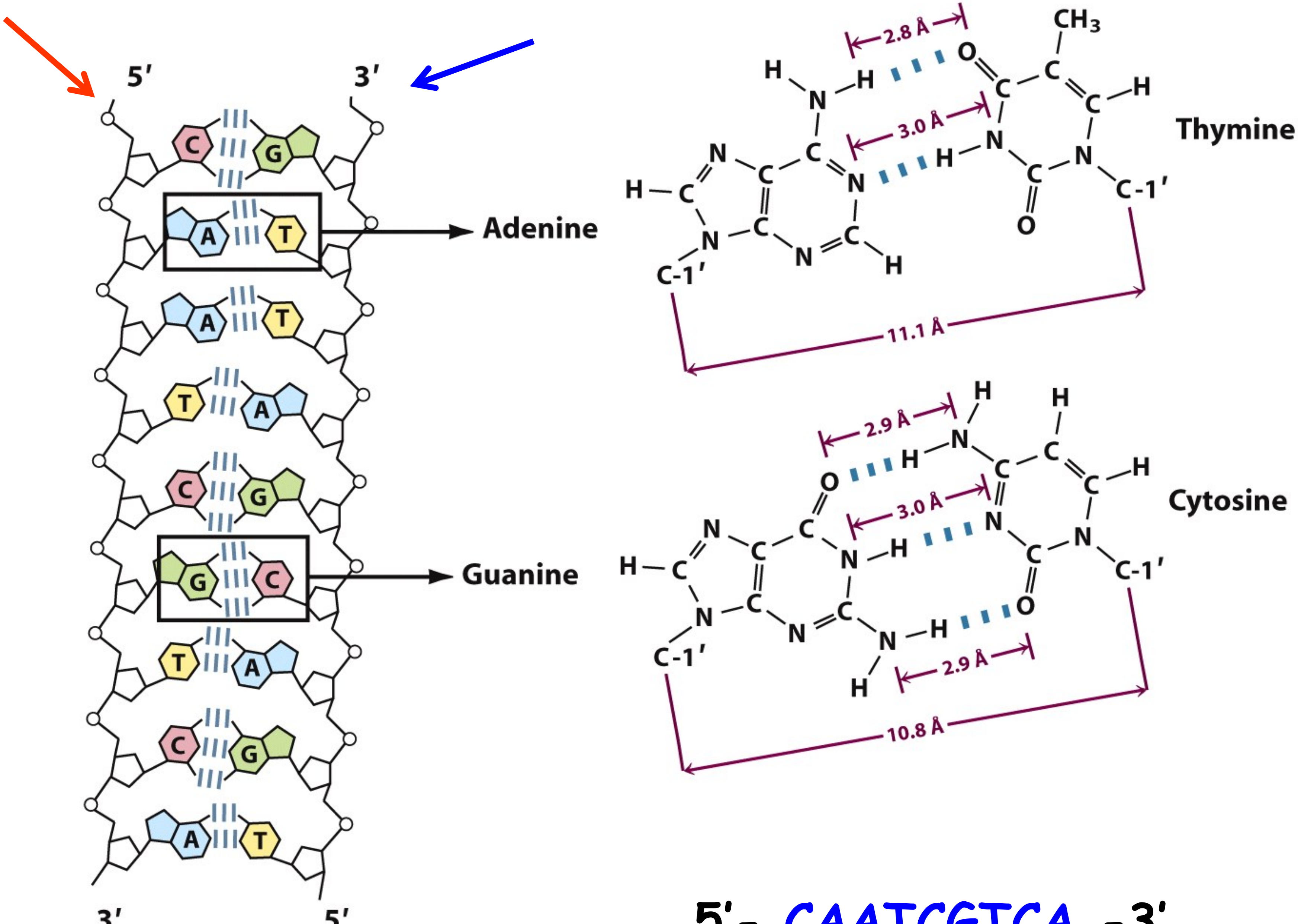


Figure 8-11
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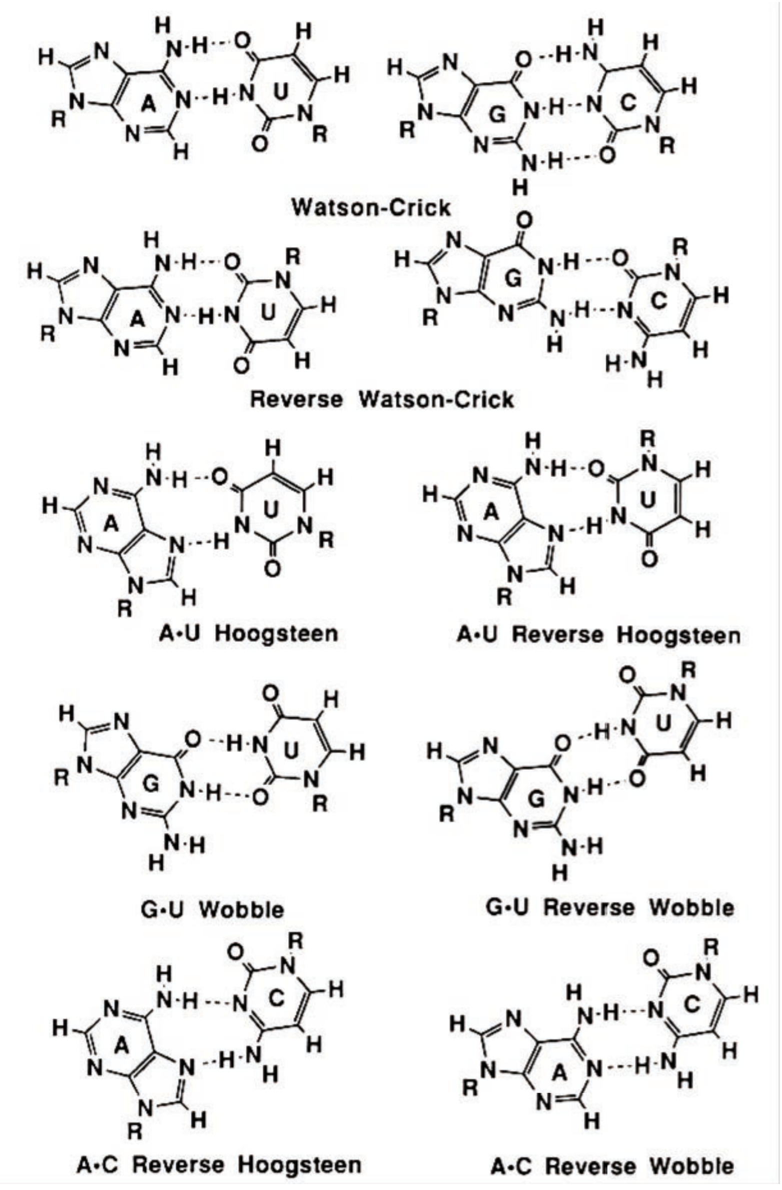
5′- CAATCGTCA -3′

3′- GTTAGCAGT -5′



There are 28 possible ways to form base-pairs with at least 2 hydrogen bonds

It is also possible to form stable triple helices

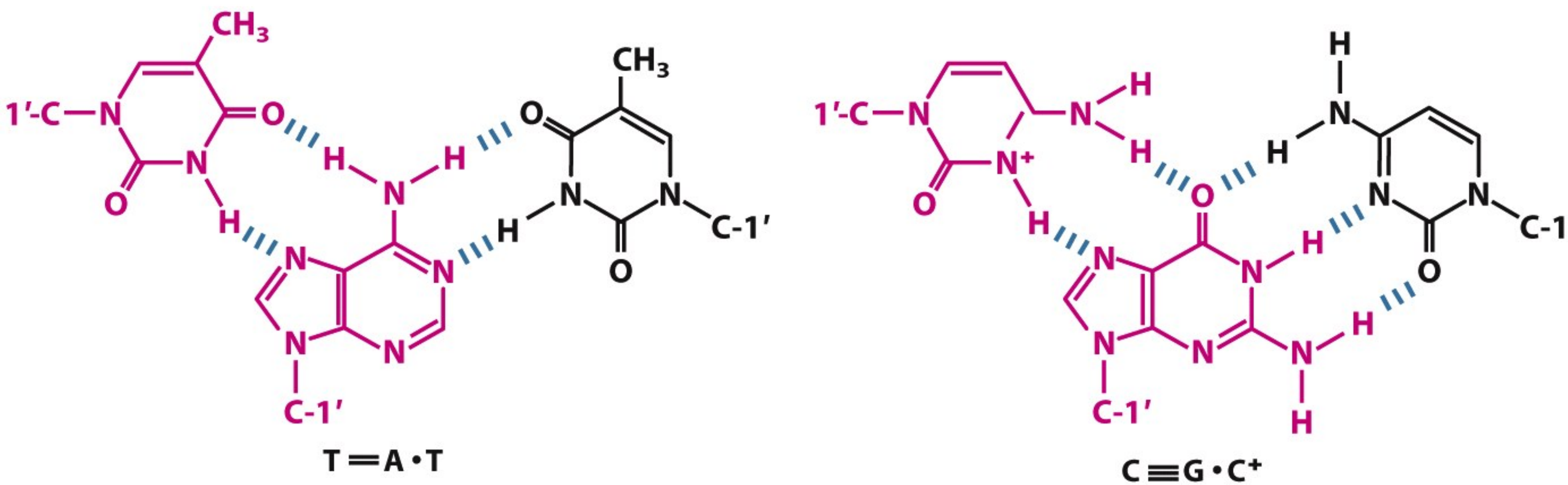


Figure 8-20a
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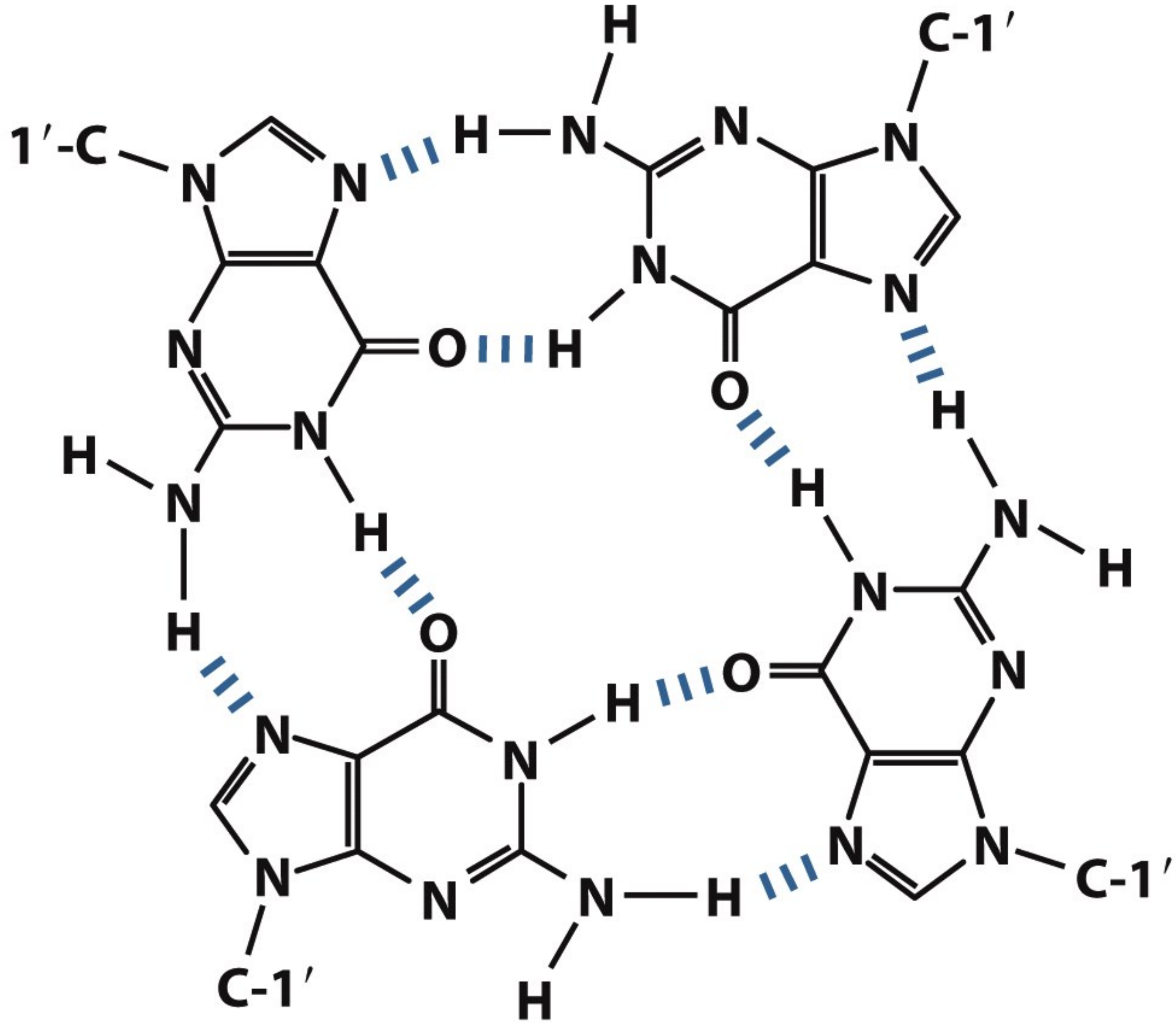

Guanosine tetraplex

Figure 8-20c
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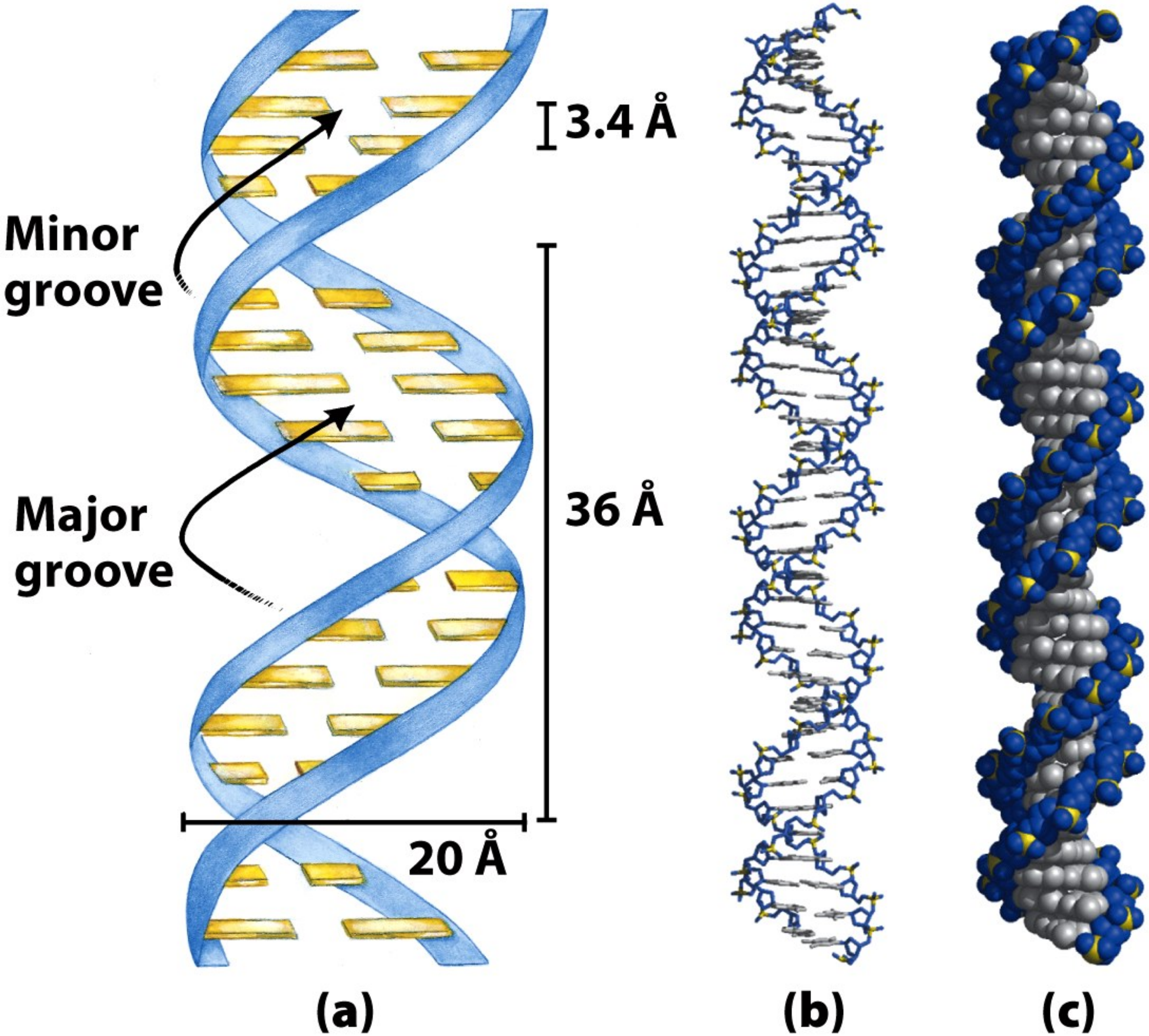

Figure 8-13
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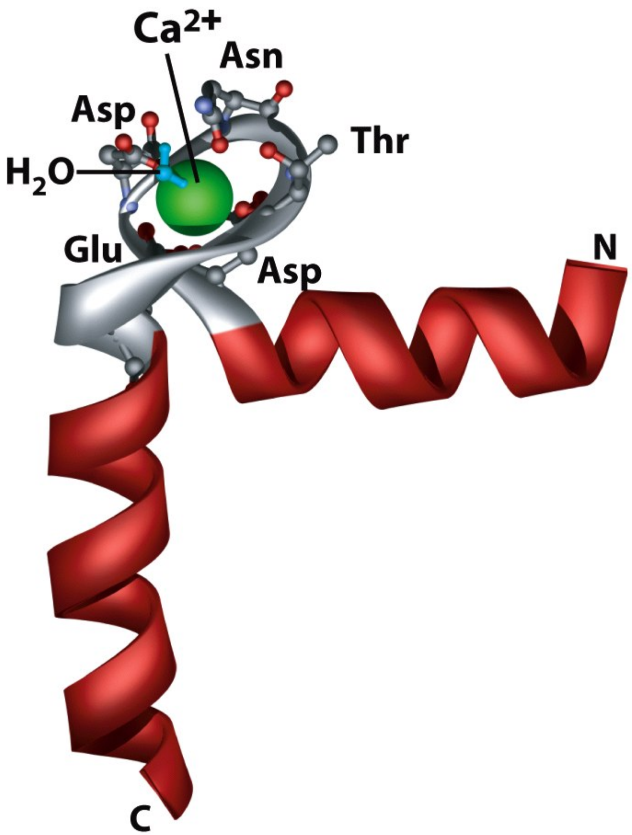
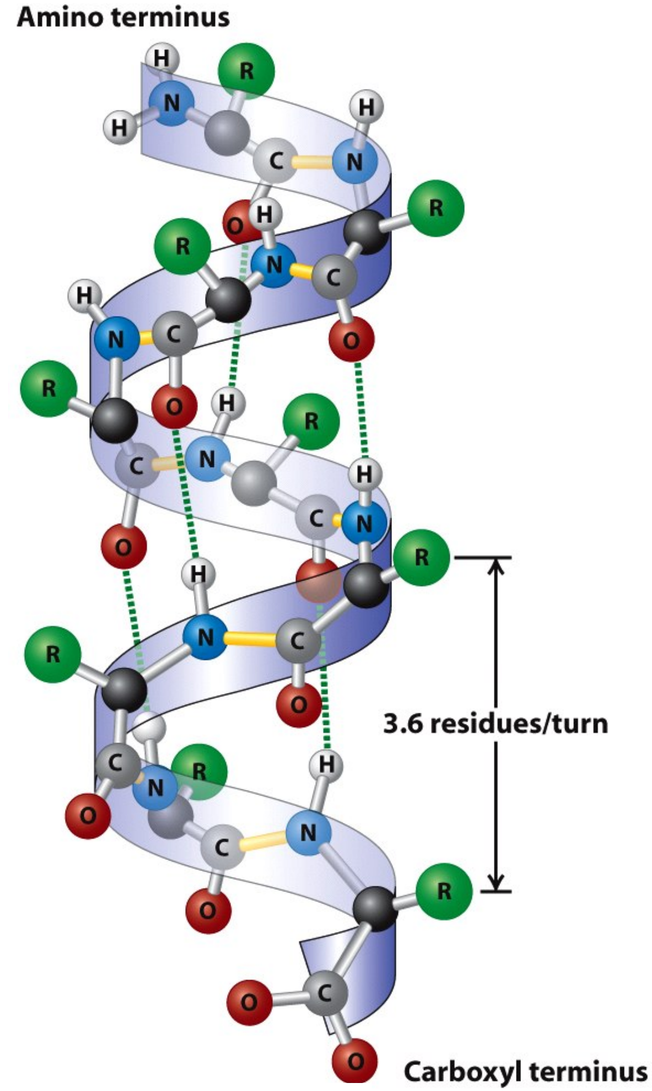
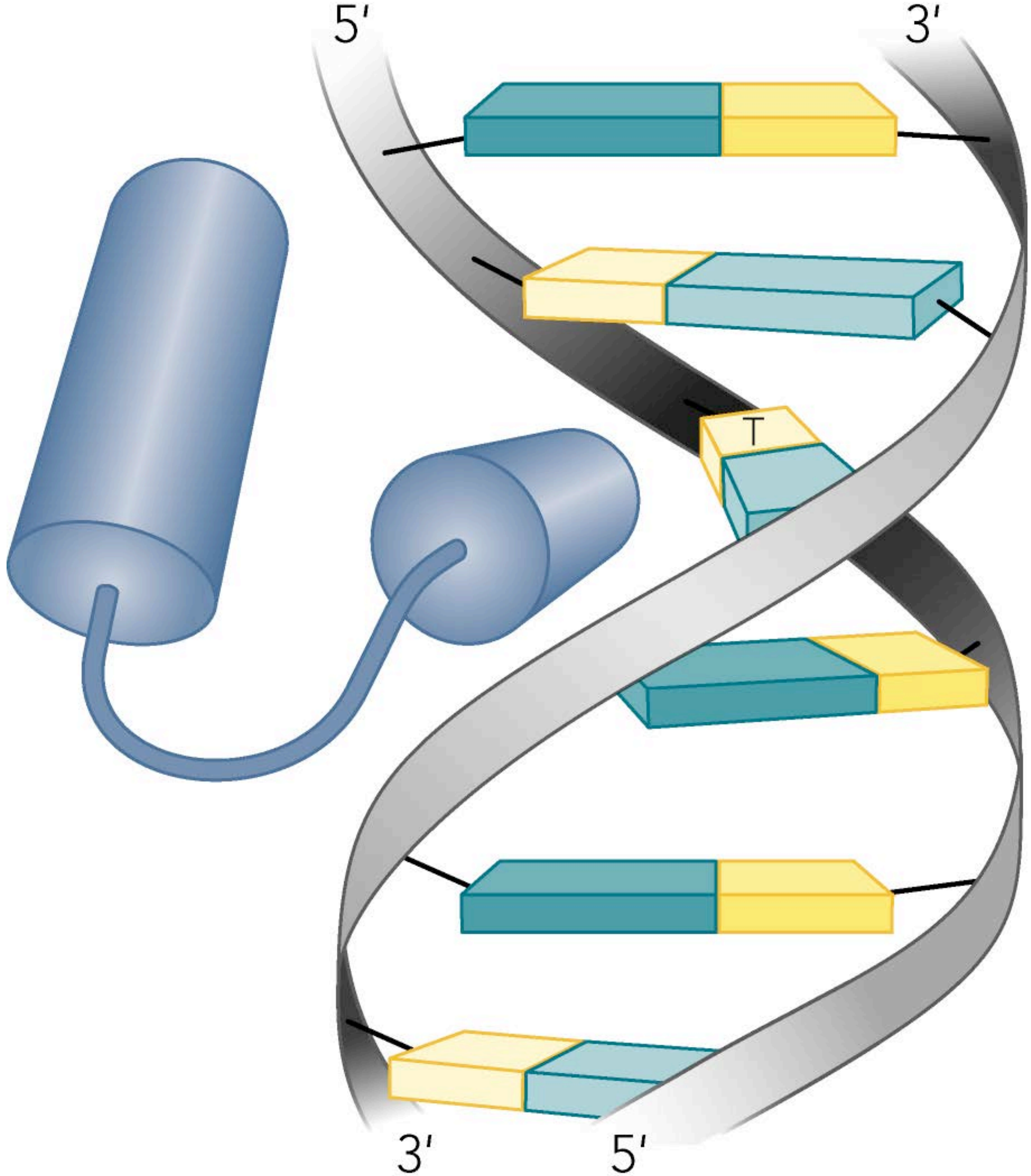
Secondary structure

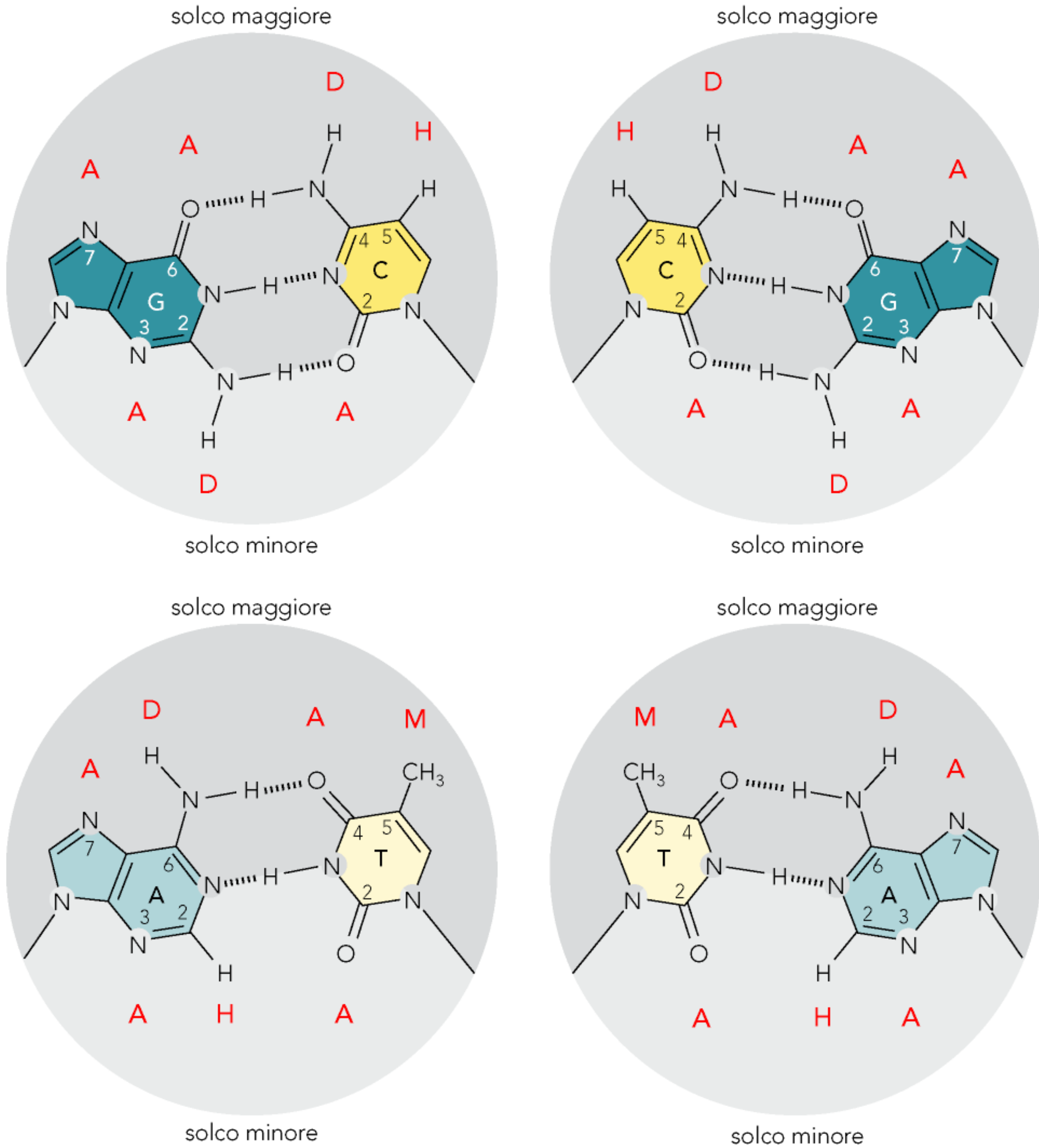
α helix

EFhand/helix-loop-helix motif





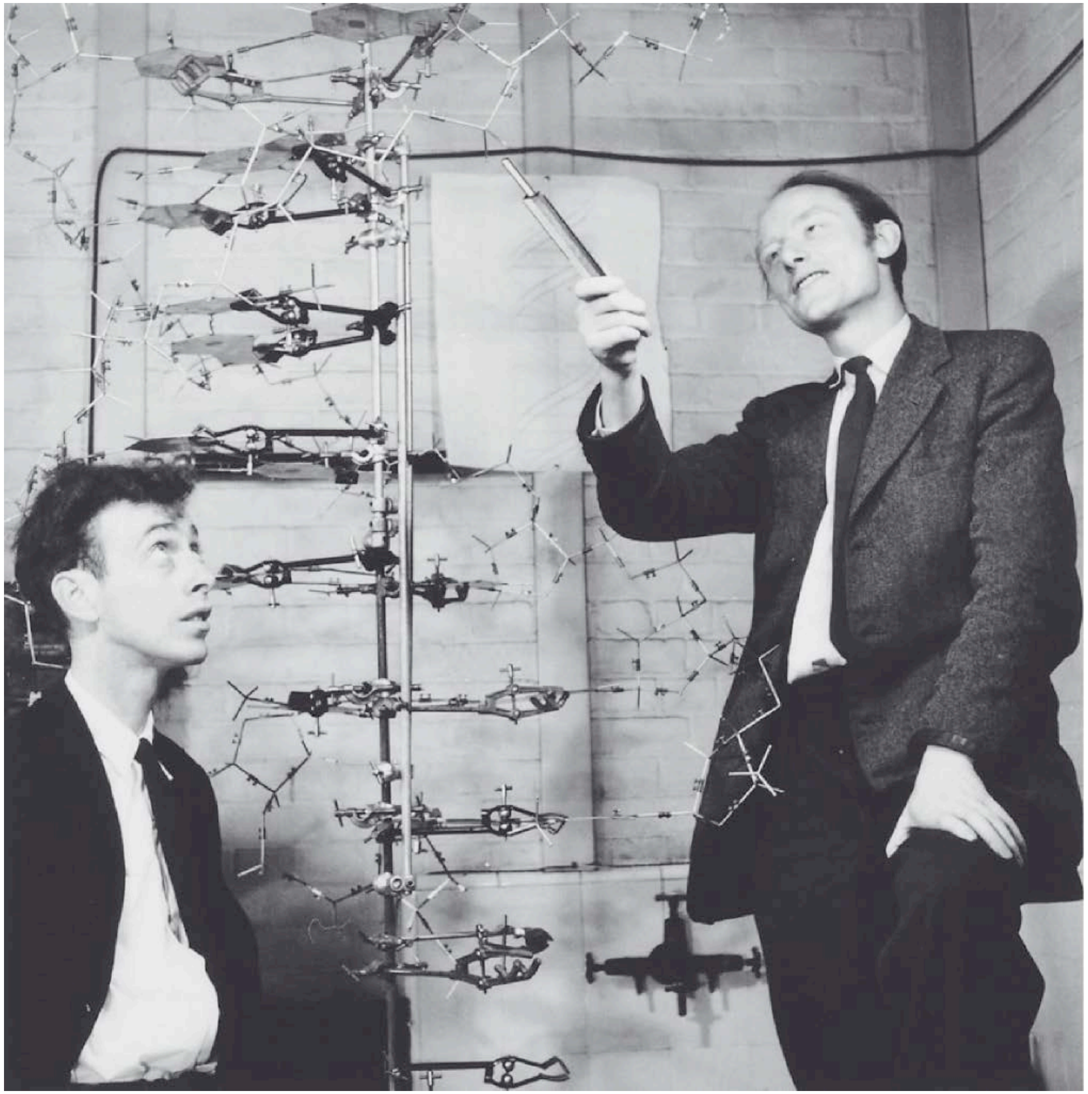

5′
3′
T
3′
5′

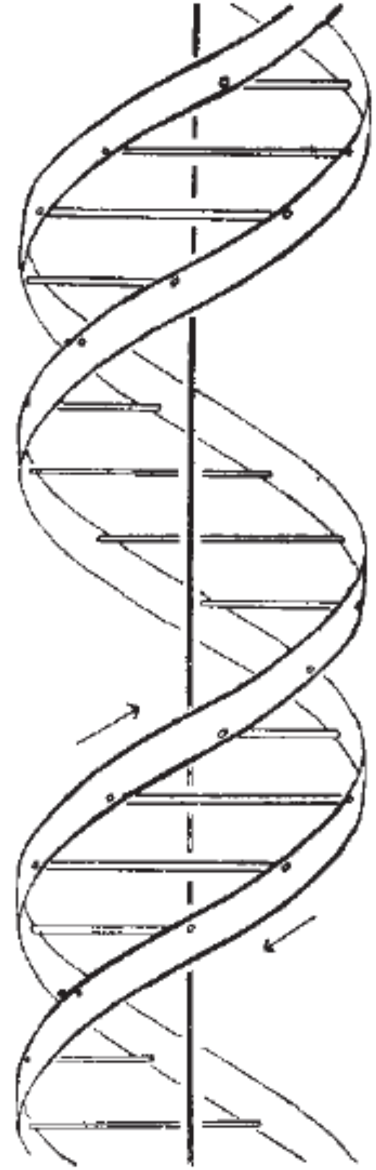

solco maggiore
D
A
A
H
G
C
solco minore
A
A
D
solco maggiore
D
H
A
A
C
G
solco minore
A
A
D
solco maggiore
D
A
M
A
A
T
solco minore
A
H
A
solco maggiore
M
A
D
A
T
A
solco minore
A
H
A

DNA Structure Discovery

Nature (1953), 171:737

"We wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest."





The last sentence of the Watson-Crick paper states"*It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.*"

Milestones in Genetics and Molecular Biology

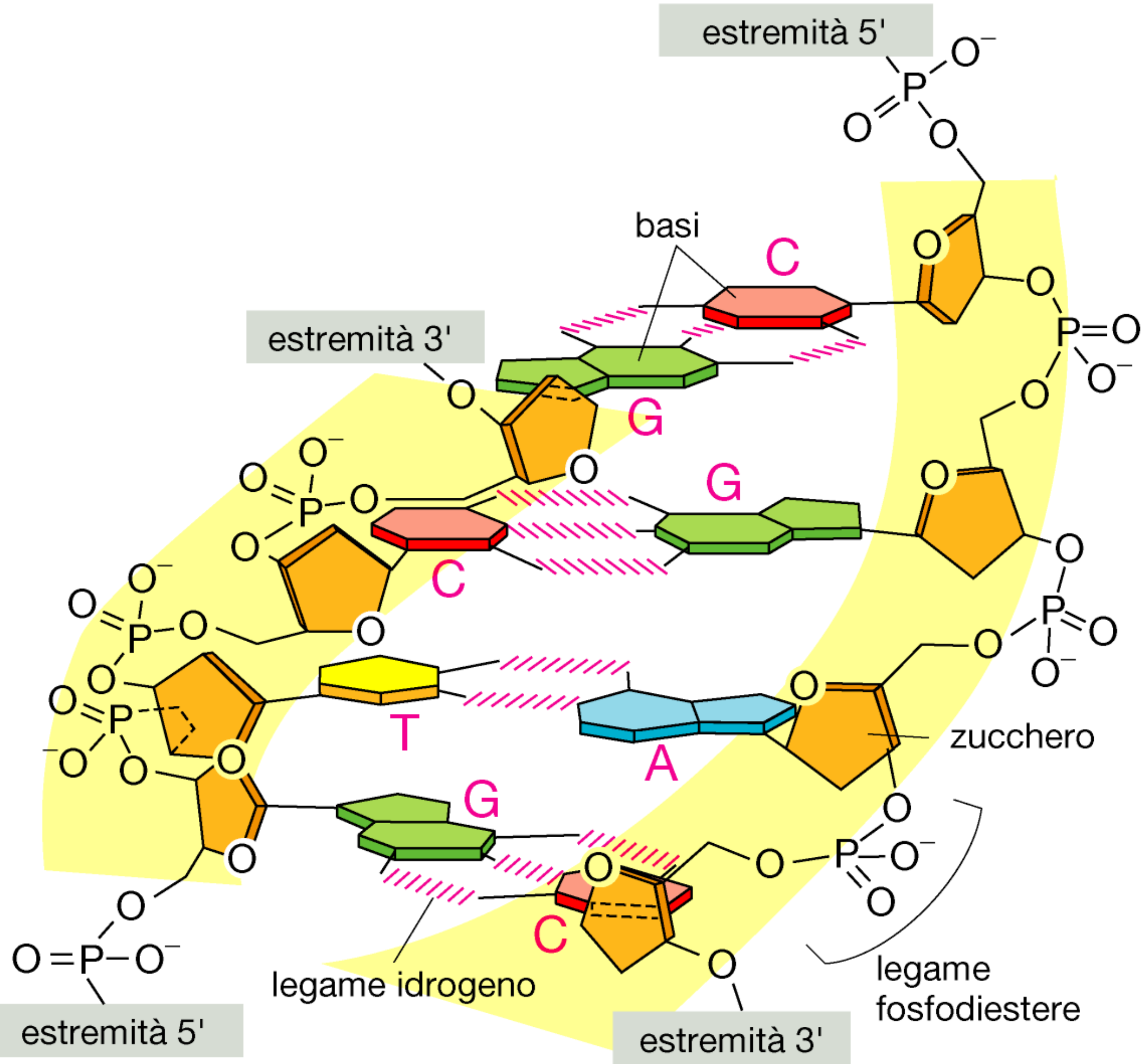
1865 Genes are particulate factors (Mendel)

1903 Genes lie on chromosomes (Morgan)

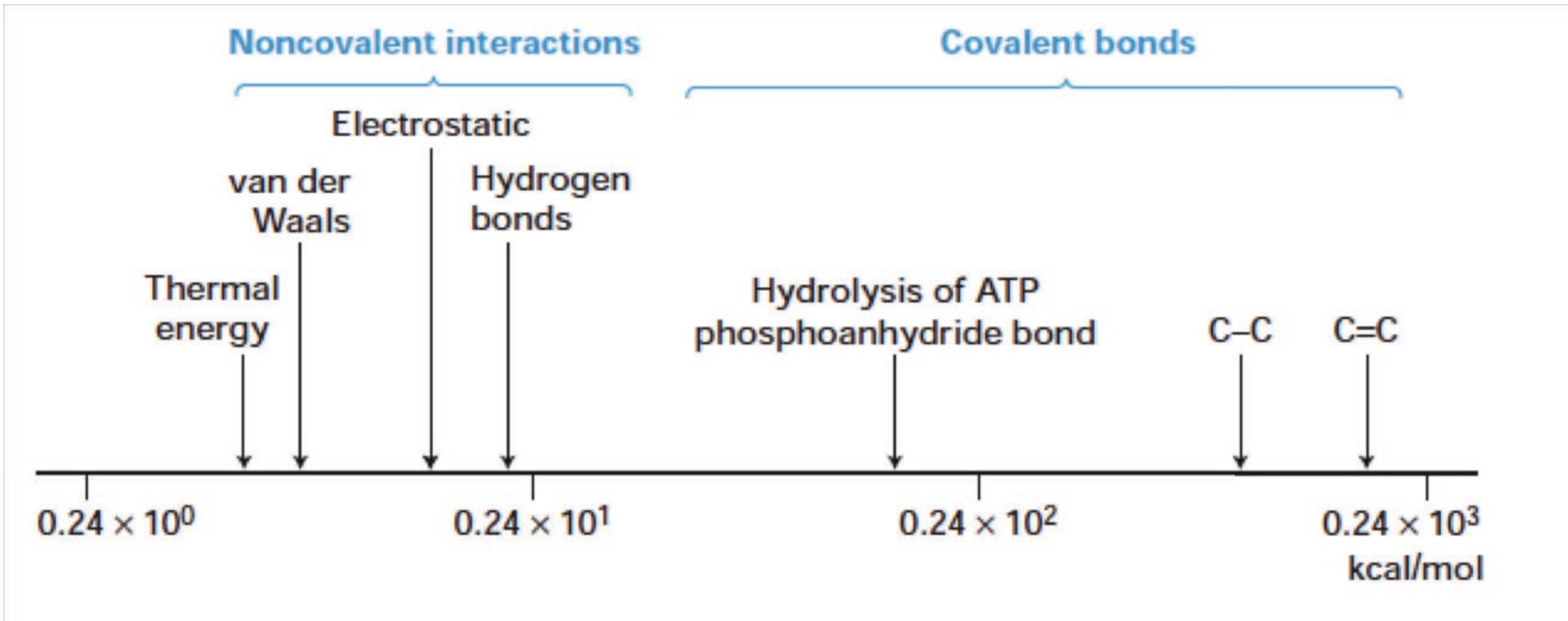
1944 DNA is the genetic material (Avery)

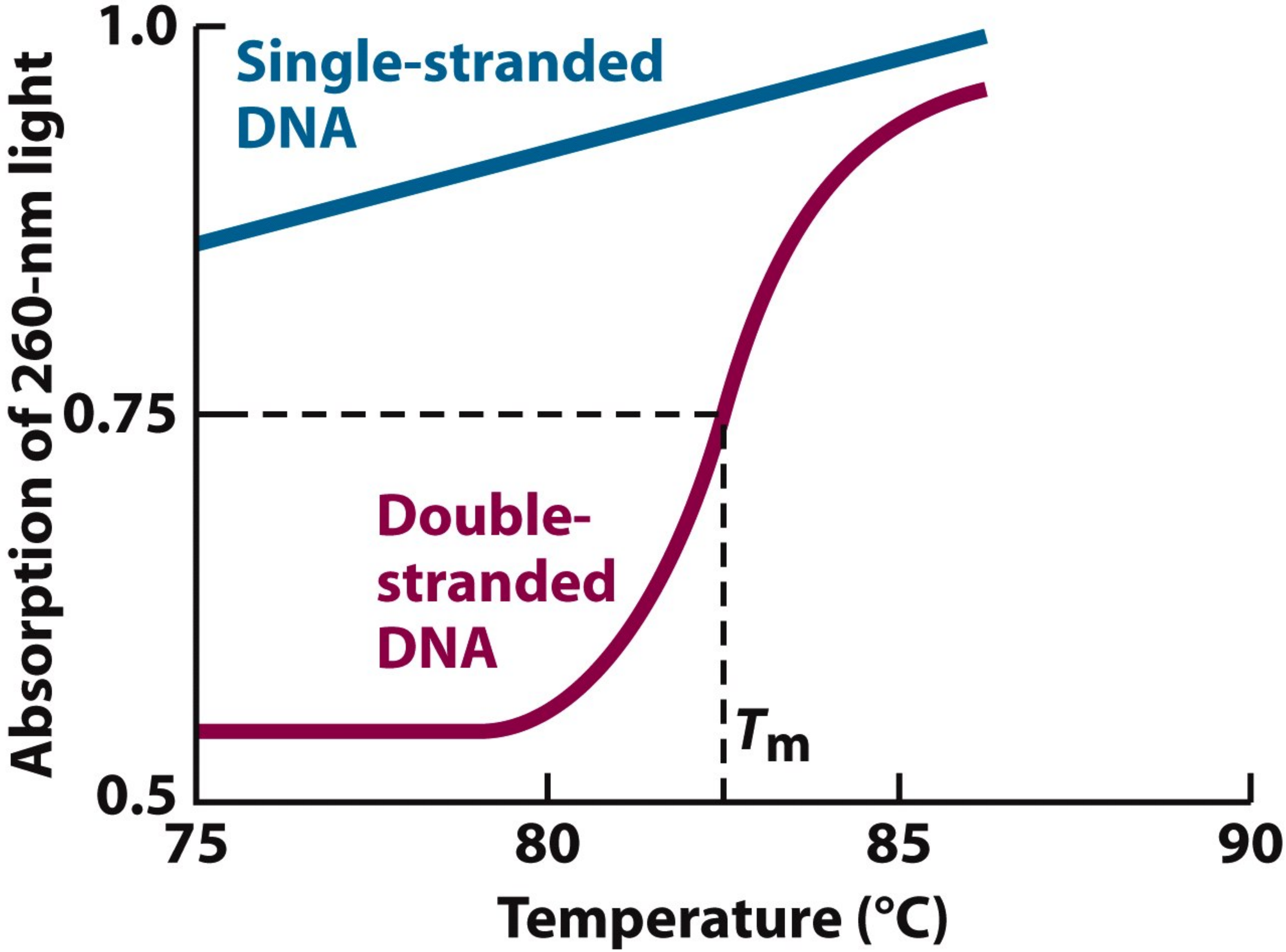
1953 DNA is a double helix (Watson, Crick, Franklin)

1958 DNA replicates semiconservatively (Meselson & Stahl)



DNA structure stability derives from the sum of a great number of weak interactions


Noncovalent interactions
Covalent bonds
Electrostatic
van der Waals
Hydrogen bonds
Thermal energy
Hydrolysis of ATP phosphoanhydride bond
C–C
C=C
0.24 × 10^0
0.24 × 10^1
0.24 × 10^2
0.24 × 10^3
kcal/mol



The two strands can be separated (denatured) with a small amount of energy

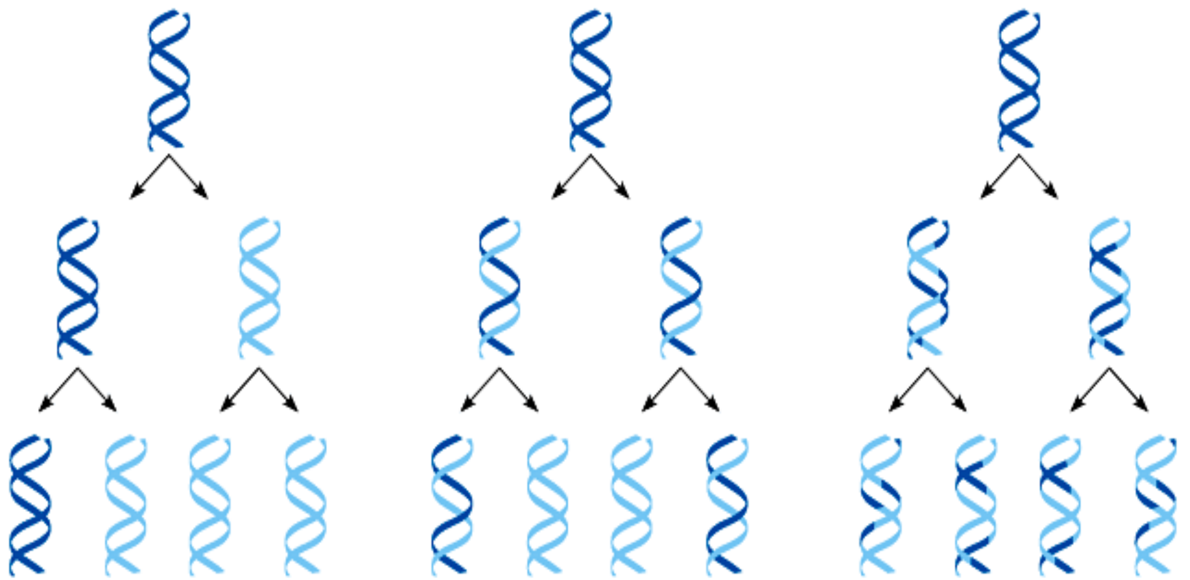
Models of DNA Replication

Conservative | Semi-conservative | Distributive

0

1

2



Parental strands stay together

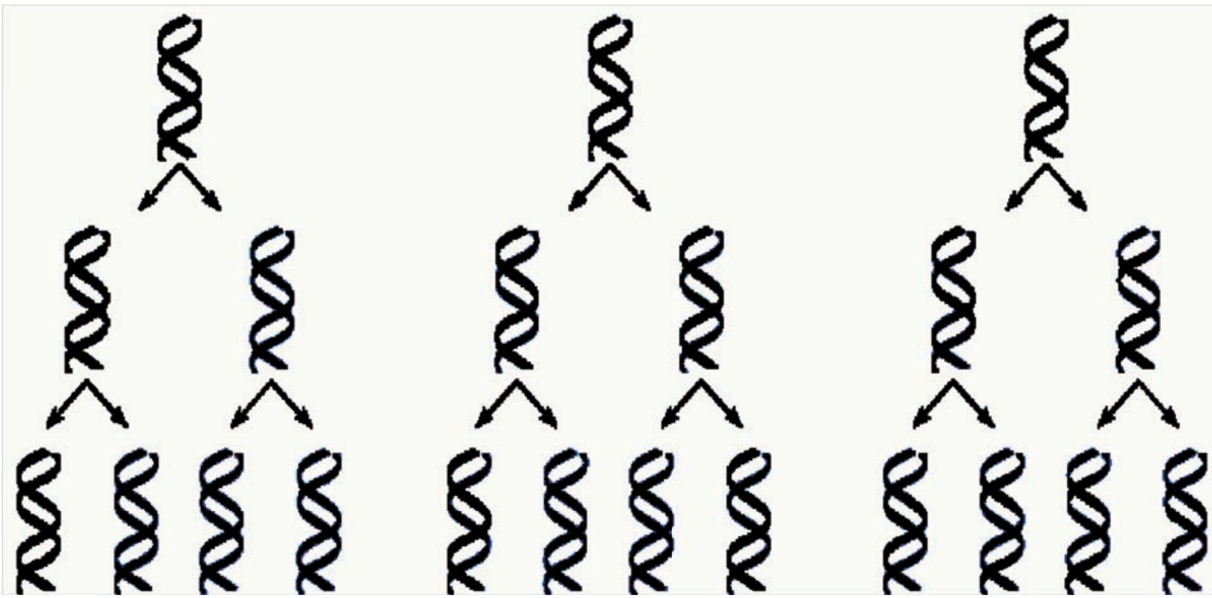
Parental strands separate every generation

Parental strands broken

Models of DNA Replication

Conservative | Semi-conservative | Distributive

0
1
2



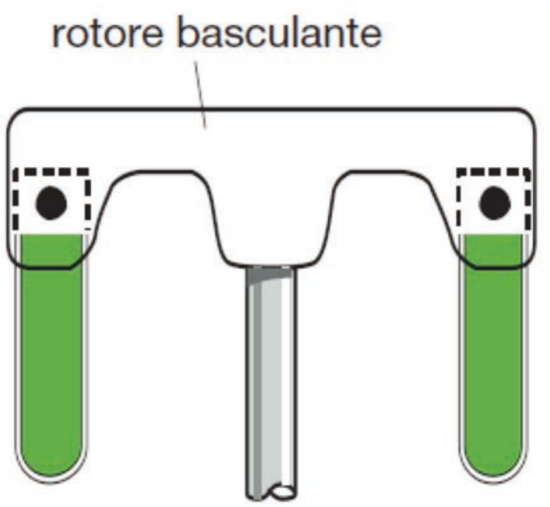
Parental strands stay together

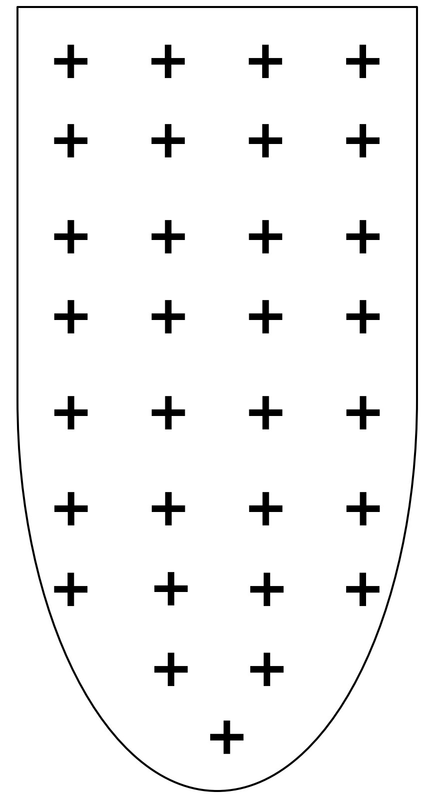
Parental strands separate every generation

Parental strands broken

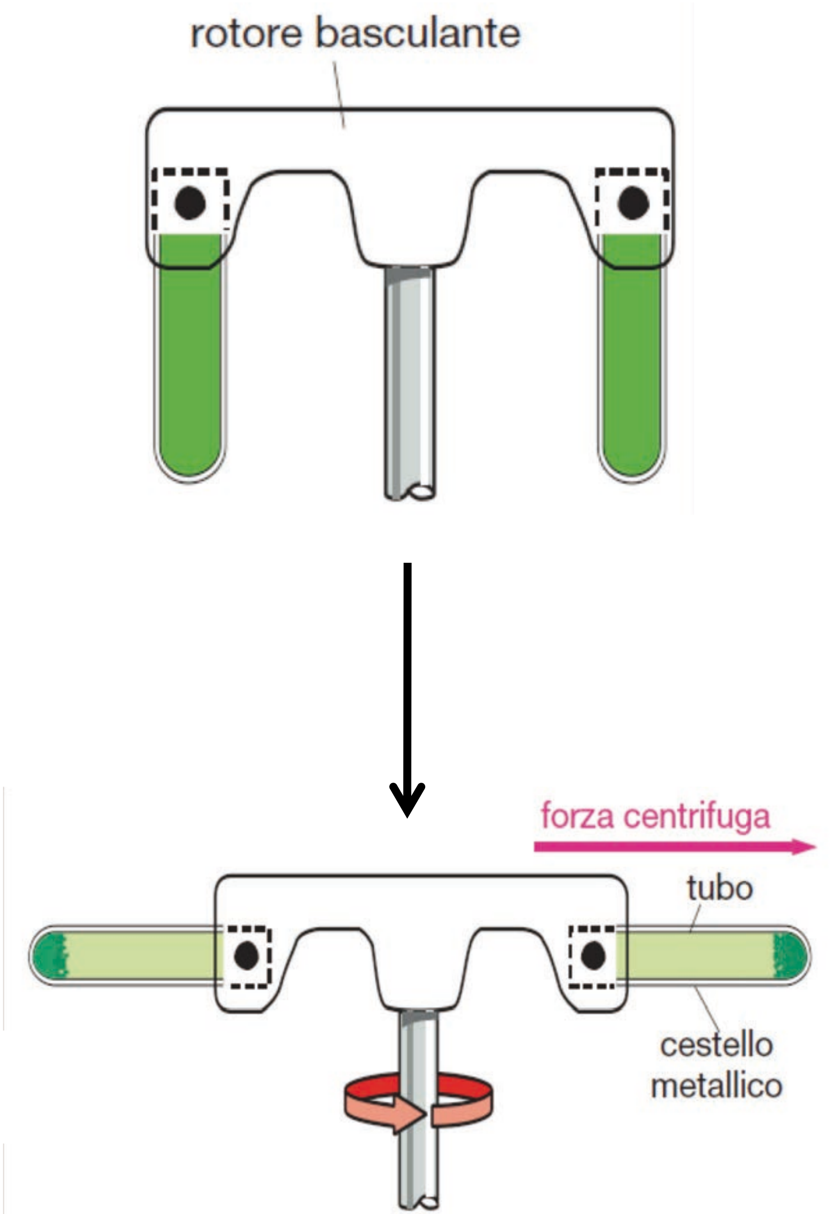
Meselson-Stahl experiment

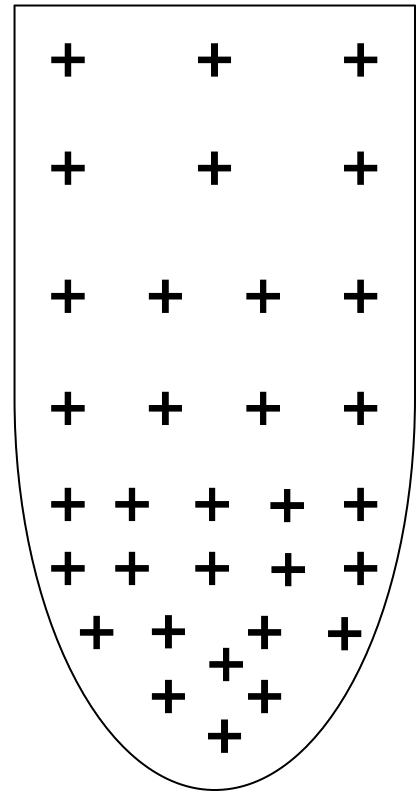
1. Grow E. coli on ^{15}N ("heavy") ammonia
2. Switch to ^{14}N (normal, "light") ammonia
3. Harvest aliquots as a function of time
4. Isolate DNA
5. Separate on the basis of DNA density using density gradient centrifugation
    - A. Pour $CsCl_2$ gradient into a tube
    - B. Layer DNA on top
    - C. Centrifuge until DNA stops moving (DNA floats when the density matches that of the salt solution)



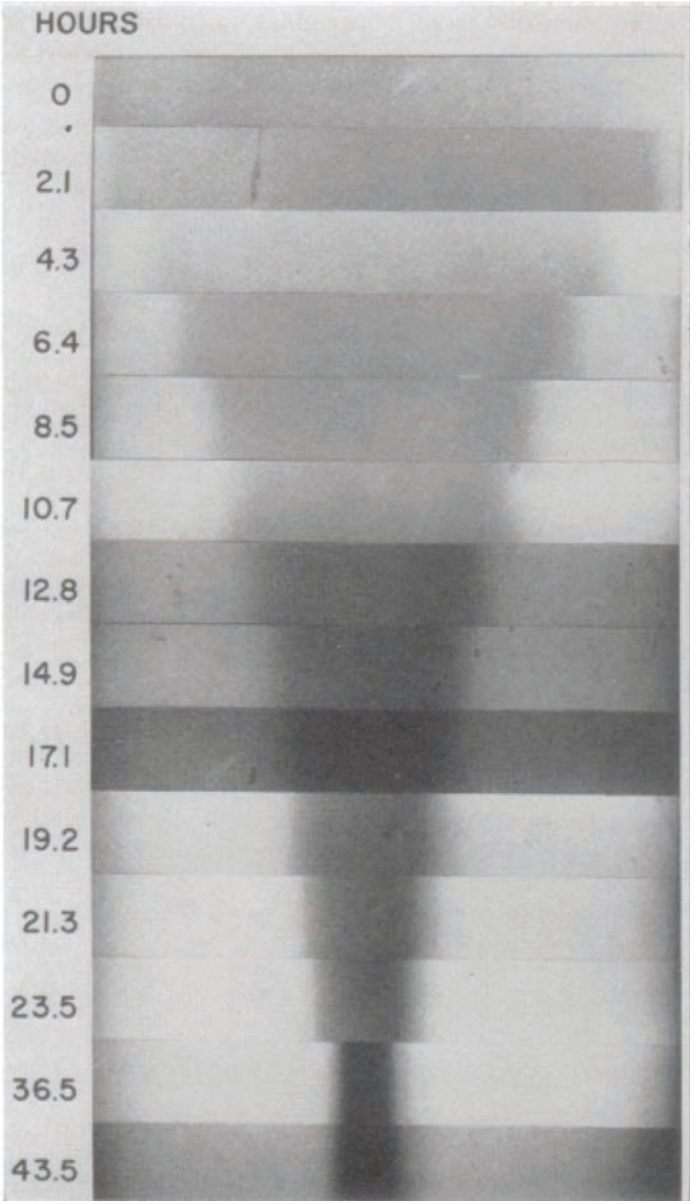


$^{137}Cs^{+}$


rotore basculante
forza centrifuga
tubo
cestello
metallico

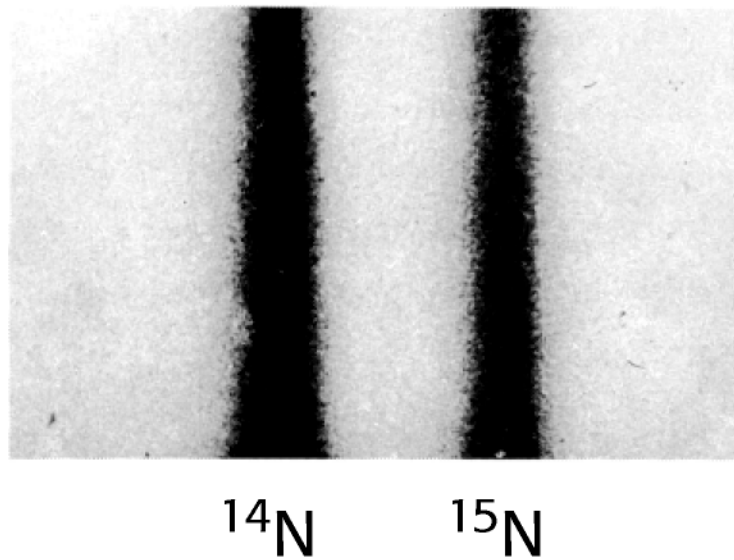


$^{137}Cs^{+}$



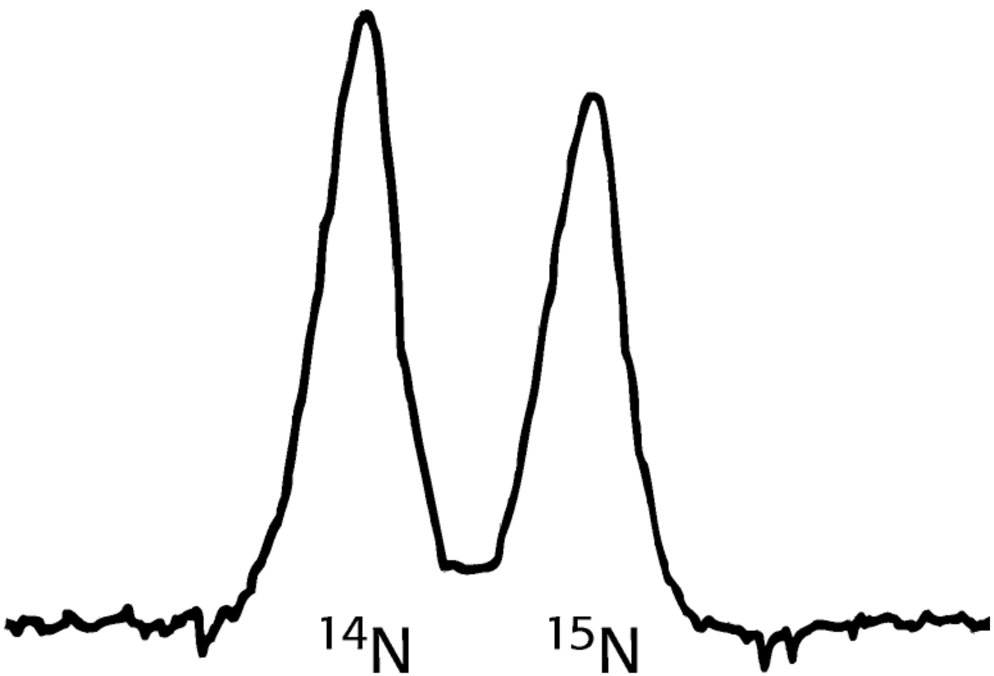
DNA molecules migrate in the gradient region according to their density

(A)

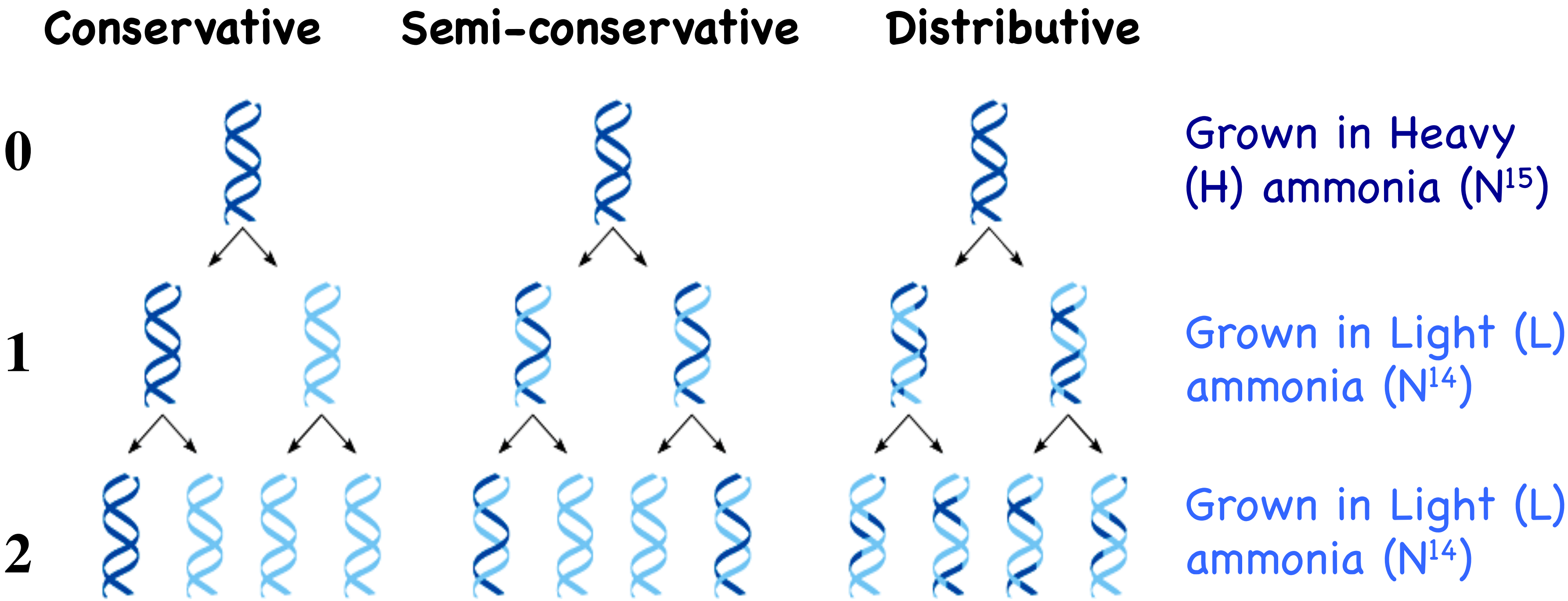


^{14}N DNA molecules can be distinguished from ^{15}N DNA molecules

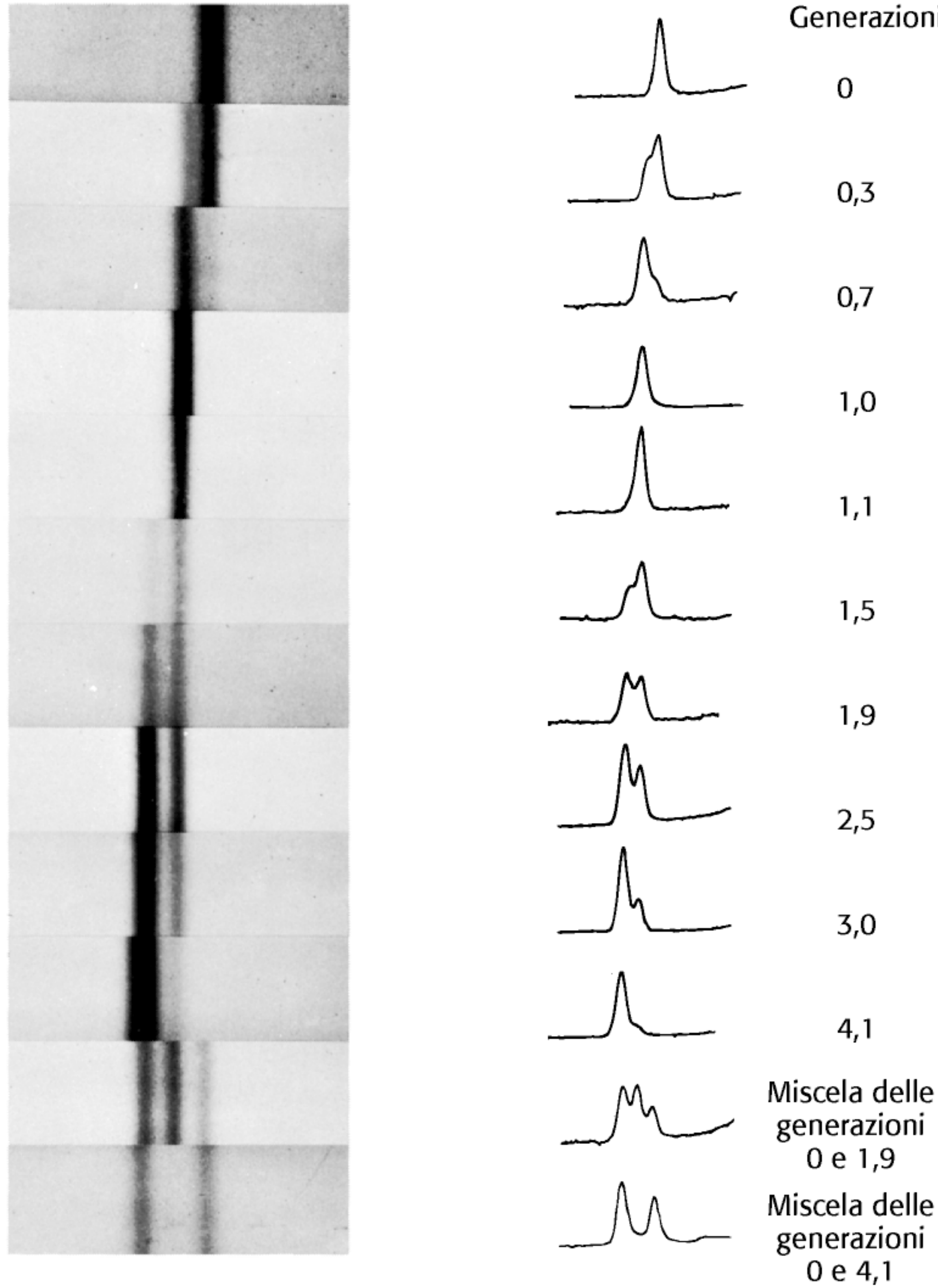
(B)



Meselson-Stahl experiment



Conservative	Semi-conservative	Distributive
Parental strands stay together -- HH maintained	Parental strands separate every generation -- no HH after 1 generation	Parental strands broken -- no LL in generation 2


Generazioni
0
0,3
0,7
1,0
1,1
1,5
1,9
2,5
3,0
4,1
Miscela delle generazioni 0 e 1,9
Miscela delle generazioni 0 e 4,1

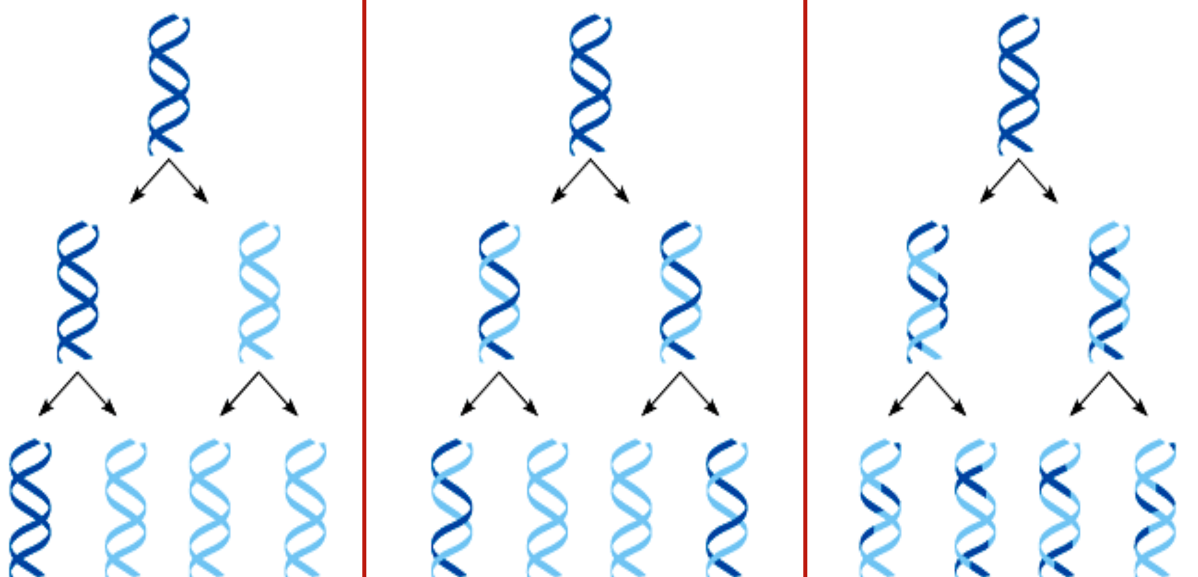
DNA replication is semi-conservative

Conservative | **Semi-conservative** | **Distributive**

0

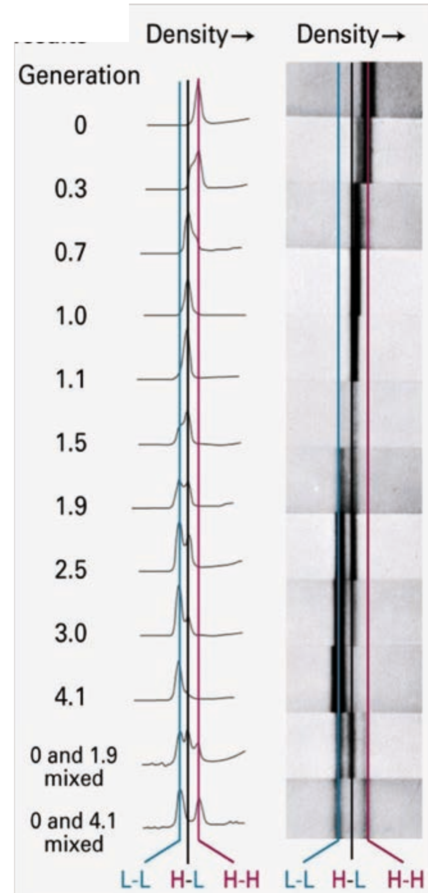
1

2



Conservative	Semi-conservative	Distributive
Parental strands stay together -- HH maintained	Parental strands separate every generation -- no HH after 1 generation	Parental strands broken -- no LL in generation 2

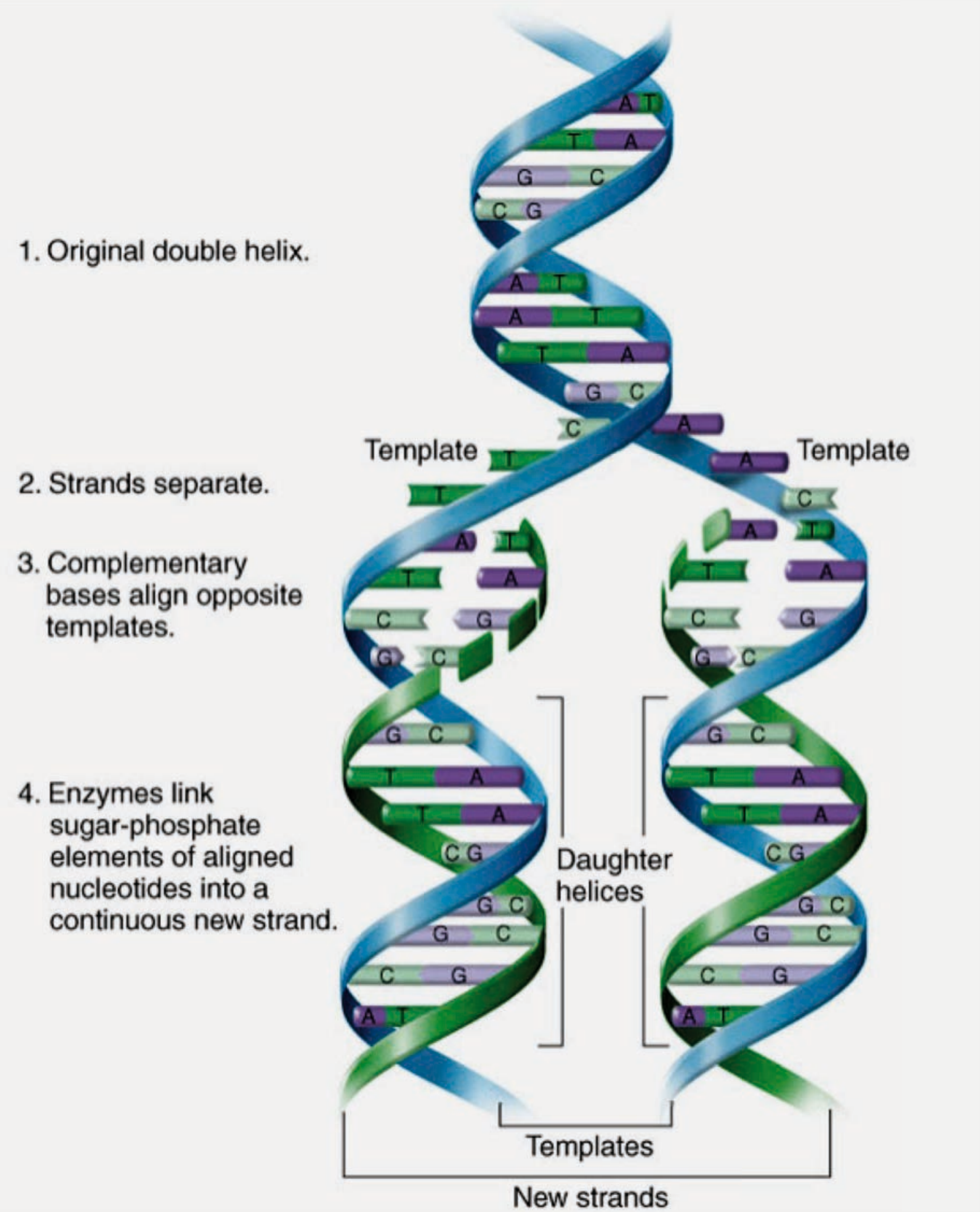
Results

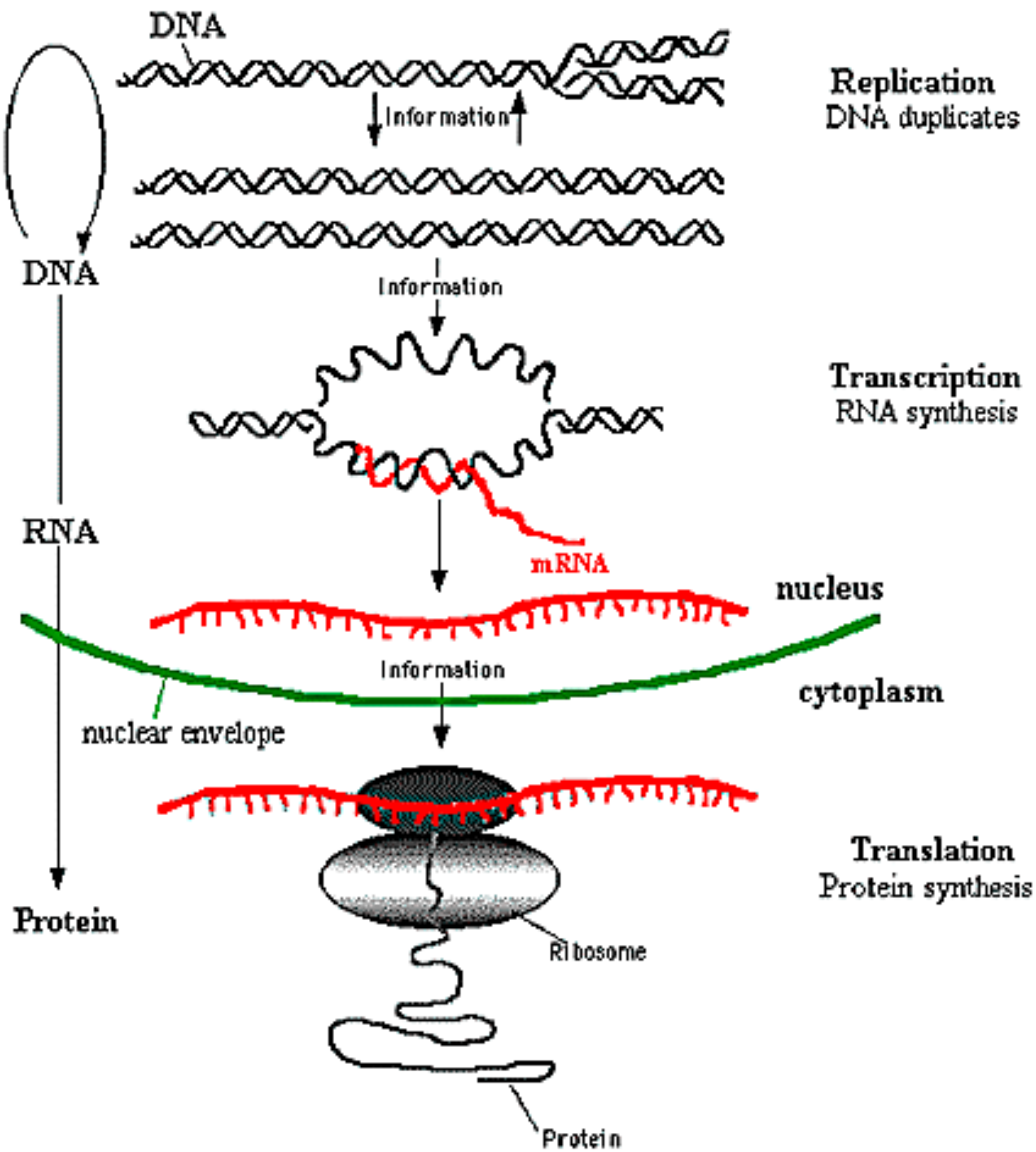


Graph Picture

Semi-conservative replication

One strand of duplex passed on unchanged to each of the daughter cells. This 'conserved' strand acts as a template for the synthesis of a new, complementary strand by the enzyme DNA polymerase





In **1956** *Francis Crick* described the scheme through which genetic information flows and named it ***The Central Dogma***

Vocabulary

- Replication -- copying DNA before cell division
- Transcription -- making an RNA copy (messenger RNA or mRNA) of DNA
- Translation -- making a protein from the mRNA

DNA --replication--> DNA

DNA --transcription--> RNA --translation--> Protein

RNA --reverse transcription--> DNA

Polymerase enzymes

DNA --replication--------> DNA

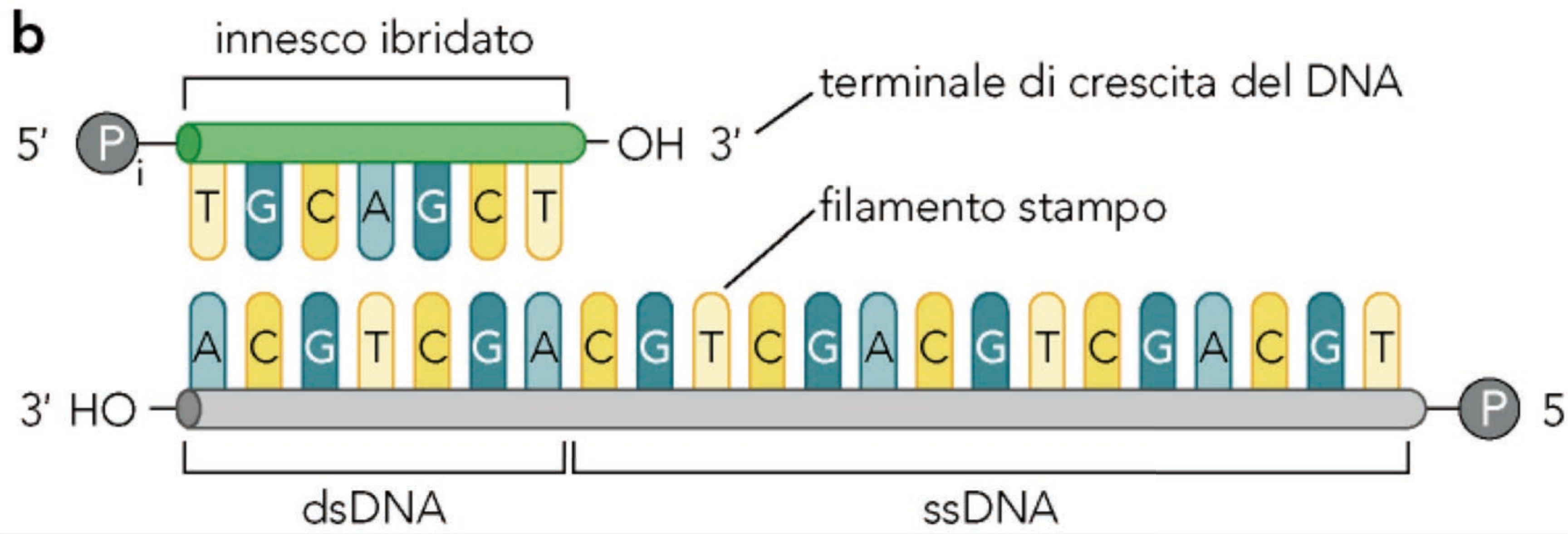
(DNA polymerase plus other proteins)

DNA --transcription--------> RNA

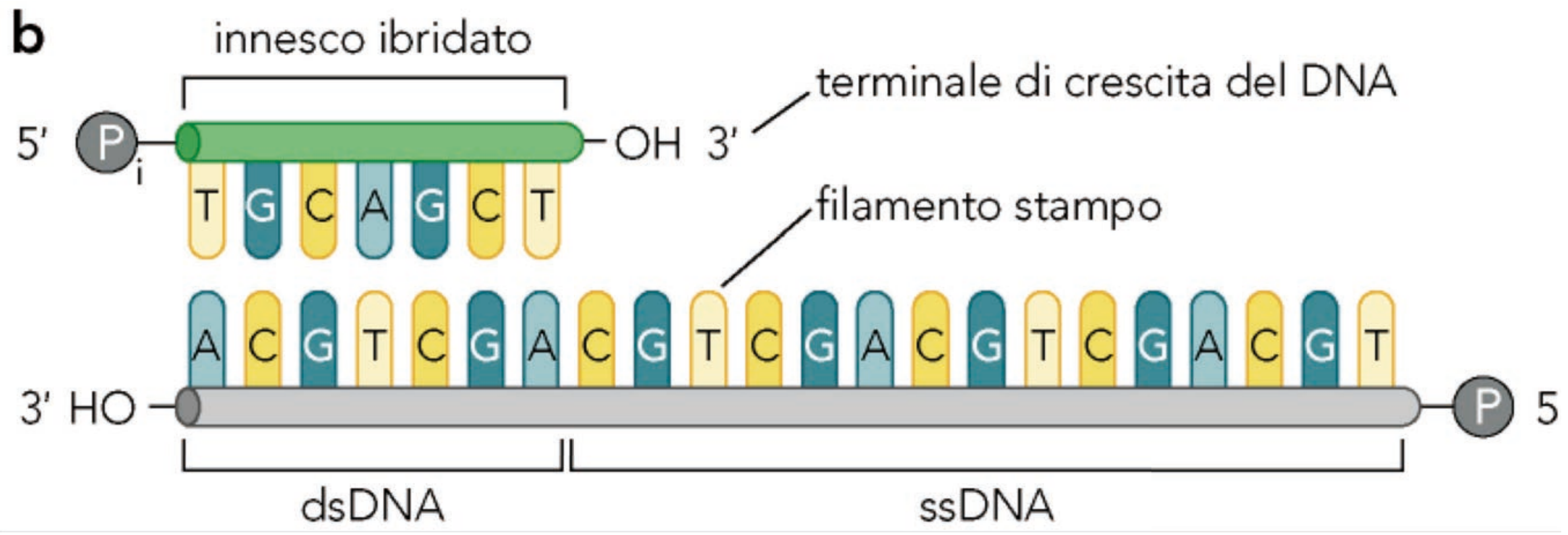
(RNA polymerase plus other proteins)

RNA --reverse transcription--------> DNA

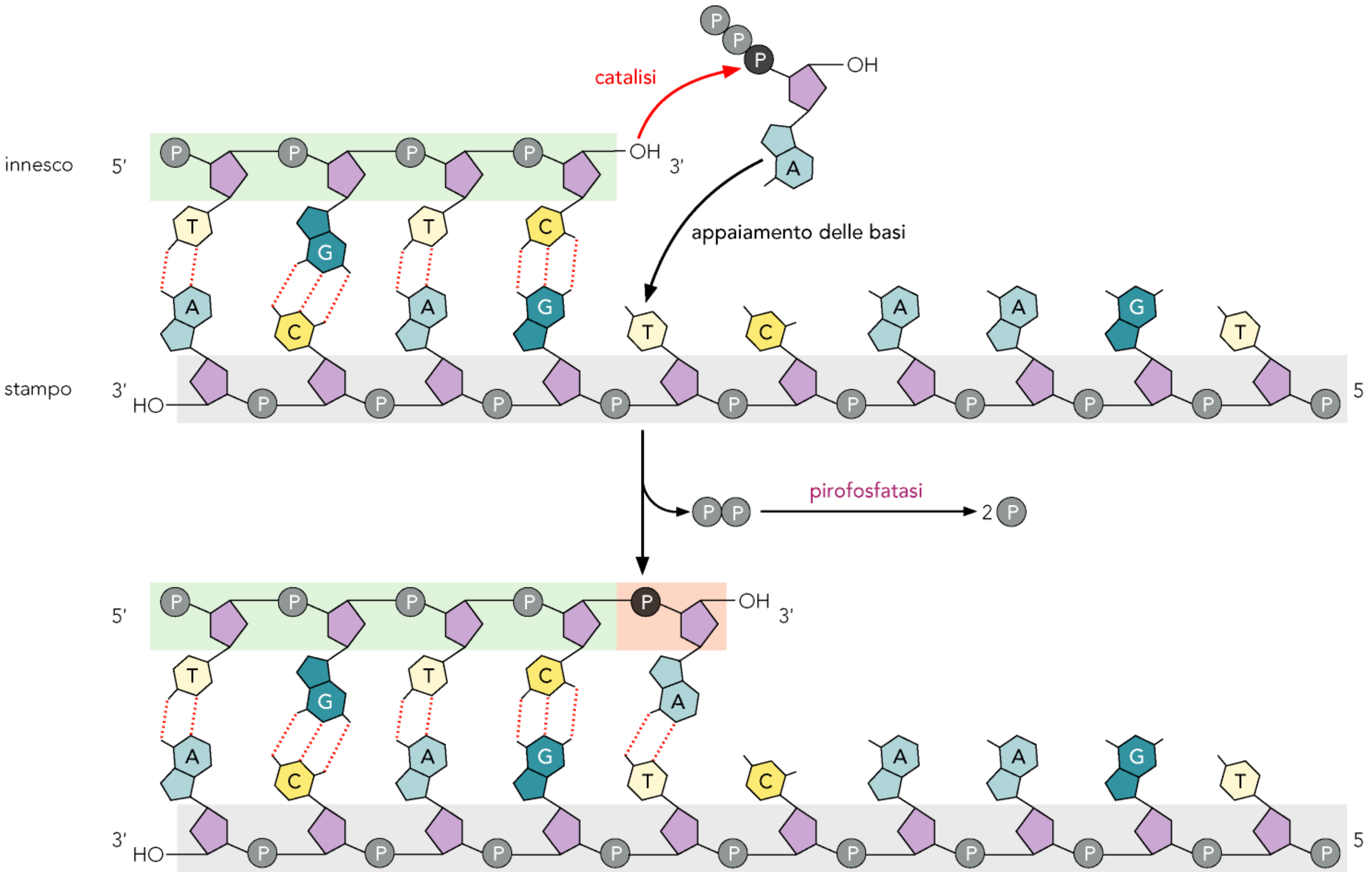
(HIV reverse transcriptase, telomerase)



DNA polymerase synthesizes a new DNA strand from a template.



- DNA polymerases add nucleotides to a ***free 3'-OH***.
- Synthesis proceeds always in ***5'→3'direction***



Precursors are nucleotide-triphosphates

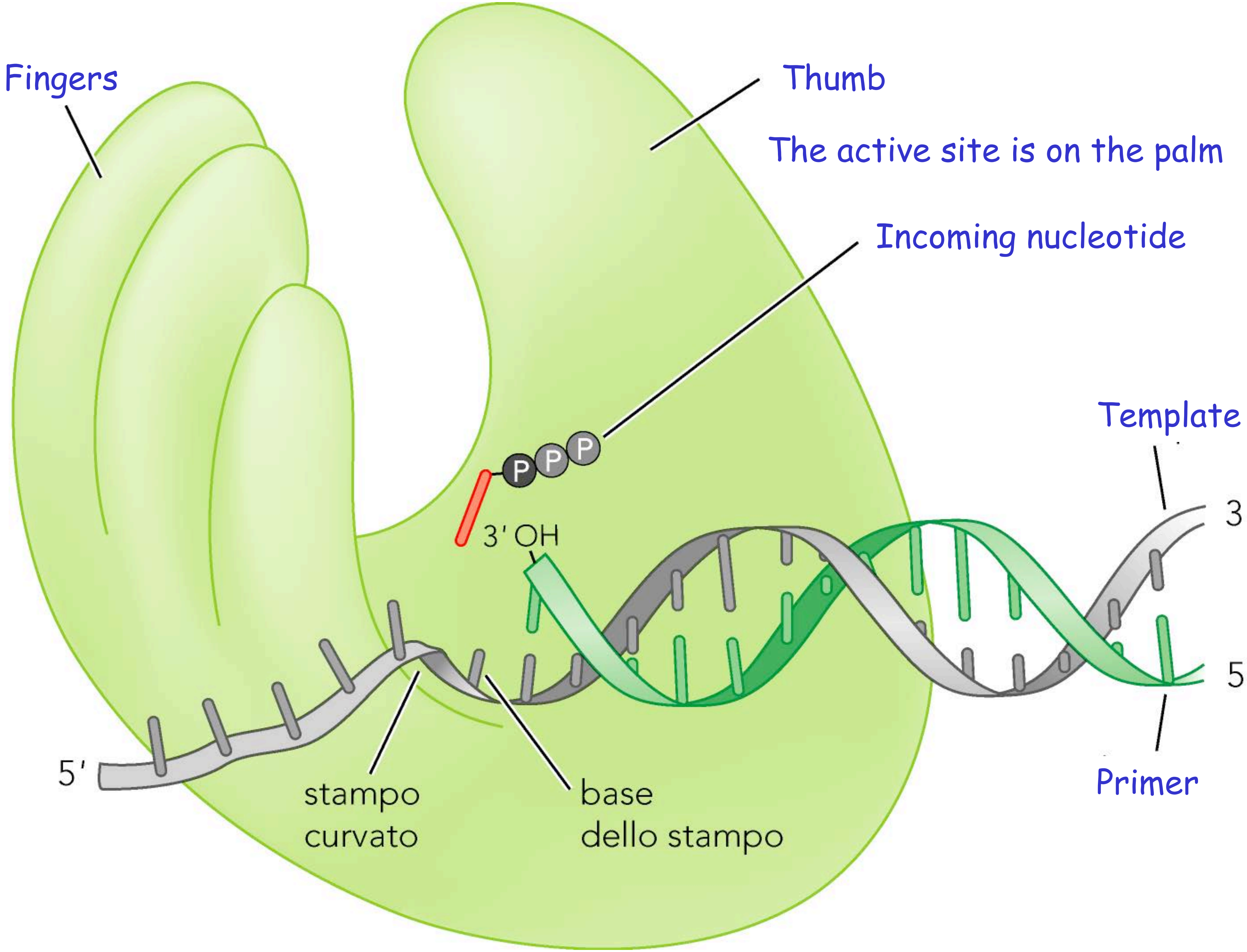
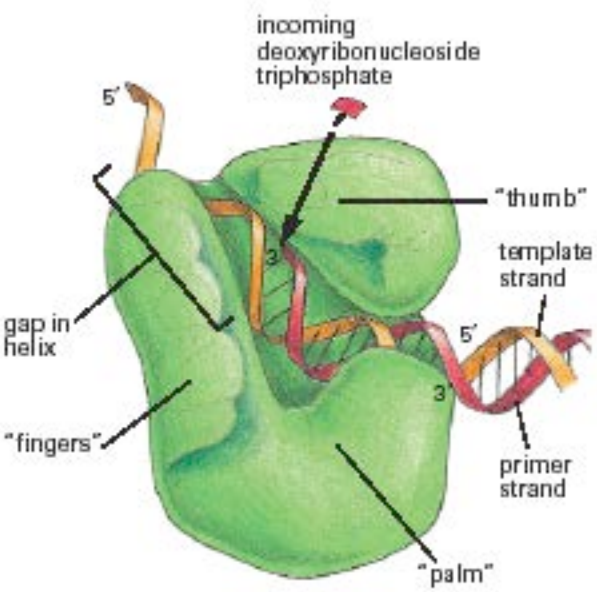

Fingers
Thumb
The active site is on the palm
Incoming nucleotide
P P P
3' OH
Template
3'
5'
Primer
5'
stampo
curvato
base
dello stampo

TABLE 5–1 The Three Steps That Give Rise To High-fidelity DNA Synthesis

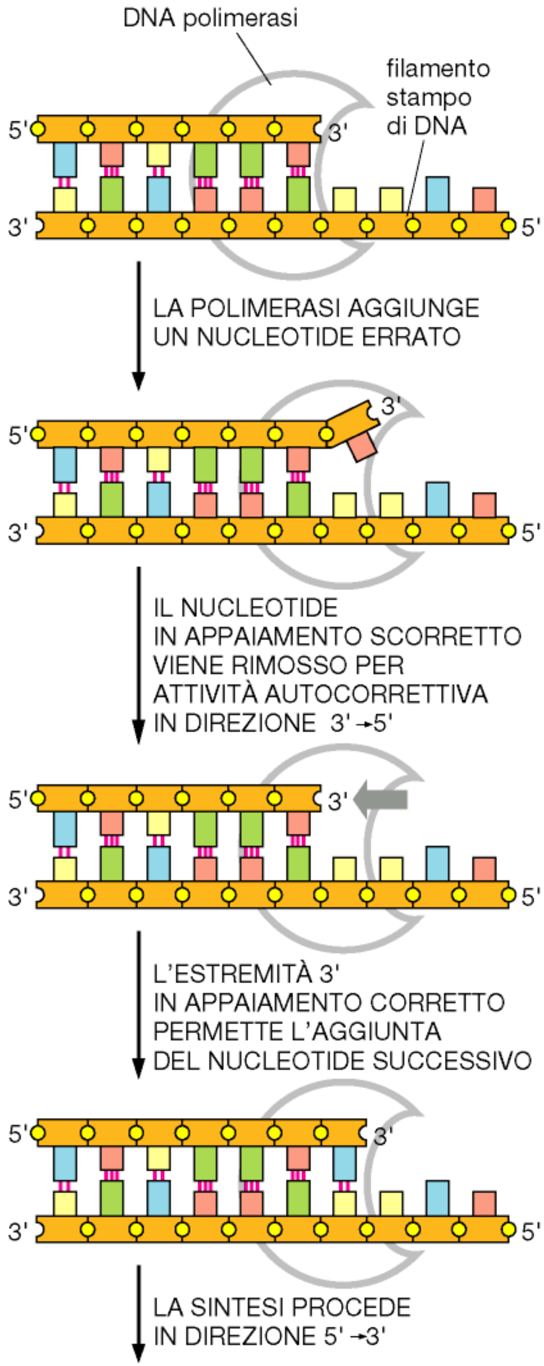
REPLICATION STEP	ERRORS PER NUCLEOTIDE POLYMERIZED
5′→3′ polymerization	1×10^5
3′→5′ exonucleolytic proofreading	1×10^2
Strand-directed mismatch repair	1×10^2
Total	1×10^9

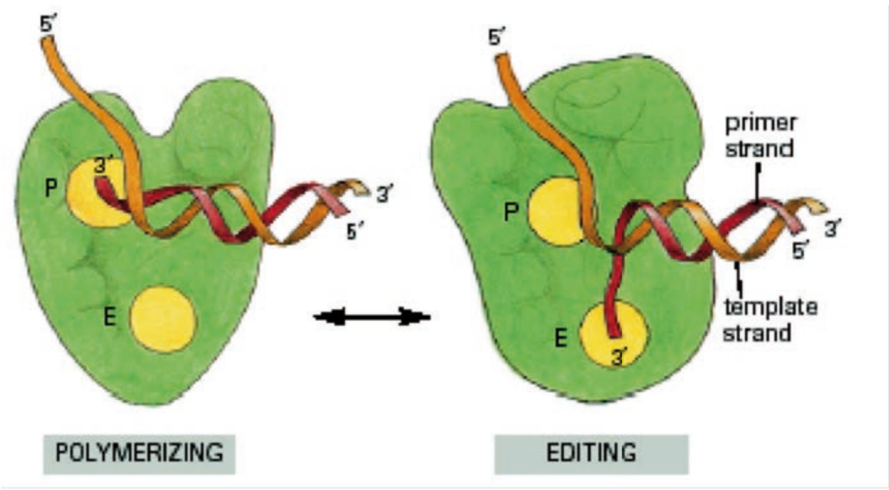
The third step, strand-directed mismatch repair, is described later in this chapter.



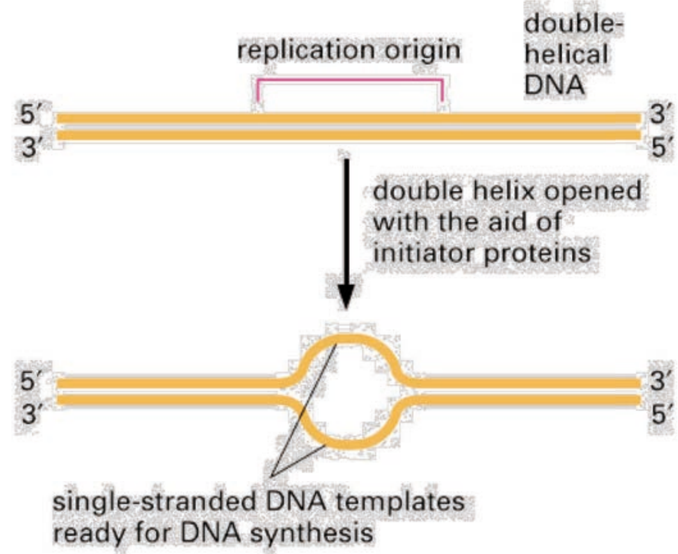
There are several proofreading steps to assure replication accuracy

Several DNA polymerases have a 3'-5'exonuclease activity that allows eliminating the last nucleotide if incorrectly incorporated





DNA replication



- Begins at an A-T rich replication origin.
- Initiator proteins bind and separate the two DNA strands.
- A protein machine containing DNA polymerase is assembled.
- DNA polymerase synthesizes new DNA using one old strand as a template.
- Replication forks move bidirectionally from origins of replication.

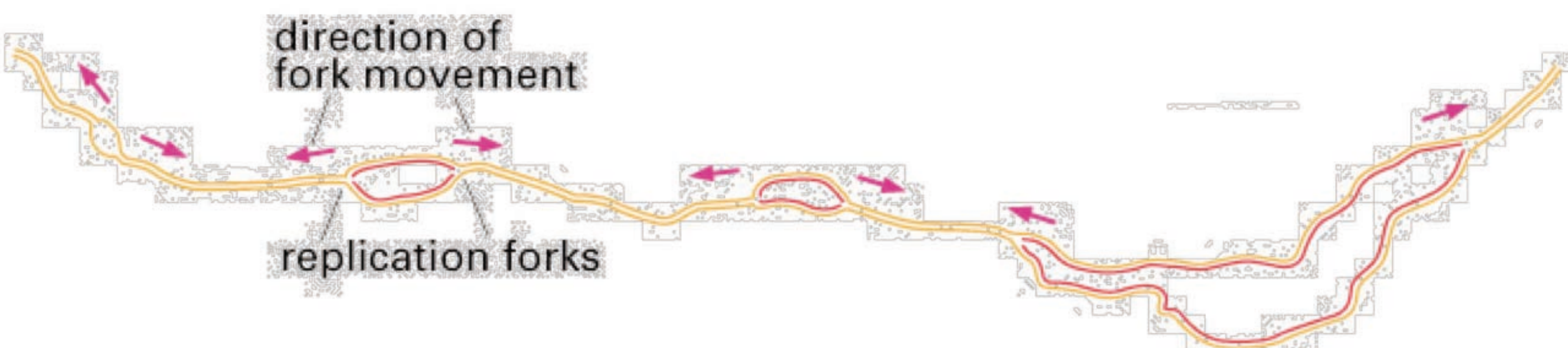
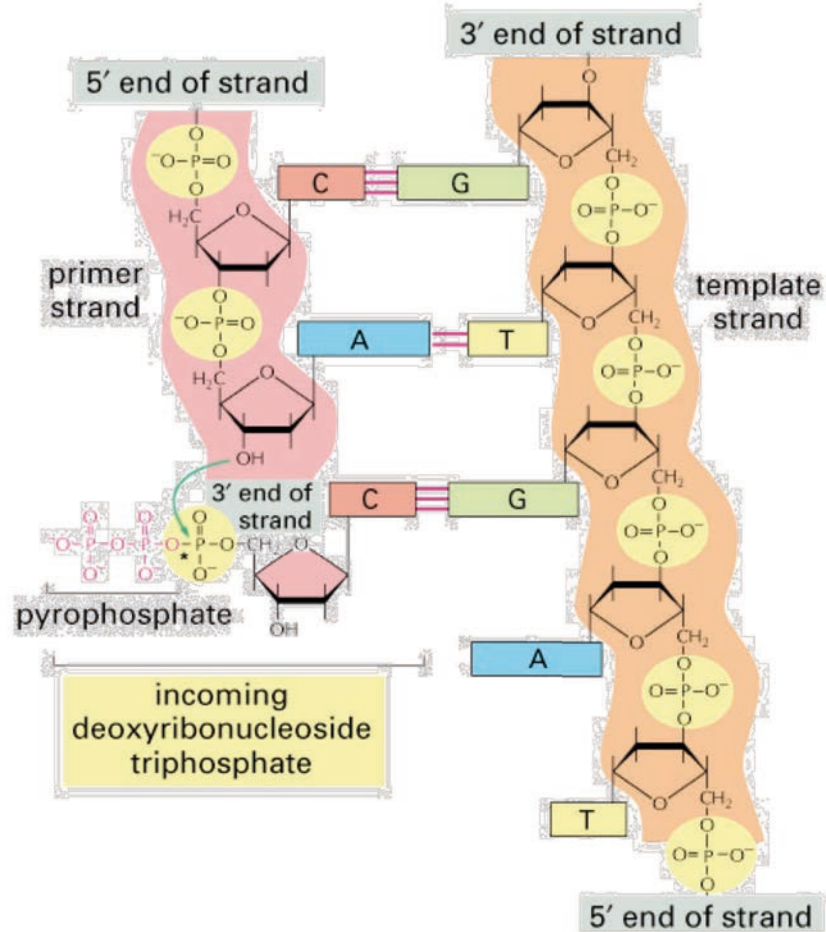
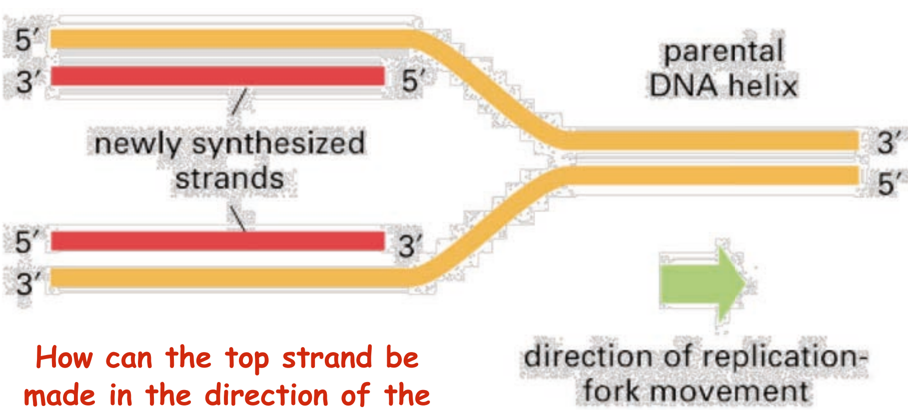


Figure 6-9 Essential Cell Biology, 2/e. (© 2004 Garland Science)

DNA always synthesized in 5' to 3' direction

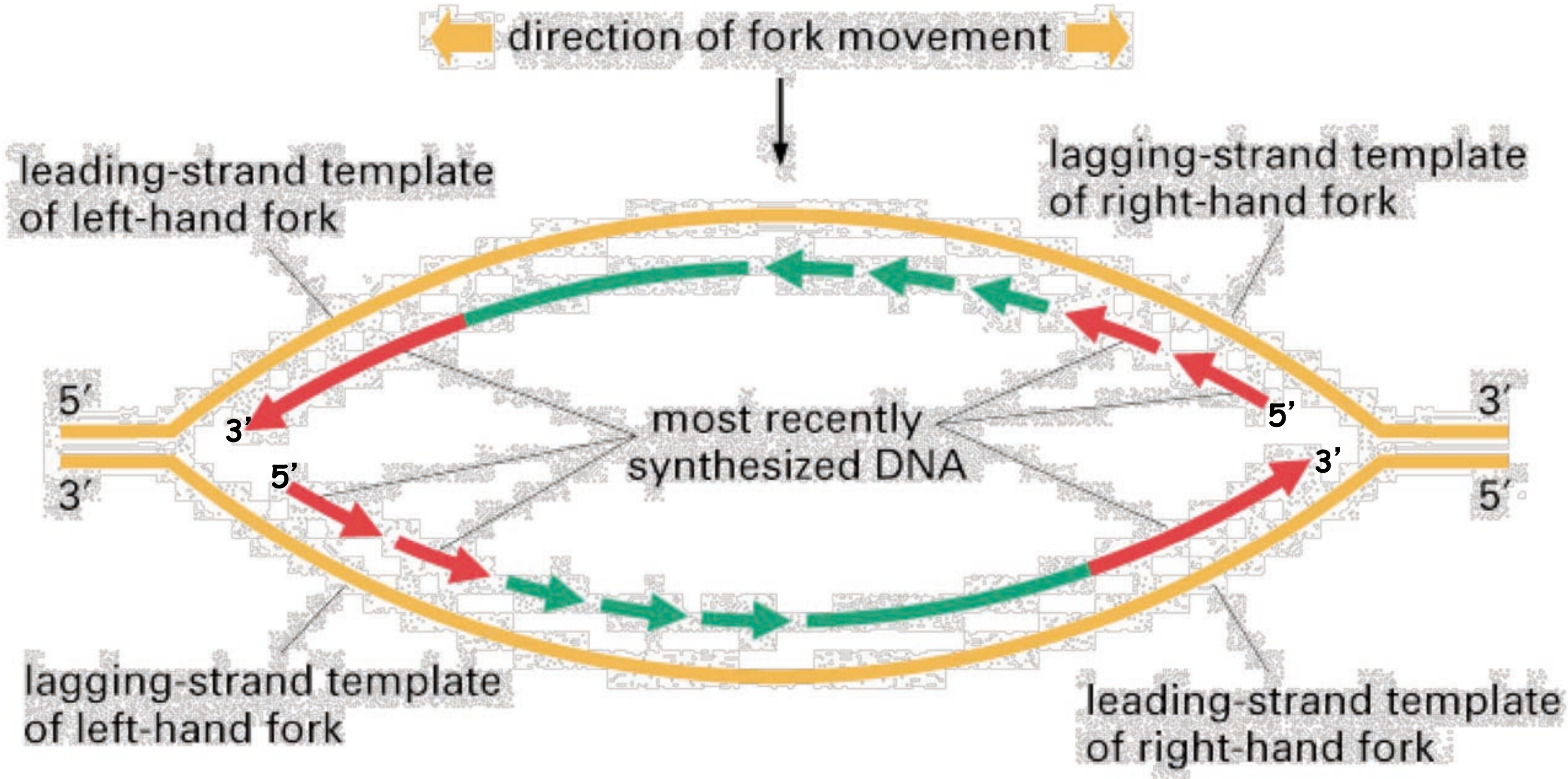
- A new deoxyribonucleotide is always added to the 3' OH end of the new strand.
- One new DNA strand at the replication fork is made on a template that runs 3' to 5'; the other strand is made on a template that runs 5' to 3'.





How can the top strand be made in the direction of the replication fork?

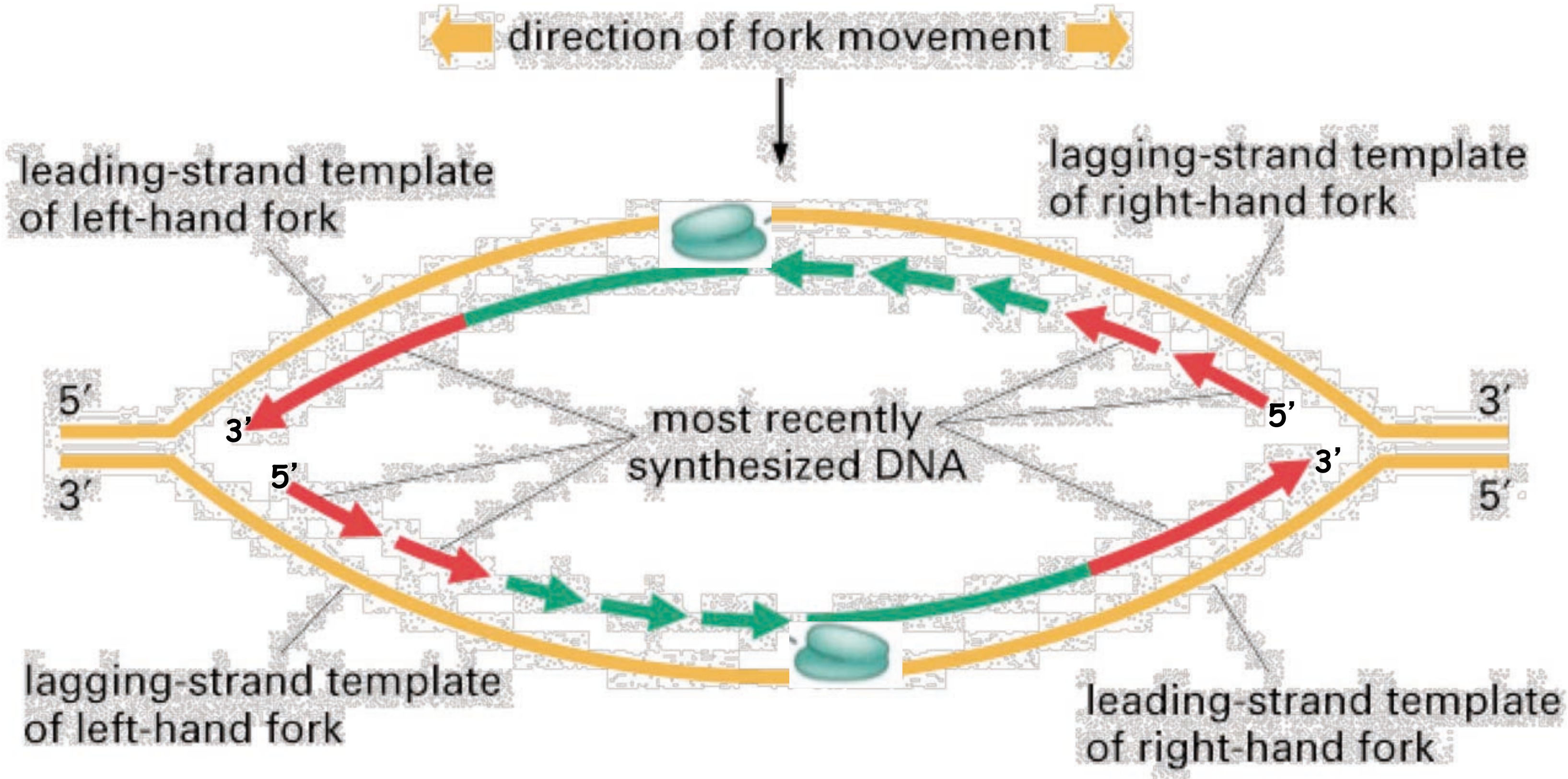
One DNA strand is synthesized continuously; the other in synthesized discontinuously and then stitched together



At the replication origin, four DNA Polymerase complexes act simultaneously

Figure 6-12 Essential Cell Biology, 2/e. (© 2004 Garland Science)

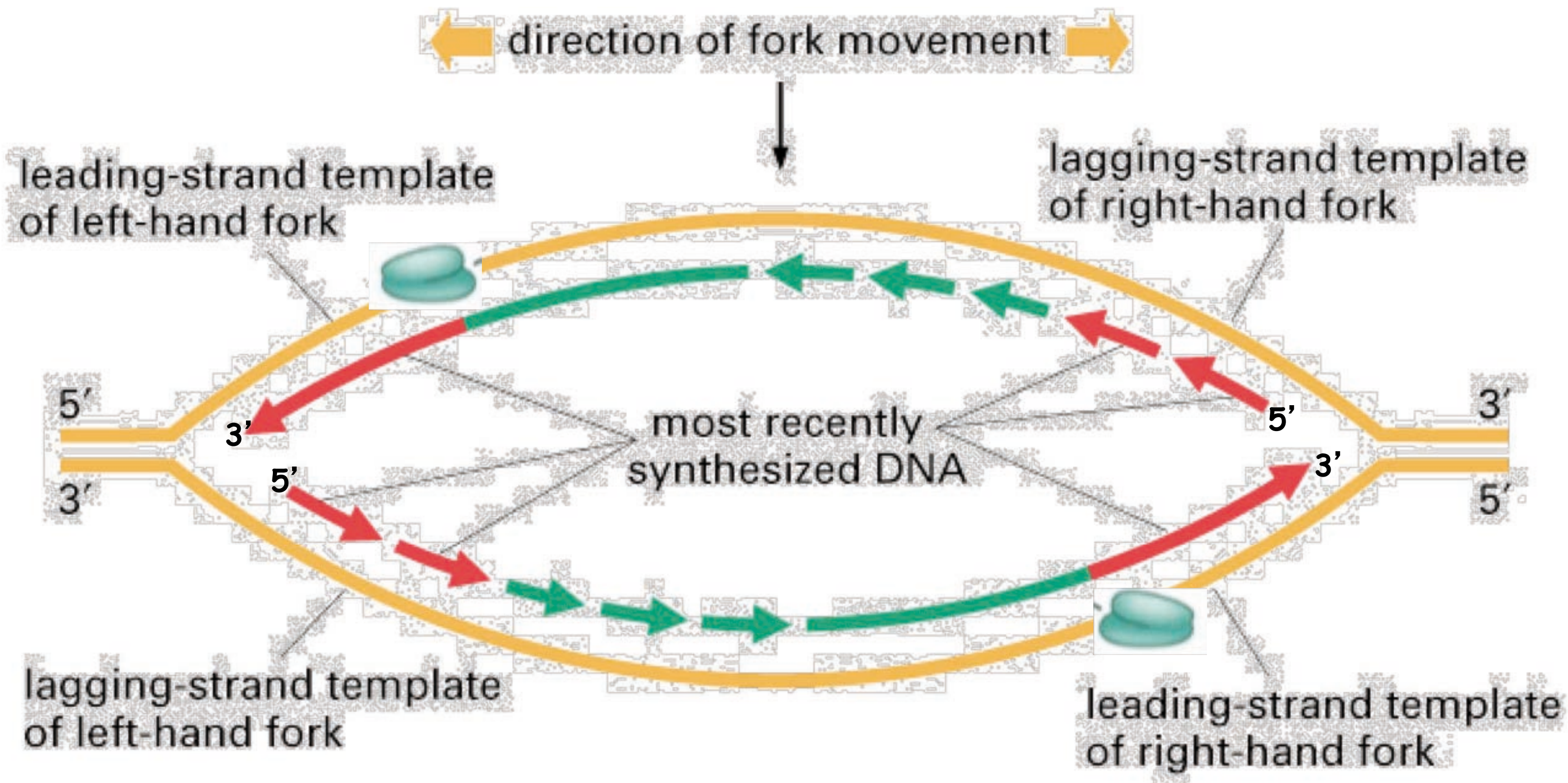
One DNA strand is synthesized continuously; the other in synthesized discontinuously and then stitched together



Leading strand: DNA strand that is synthesized continuously in the direction of replication fork.

Figure 6-12 Essential Cell Biology, 2/e. (© 2004 Garland Science)

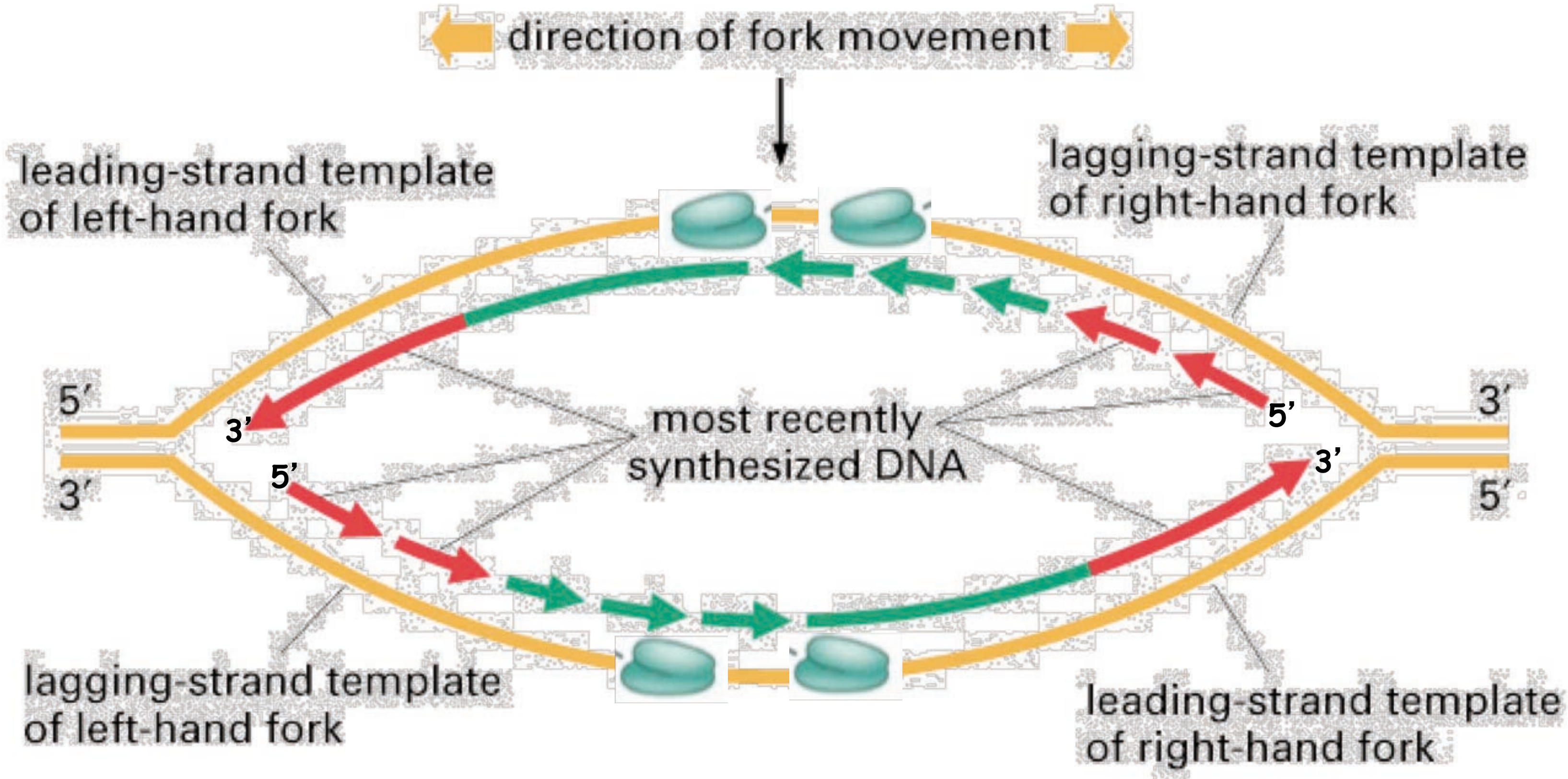
One DNA strand is synthesized continuously; the other in synthesized discontinuously and then stitched together



Leading strand: DNA strand that is synthesized continuously in the direction of replication fork.

Figure 6-12 Essential Cell Biology, 2/e. (© 2004 Garland Science)

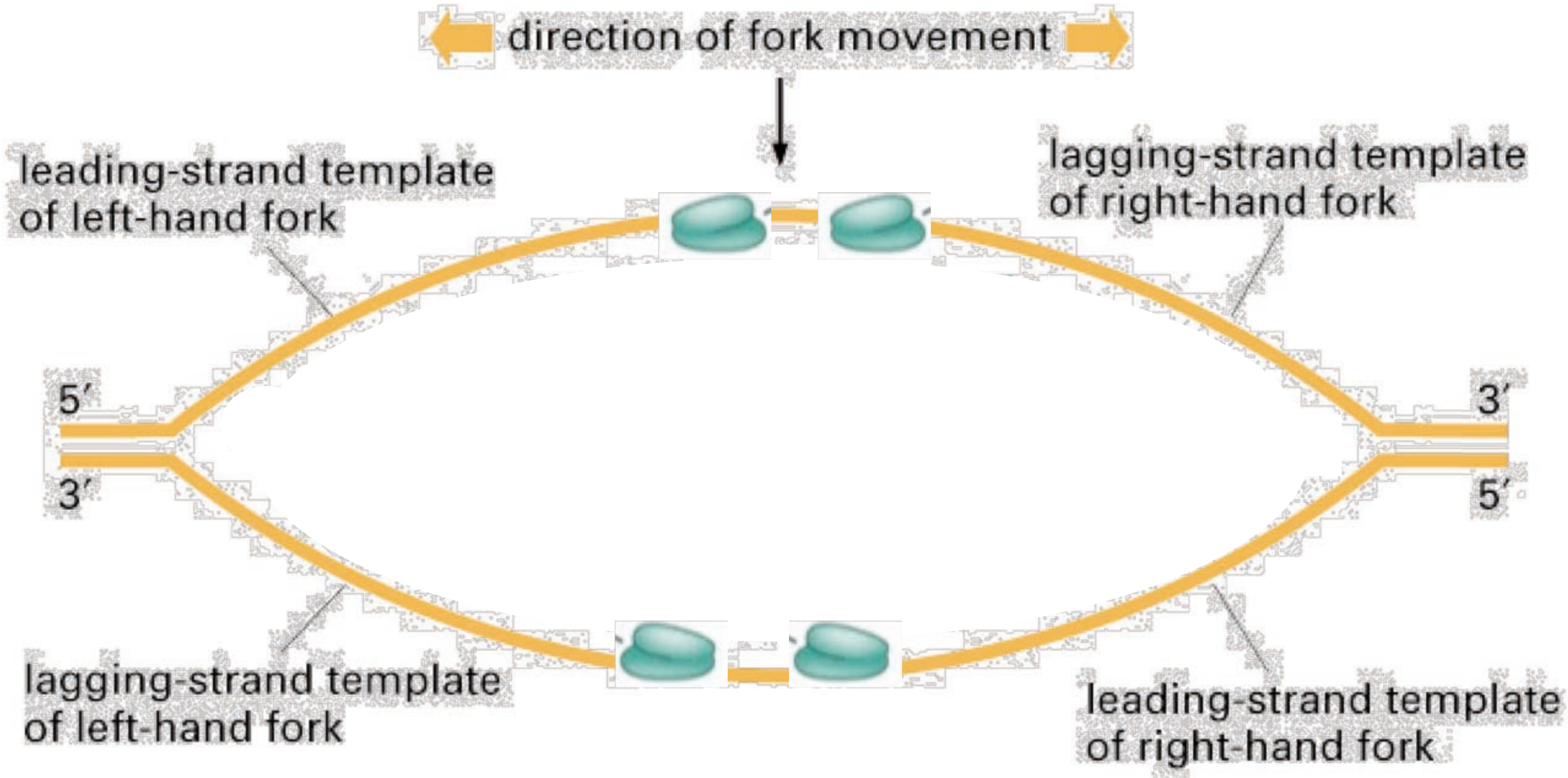
One DNA strand is synthesized continuously; the other in synthesized discontinuously and then stitched together



Leading strand: DNA strand that is synthesized continuously in the direction of replication fork.
Lagging strand: DNA strand that is synthesized discontinuously (Okazaki fragments), then stitched together.

Figure 6-12 Essential Cell Biology, 2/e. (© 2004 Garland Science)

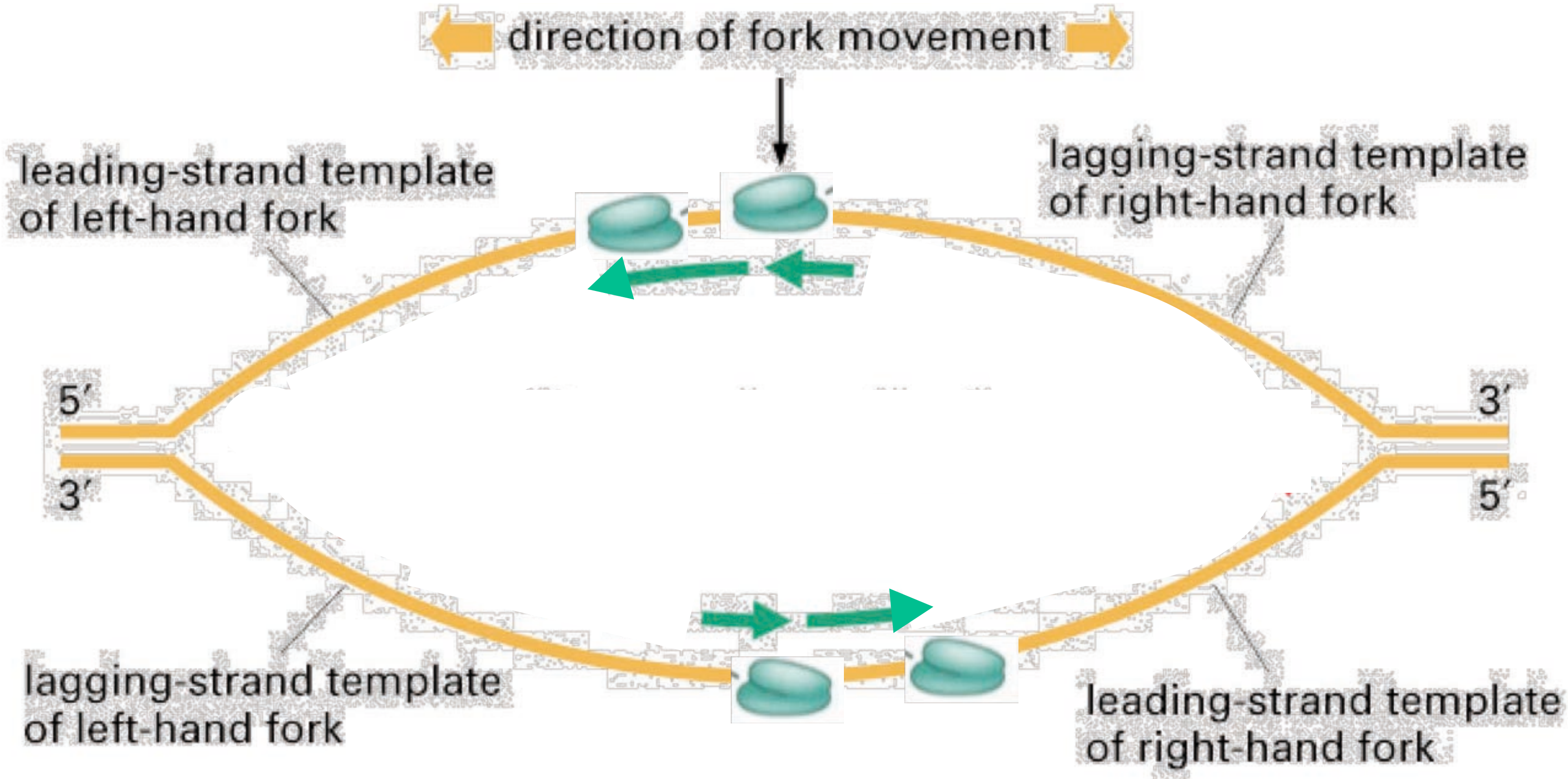
One DNA strand is synthesized continuously; the other in synthesized discontinuously and then stitched together



Leading strand: DNA strand that is synthesized continuously in the direction of replication fork.
Lagging strand: DNA strand that is synthesized discontinuously (Okazaki fragments), then stitched together.

Figure 6-12 Essential Cell Biology, 2/e. (© 2004 Garland Science)

One DNA strand is synthesized continuously; the other in synthesized discontinuously and then stitched together

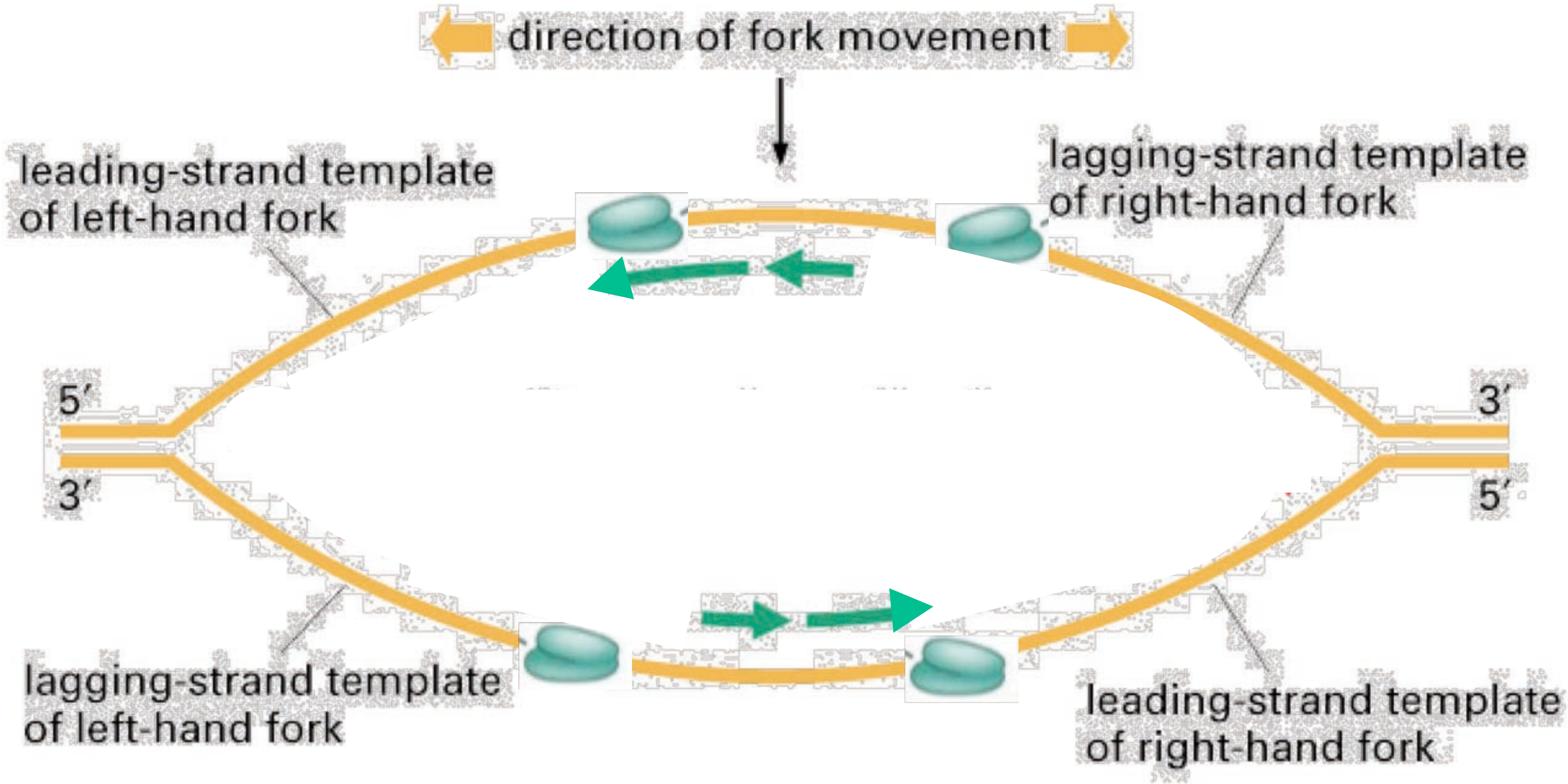


Leading strand: DNA strand that is synthesized continuously in the direction of replication fork.

Lagging strand: DNA strand that is synthesized discontinuously (Okazaki fragments), then stitched together.

Figure 6-12 Essential Cell Biology, 2/e. (© 2004 Garland Science)

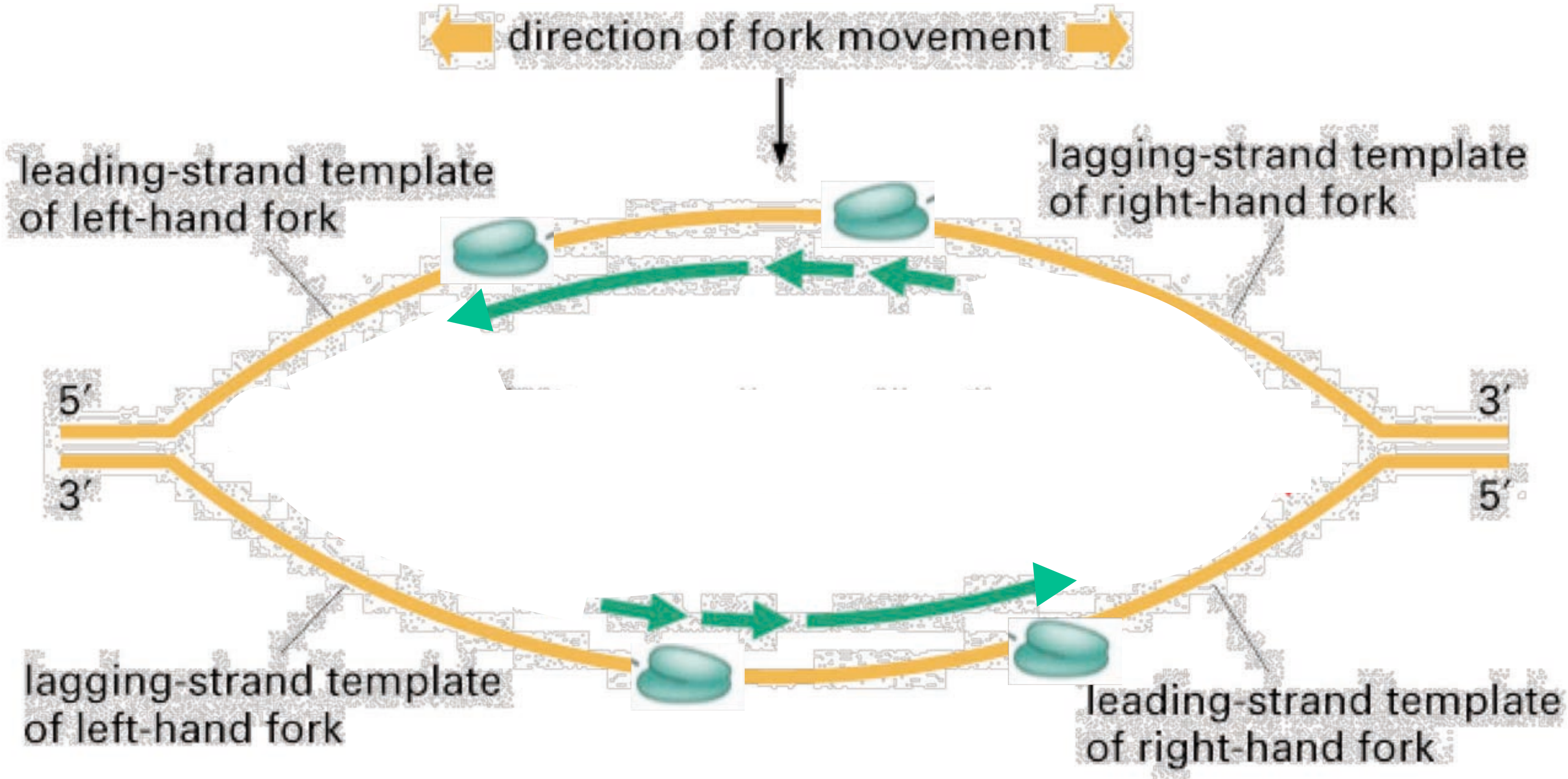
One DNA strand is synthesized continuously; the other in synthesized discontinuously and then stitched together



Leading strand: DNA strand that is synthesized continuously in the direction of replication fork.
Lagging strand: DNA strand that is synthesized discontinuously (Okazaki fragments), then stitched together.

Figure 6-12 Essential Cell Biology, 2/e. (© 2004 Garland Science)

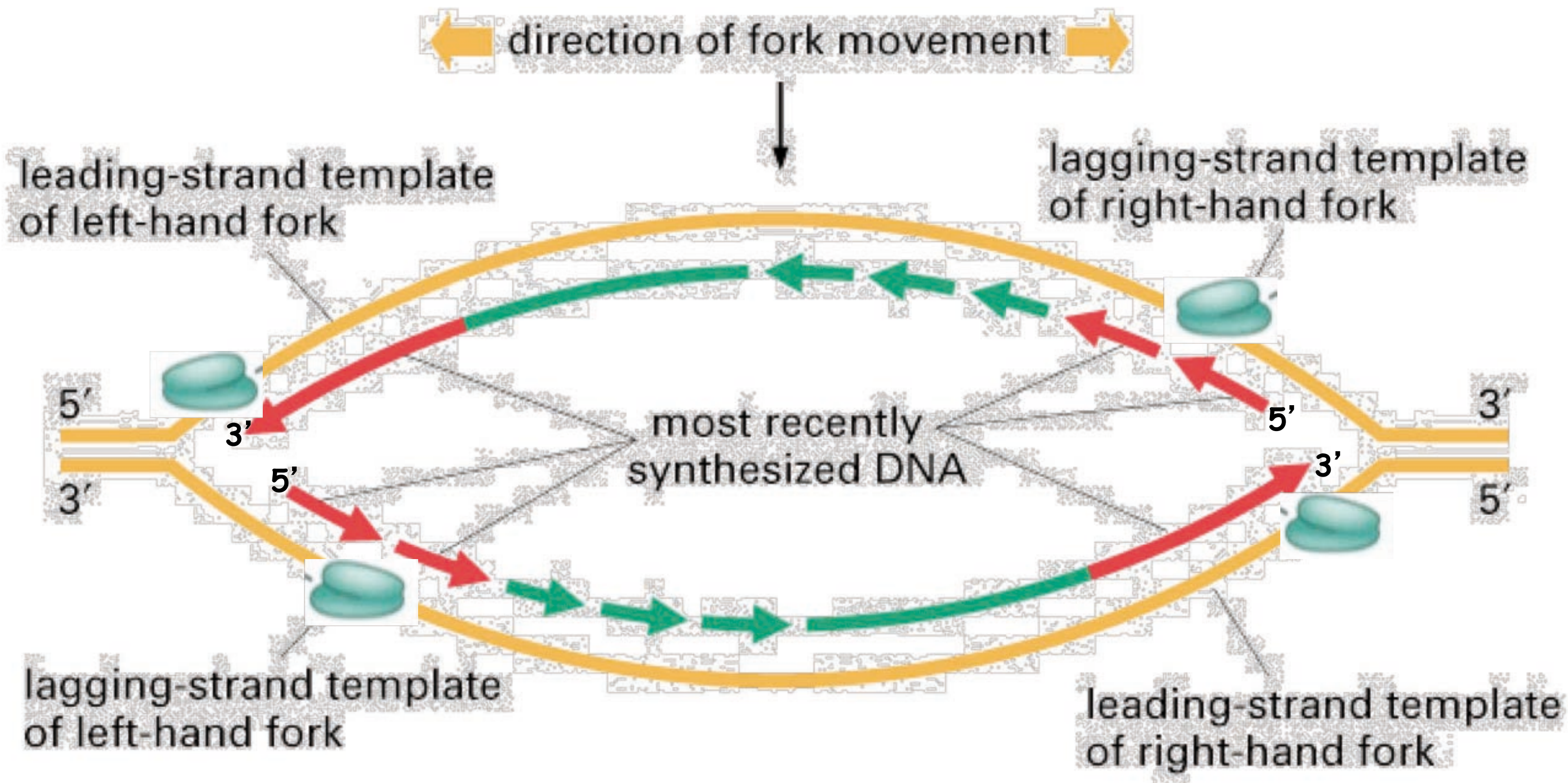
One DNA strand is synthesized continuously; the other in synthesized discontinuously and then stitched together



Leading strand: DNA strand that is synthesized continuously in the direction of replication fork.
Lagging strand: DNA strand that is synthesized discontinuously (Okazaki fragments), then stitched together.

Figure 6-12 Essential Cell Biology, 2/e. (© 2004 Garland Science)

One DNA strand is synthesized continuously; the other in synthesized discontinuously and then stitched together

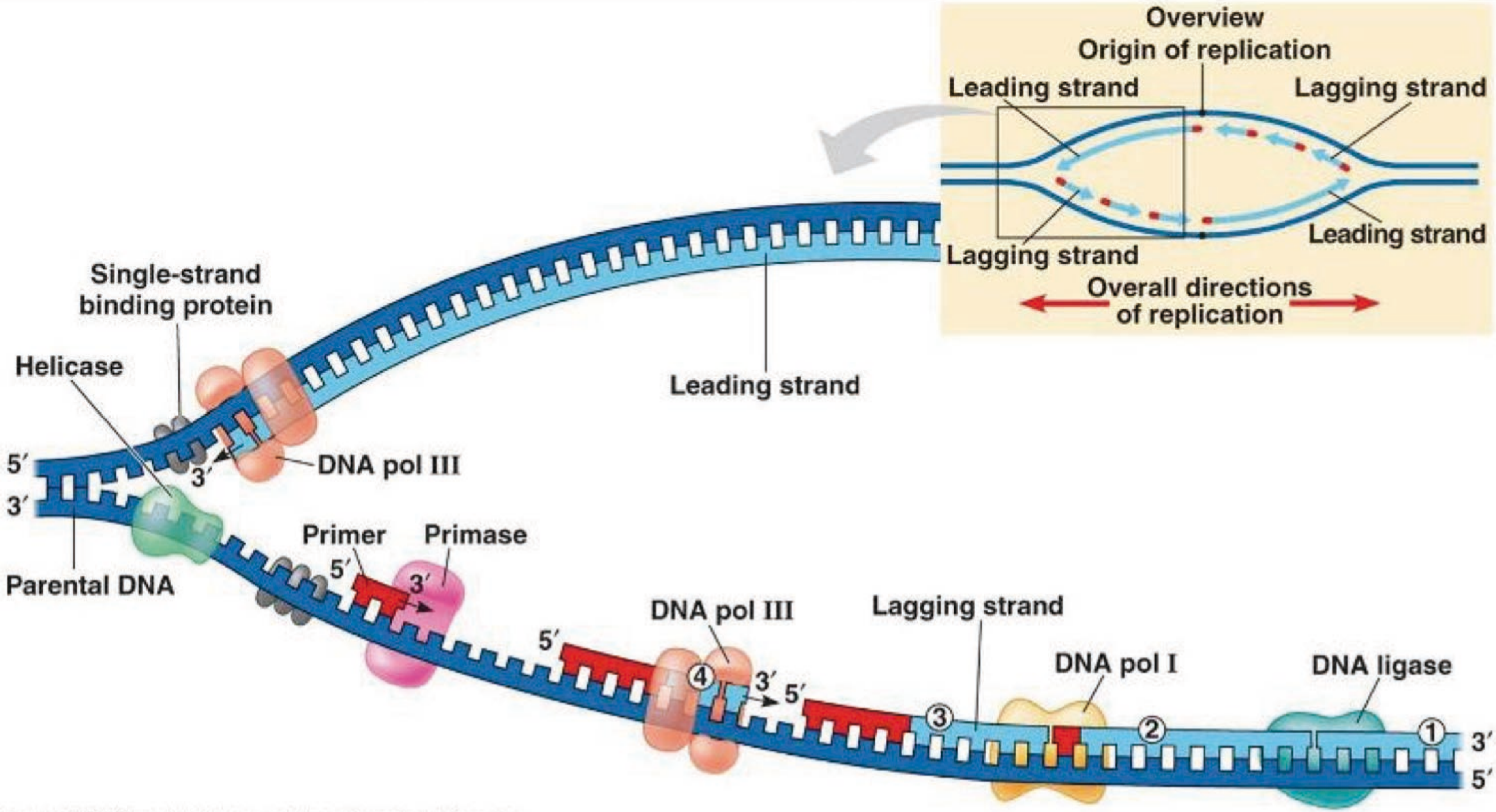


Leading strand: DNA strand that is synthesized continuously in the direction of replication fork.
Lagging strand: DNA strand that is synthesized discontinuously (Okazaki fragments), then stitched together.

Figure 6-12 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Core proteins at the replication fork

Topoisomerases	- Prevents torsion by DNA breaks
Helicases	- separates 2 strands
Primase	- RNA primer synthesis
Single strand binding proteins	- prevent reannealing of single strands
DNA polymerase	- synthesis of new strand
DNA ligase	- seals nick via phosphodiester linkage



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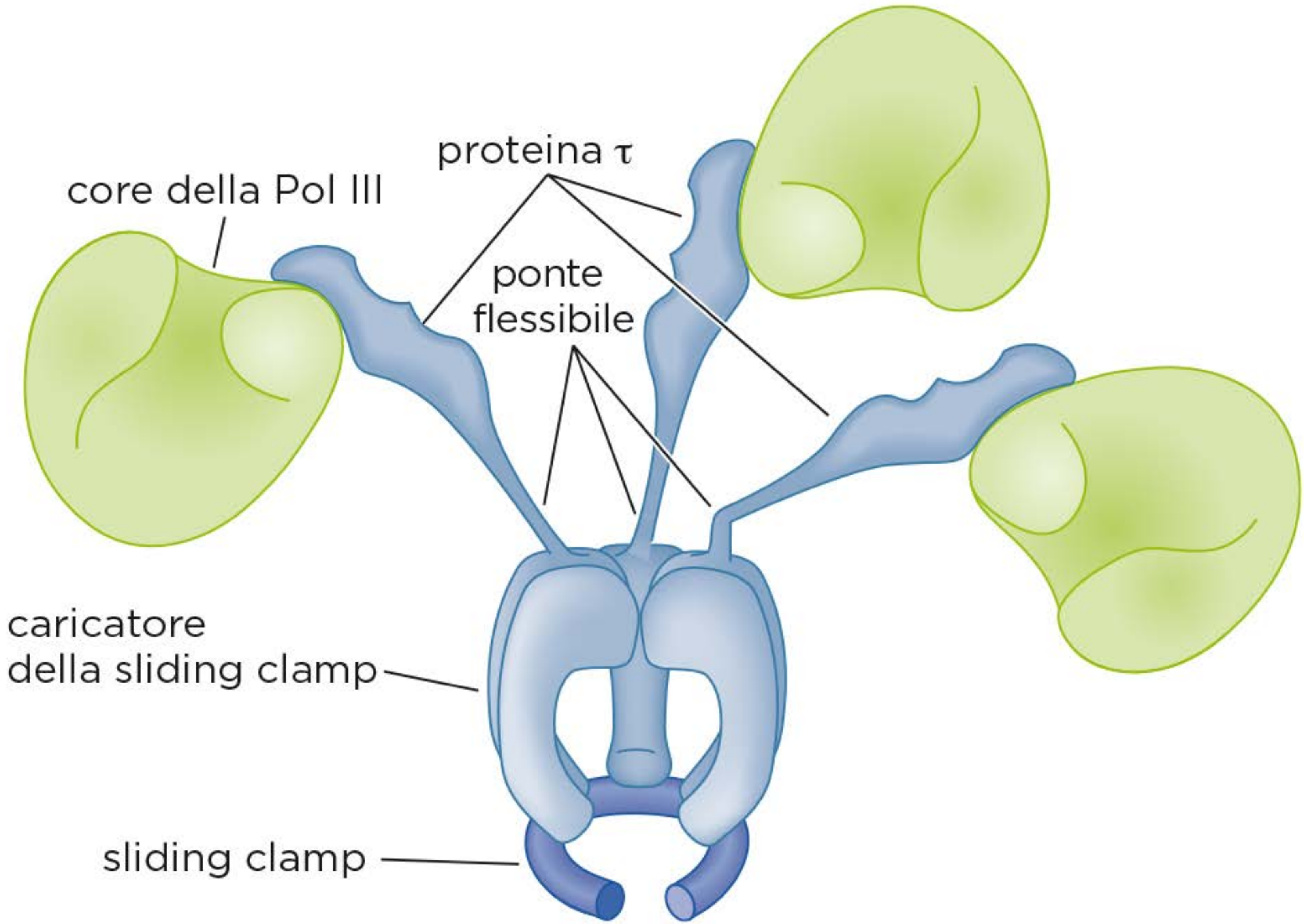
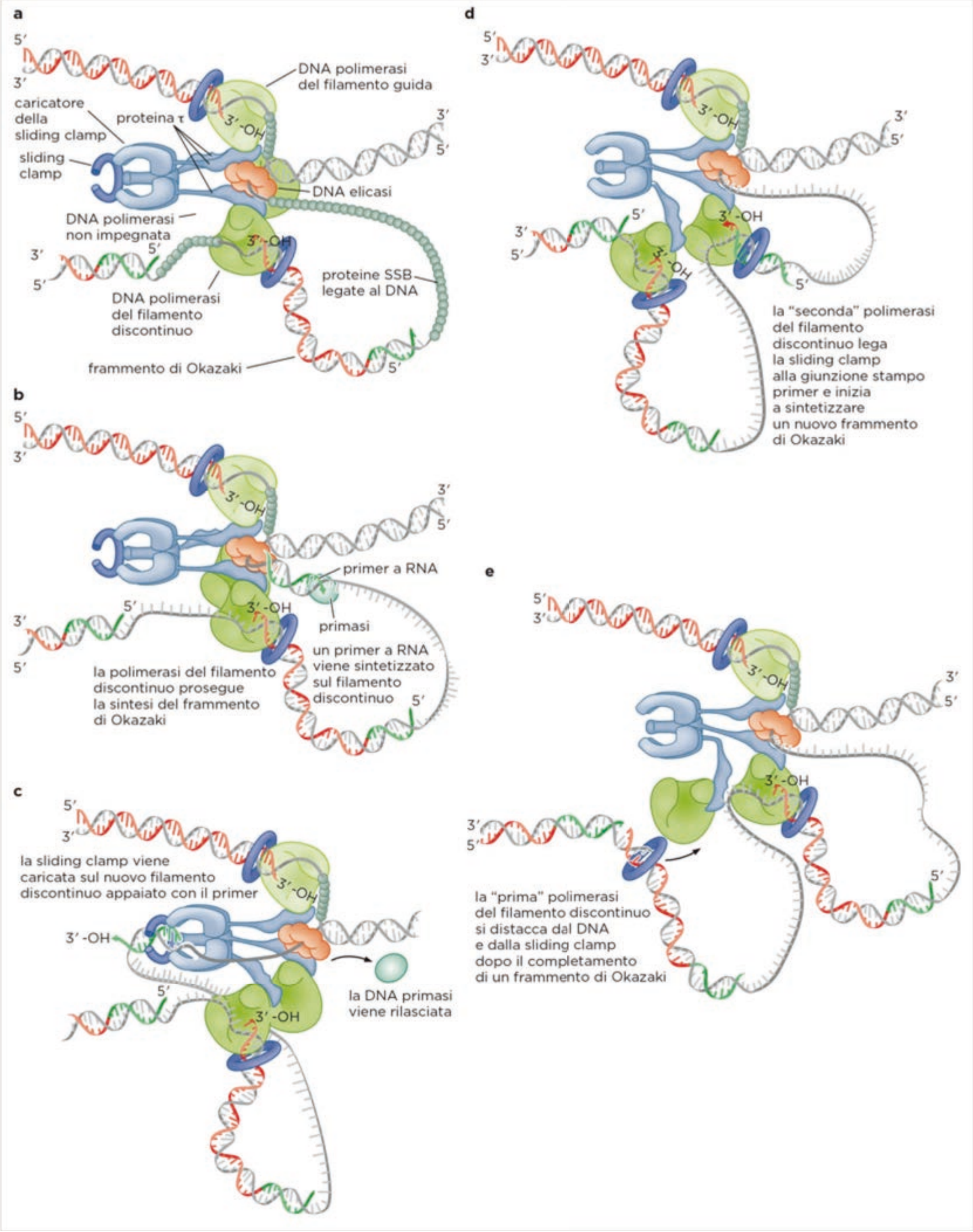
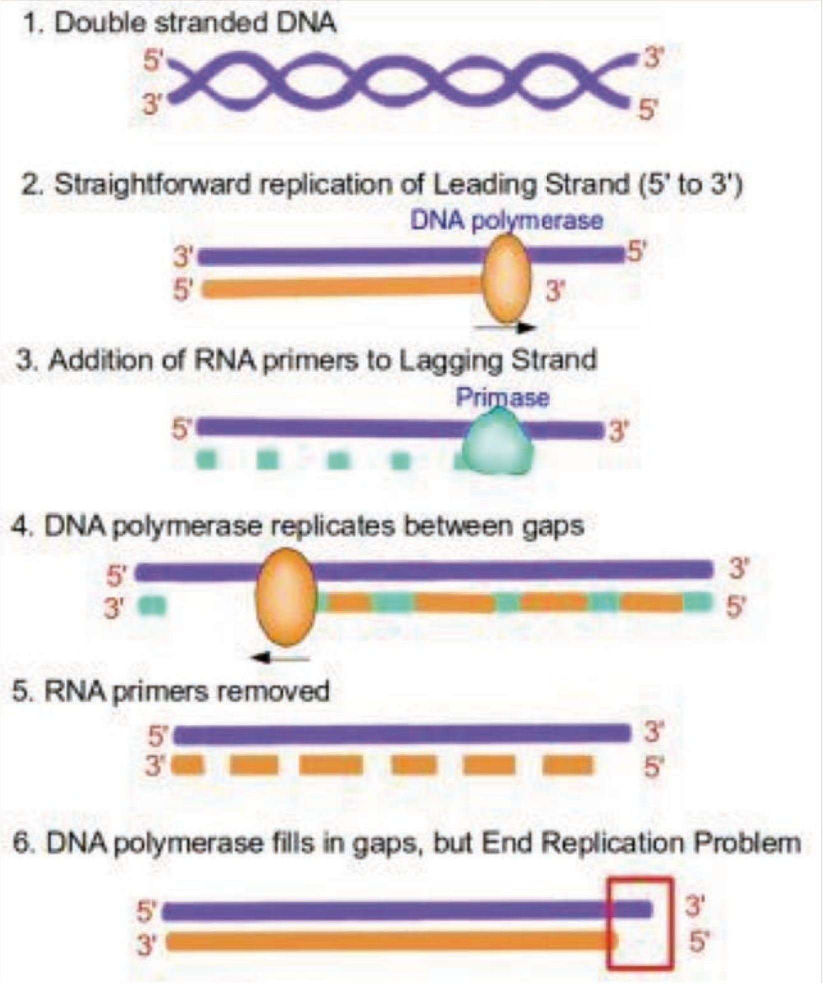

proteina τ
core della Pol III
ponte
flessibile
caricatore
della sliding clamp
sliding clamp

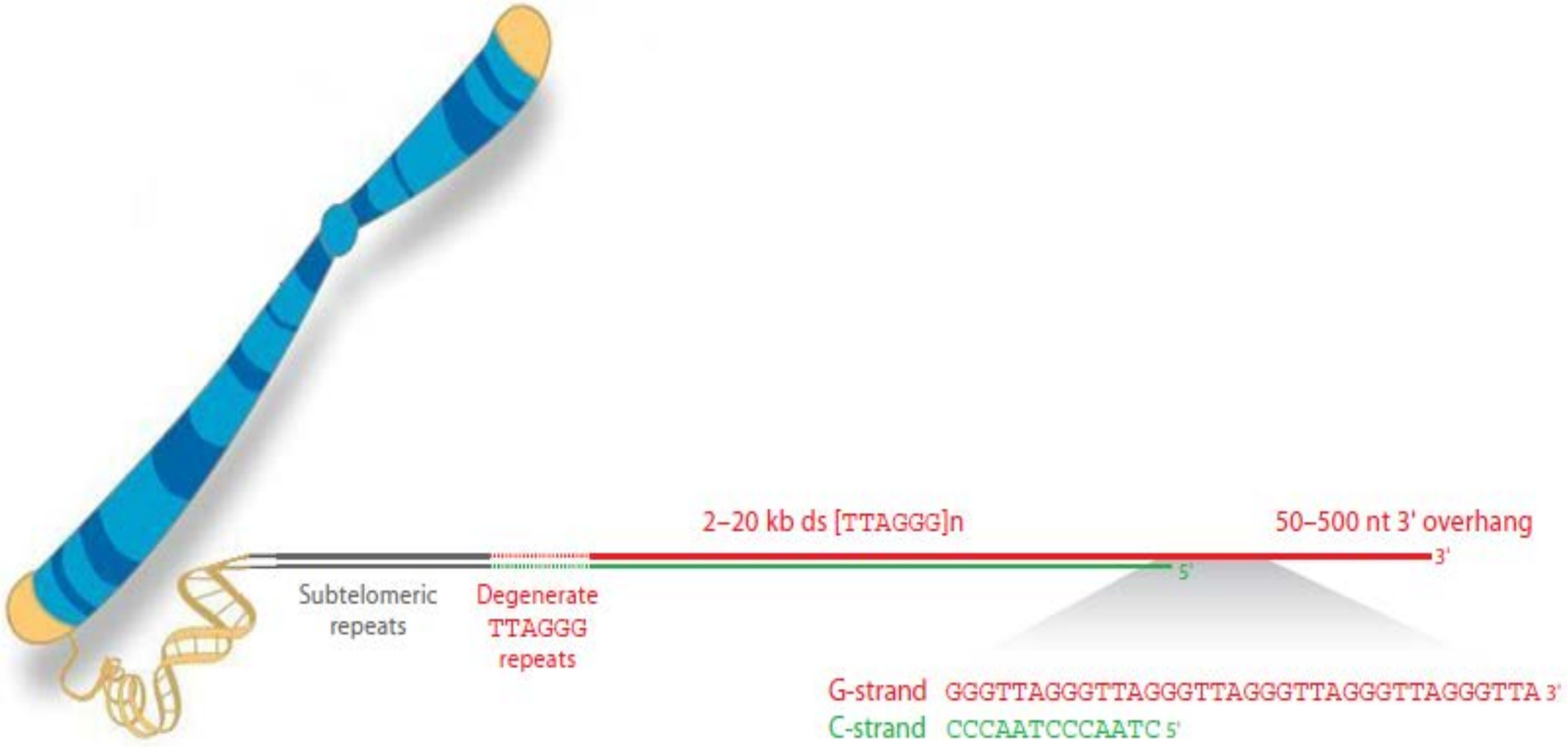


a
5′
3′
DNA polimerasi
del filamento guida
caricatore
della
sliding clamp
proteina τ
3′-OH
3′
5′
sliding
clamp
DNA elicasi
DNA polimerasi
non impegnata
3′
5′
3′-OH
5′
proteine SSB
legate al DNA
DNA polimerasi
del filamento
discontinuo
frammento di Okazaki
5′
b
5′
3′
3′-OH
3′
5′
primer a RNA
3′
5′
3′-OH
primasi
5′
un primer a RNA
viene sintetizzato
sul filamento
discontinuo
la polimerasi del filamento
discontinuo prosegue
la sintesi del frammento
di Okazaki
5′
c
5′
3′
la sliding clamp viene
caricata sul nuovo filamento
discontinuo appaiato con il primer
3′-OH
3′-OH
5′
3′-OH
la DNA primasi
viene rilasciata
d
5′
3′
3′-OH
3′
5′
5′
3′-OH
3′
5′
3′-OH
5′
la “seconda” polimerasi
del filamento
discontinuo lega
la sliding clamp
alla giunzione stampo
primer e inizia
a sintetizzare
un nuovo frammento
di Okazaki
e
5′
3′
3′-OH
3′
5′
3′-OH
3′
5′
5′
la “prima” polimerasi
del filamento discontinuo
si distacca dal DNA
e dalla sliding clamp
dopo il completamento
di un frammento di Okazaki

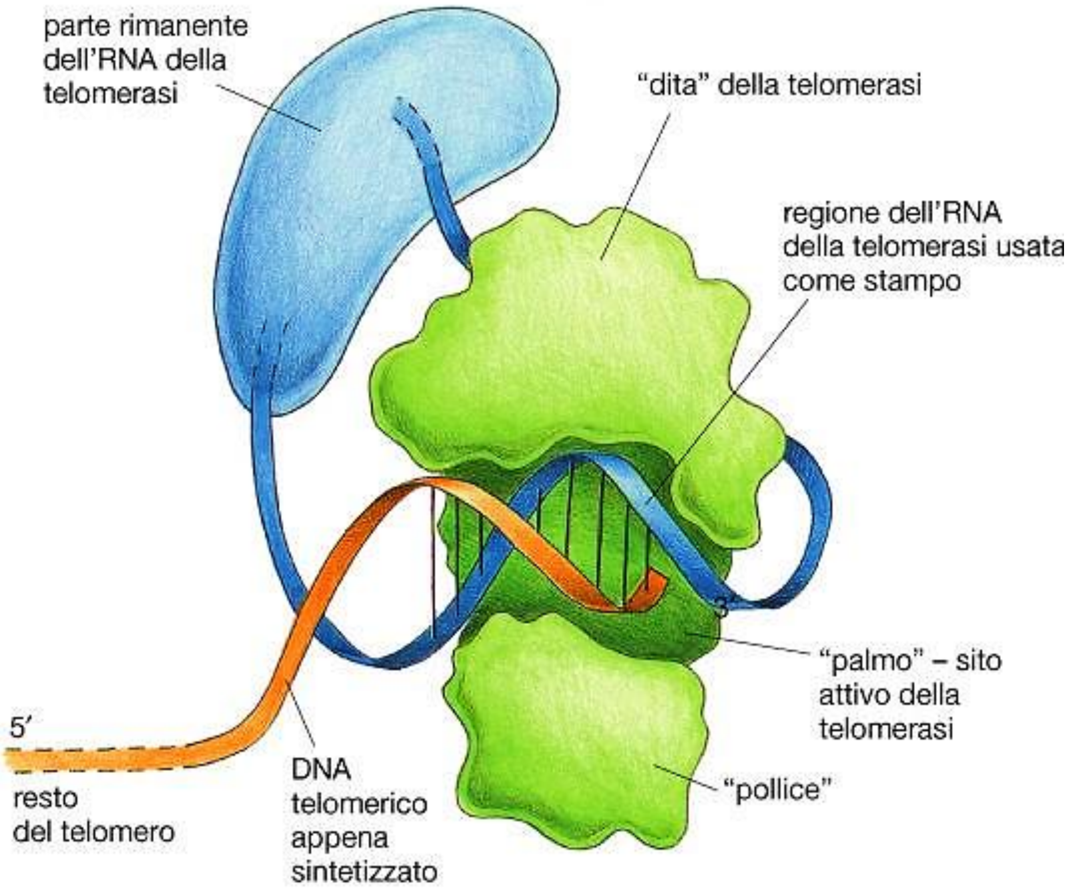
End-replication problem



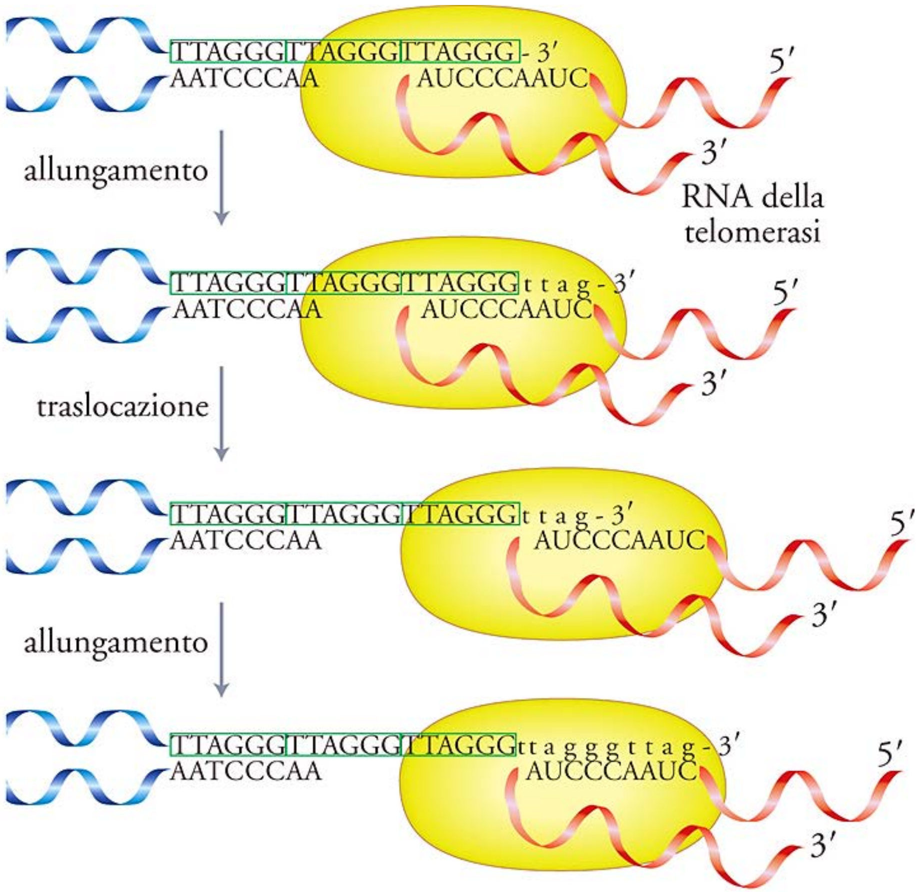
Human Telomeres



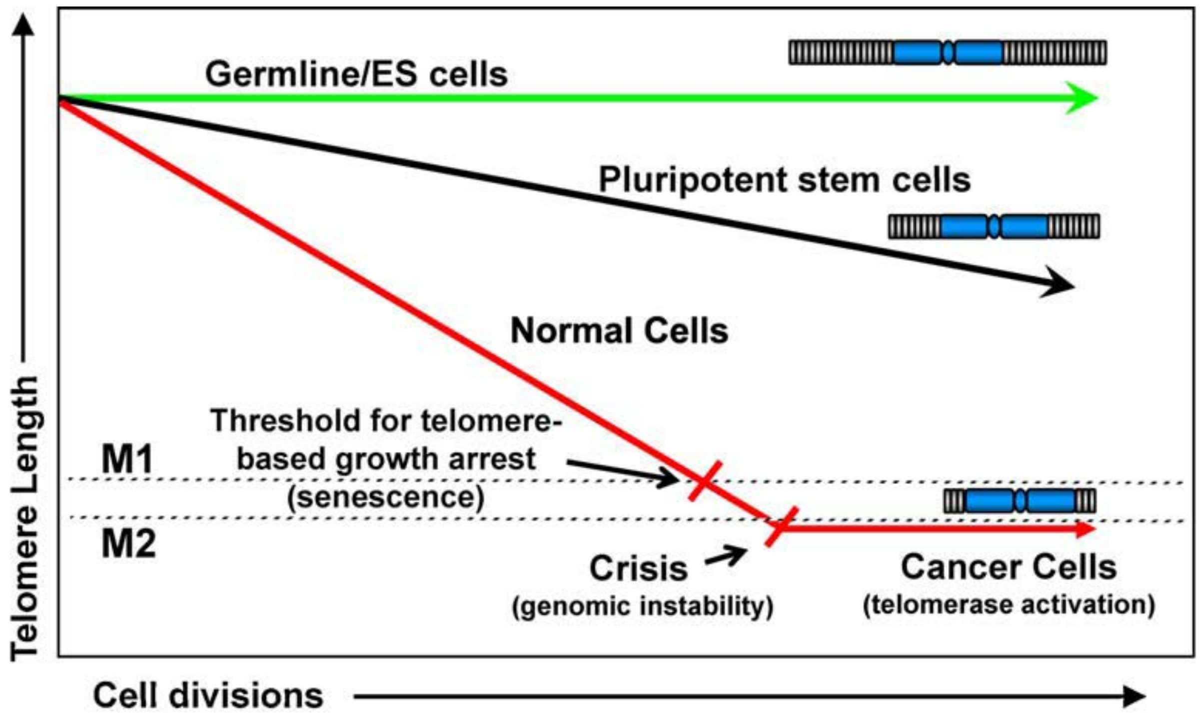
Telomerase

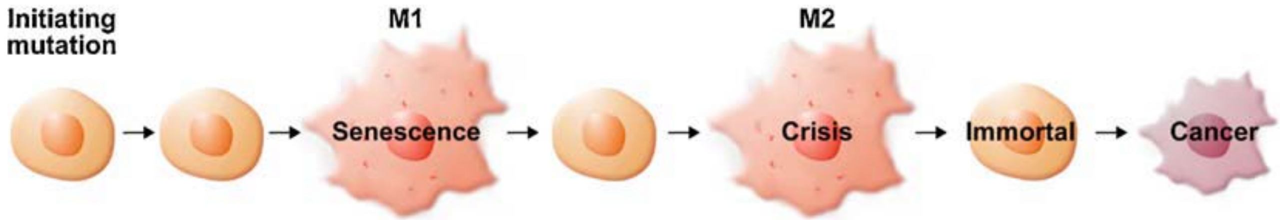

parte rimanente
dell'RNA della
telomerasi
"dita" della telomerasi
regione dell'RNA
della telomerasi usata
come stampo
3'
"palmo" – sito
attivo della
telomerasi
"pollice"
5'
resto
del telomero
DNA
telomerico
appena
sintetizzato

Telomerase in action



Telomerase and cancer





Polymerase enzymes

DNA --replication--> DNA

(DNA polymerase plus other proteins)

DNA --transcription--> RNA

(RNA polymerase plus other proteins)

RNA --reverse transcription--> DNA

(HIV reverse transcriptase, telomerase)

Duplication of eukaryotic genomes requires the three different polymerization activities

Four requirements for DNA to be genetic material

Must carry information

Must replicate itself

Must allow for information to change

Must regulate the expression of the phenotipe

DNA has the ability to mutate (change). This allows for new characteristics and abilities to appear which may help an individual to survive and reproduce (EVOLUTION).