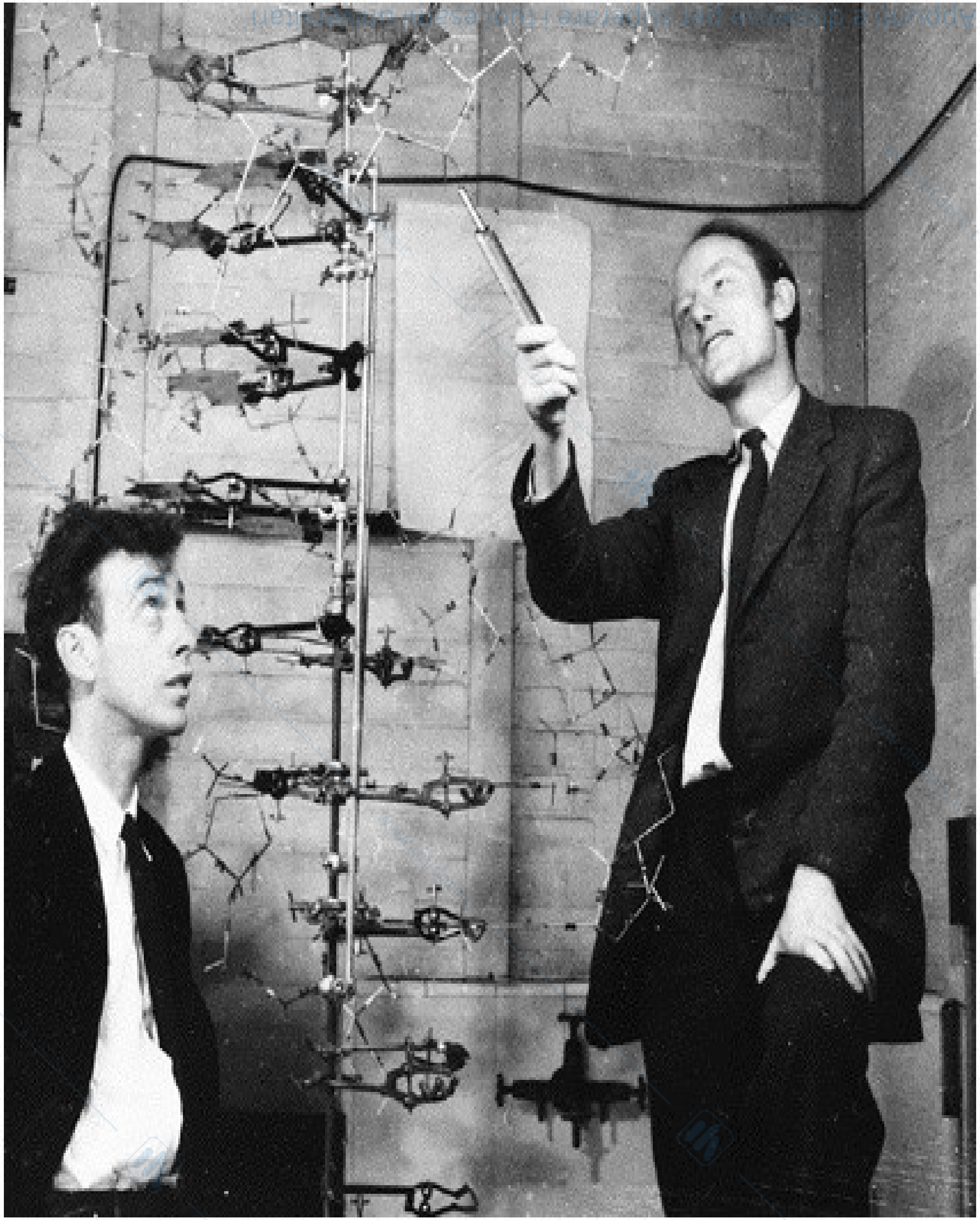
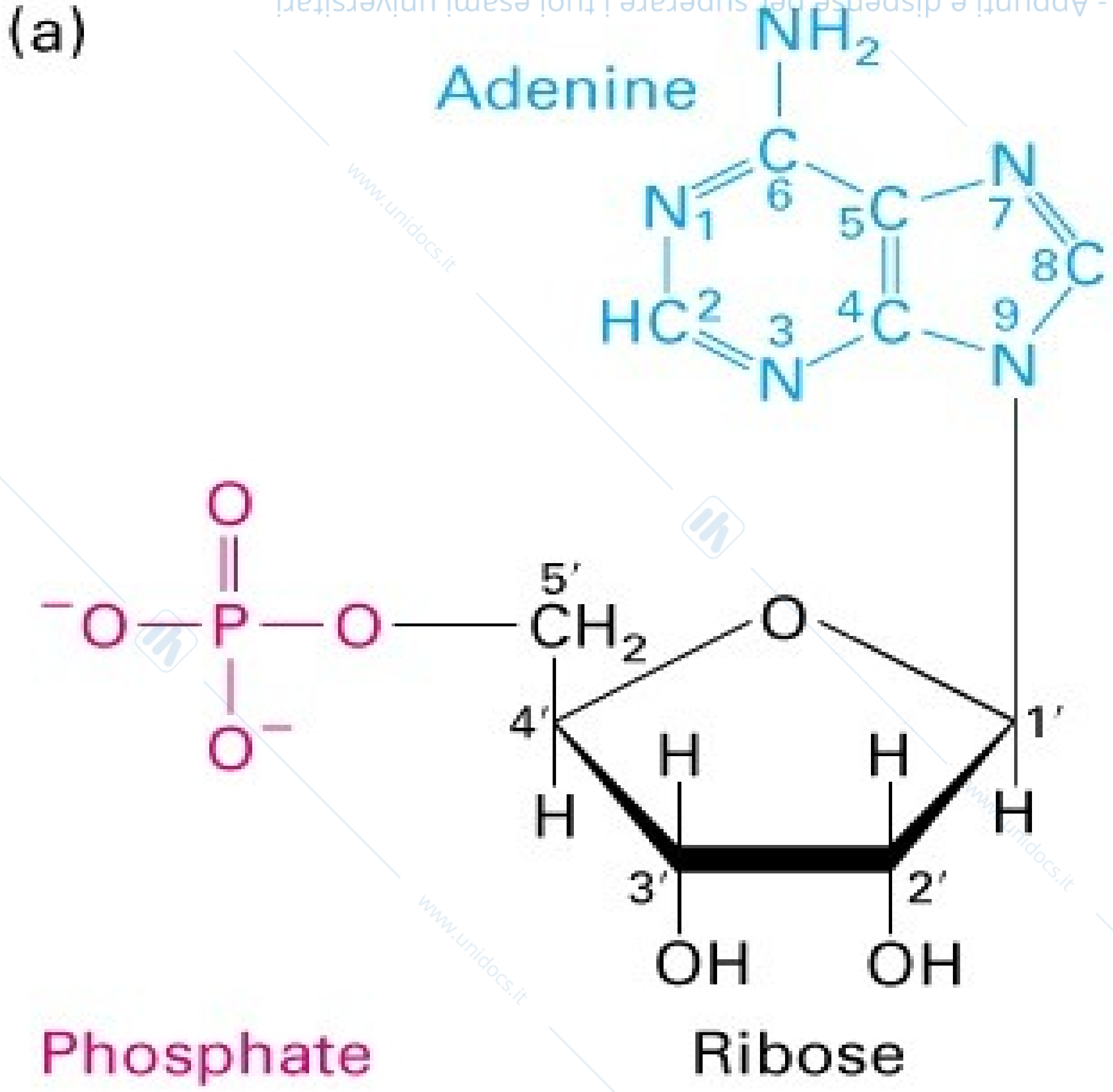
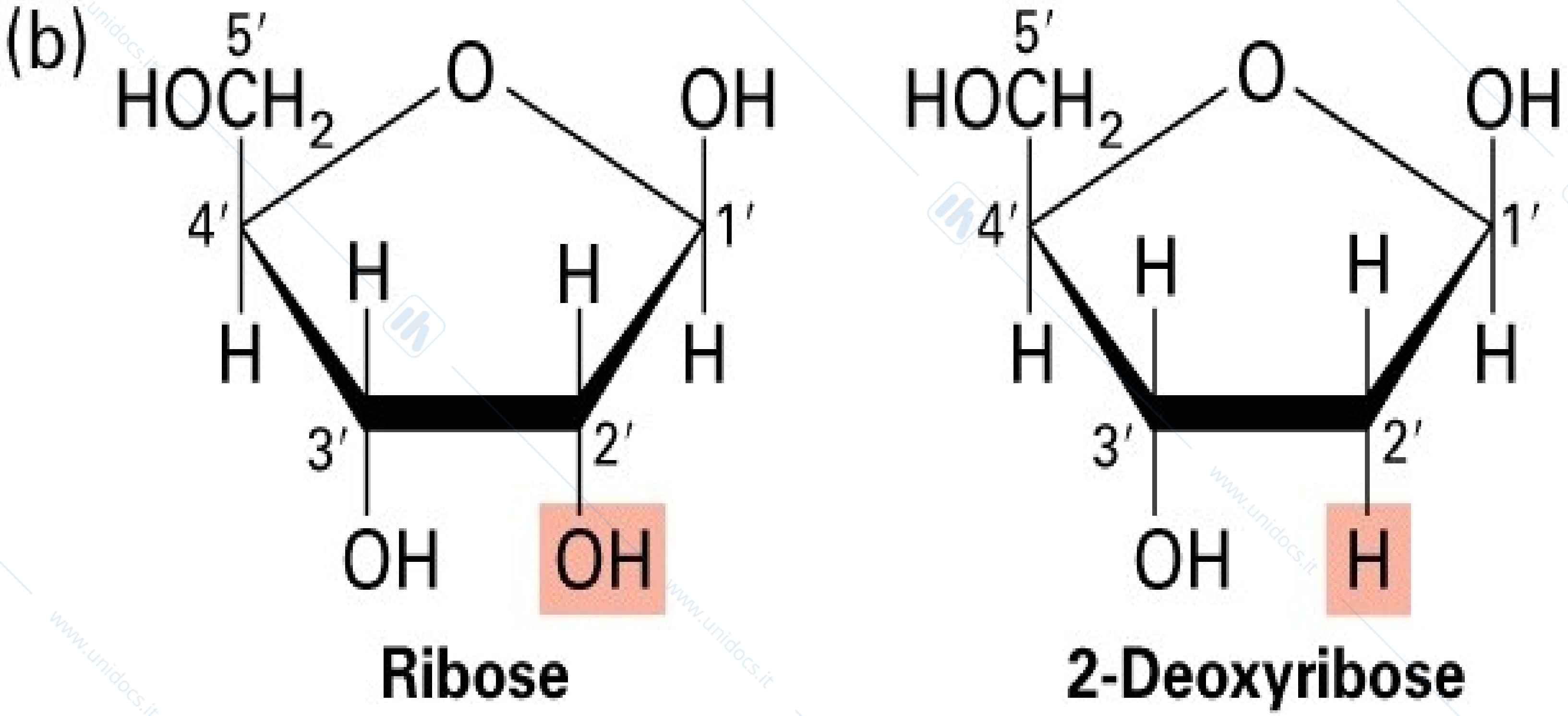


Figure 1.1 A brief history of genetics.

1865	Genes are particulate factors
1903	Chromosomes are hereditary units
1910	Genes lie on chromosomes
1913	Chromosomes contain linear arrays of genes
1927	Mutations are physical changes in genes
1931	Recombination is caused by crossing over
1944	DNA is the genetic material
1945	A gene codes for a protein
1953	DNA is a double helix
1958	DNA replicates semiconservatively
1961	Genetic code is triplet
1977	DNA can be sequenced
1997	Genomes can be sequenced

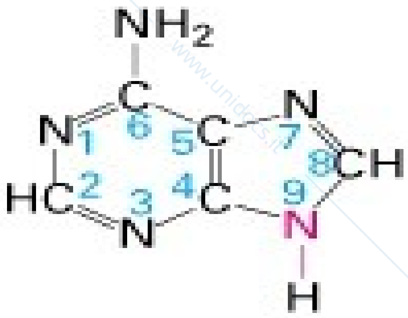


Adenosine
5′-monophosphate
(AMP)

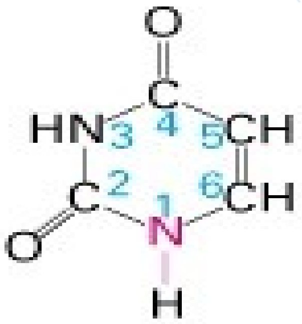

(b)
5′
HOCH2
O
OH
4′
1′
H
H
H
H
3′
2′
OH
OH
Ribose
5′
HOCH2
O
OH
4′
1′
H
H
H
H
3′
2′
OH
H
2-Deoxyribose

PURINES

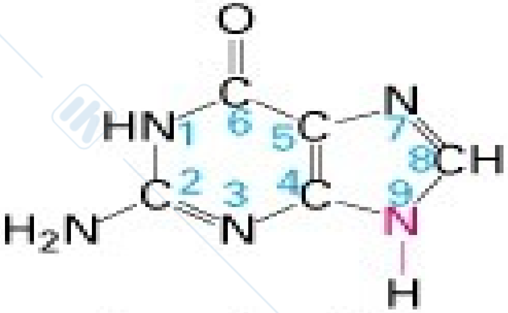
PYRIMIDINES



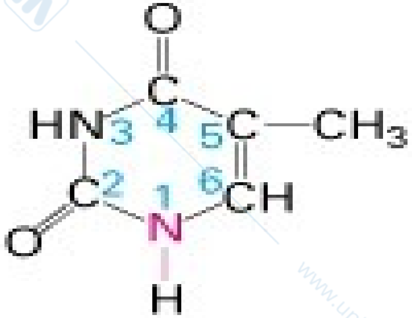
Adenine (A)



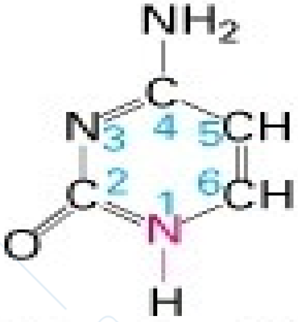
Uracil (U)



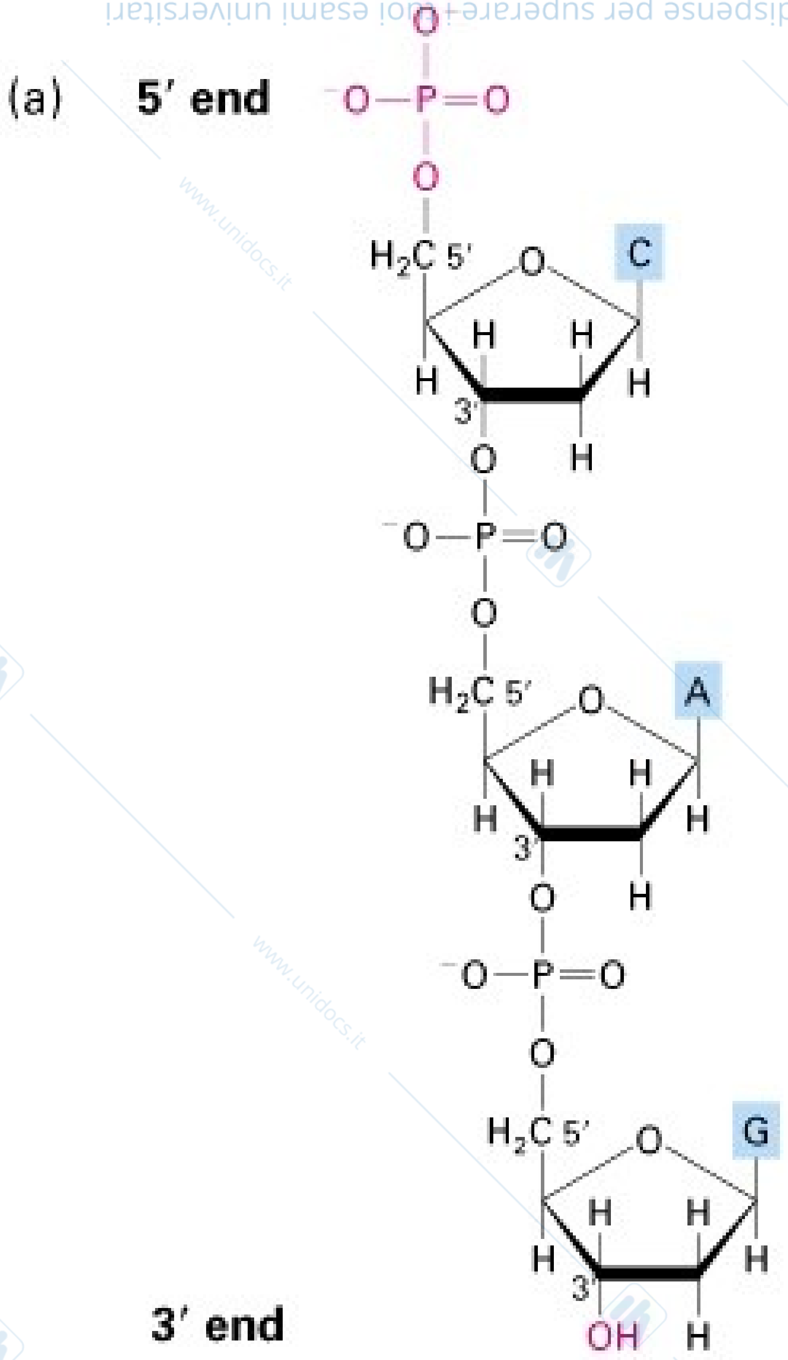
Guanine (G)



Thymine (T)



Cytosine (C)


(a)
5′ end
C
A
G
3′ end
OH

(b)

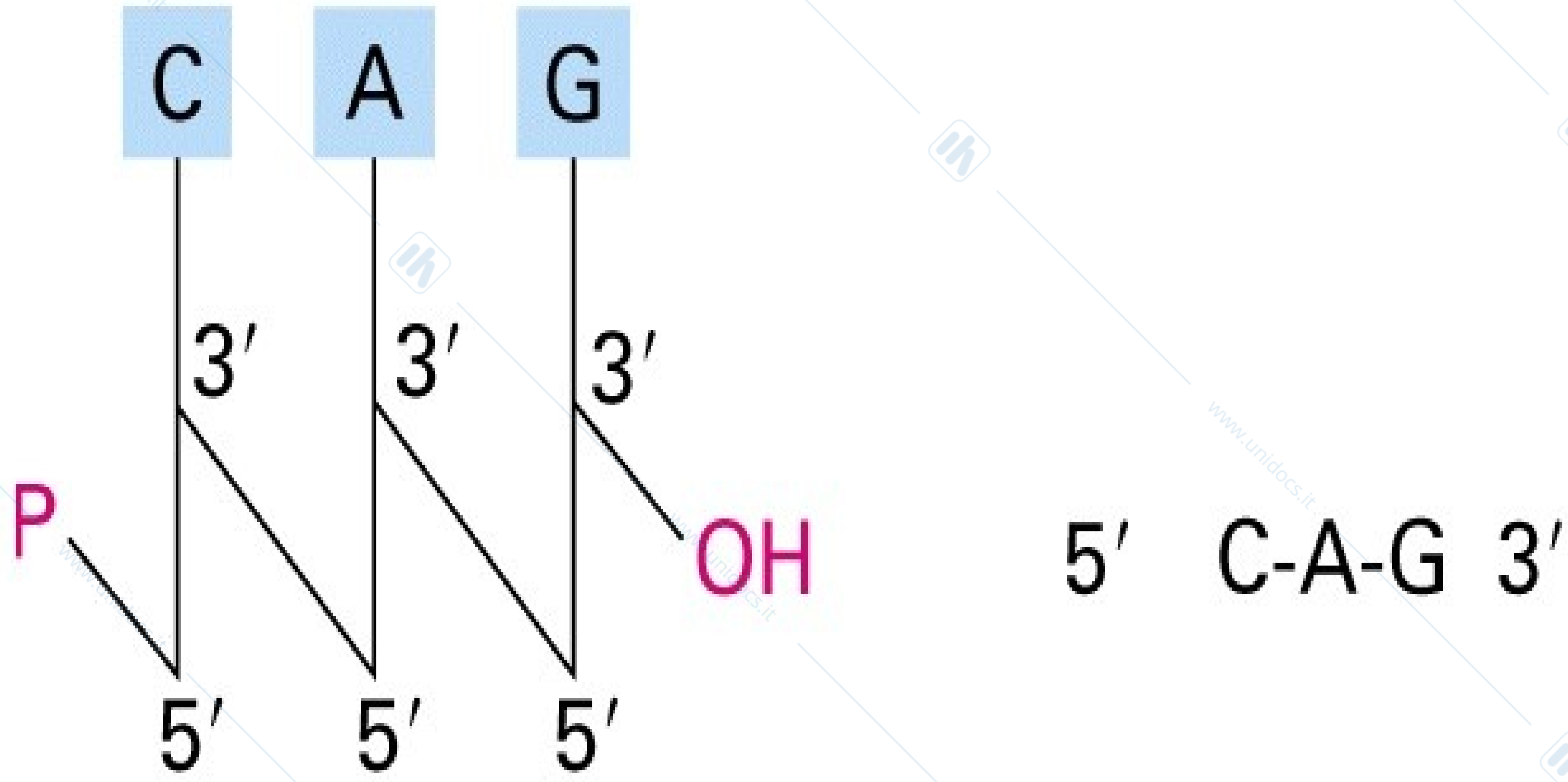


Figure 1.5 A polynucleotide chain consists of a series of 5'-3' sugar-phosphate links that form a backbone from which the bases protrude.

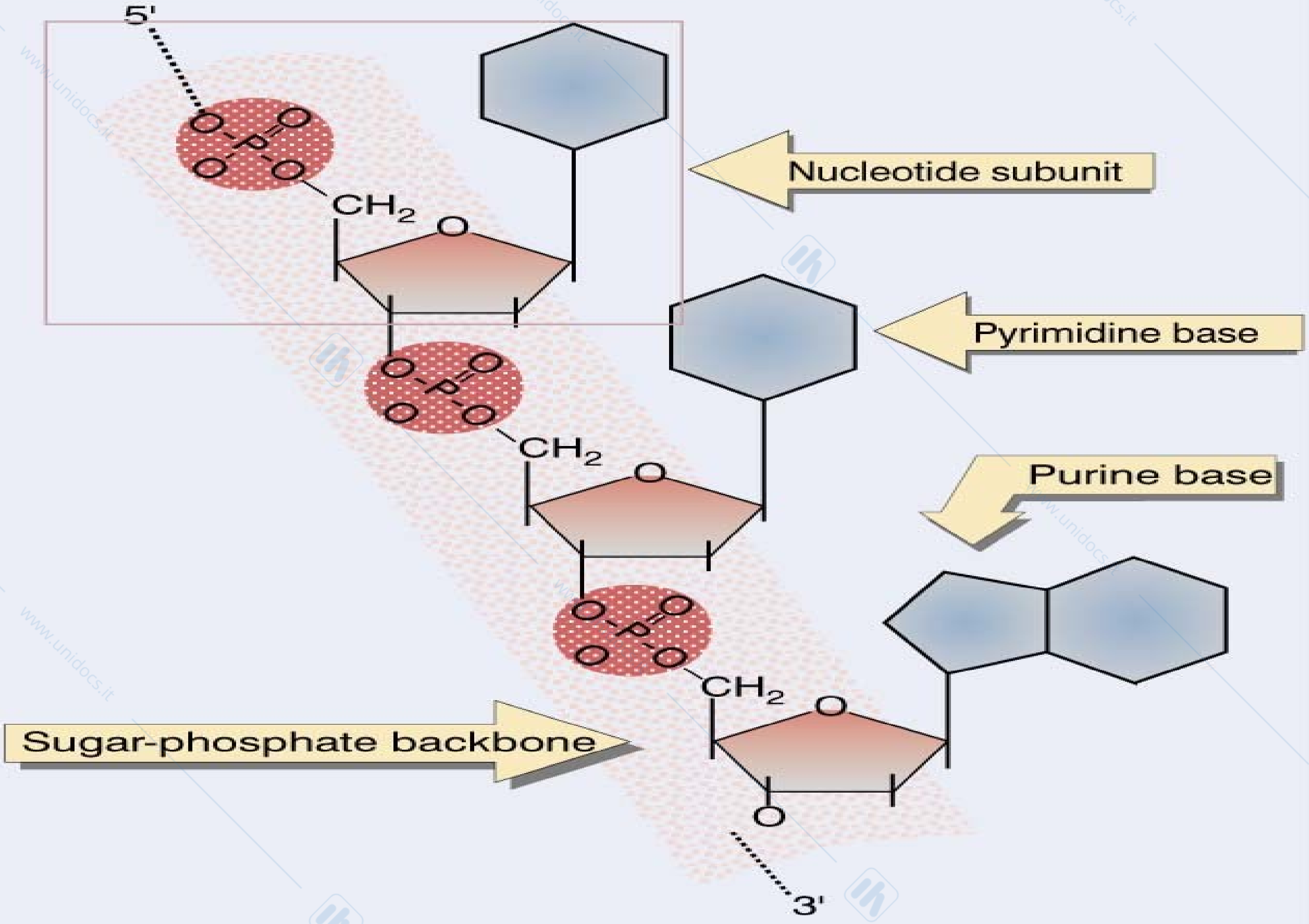


Figure 3.1 DNA content of the haploid genome is related to the morphological complexity of lower eukaryotes, but varies extensively among the higher eukaryotes. The range of DNA values within a phylum is indicated by the shaded area.

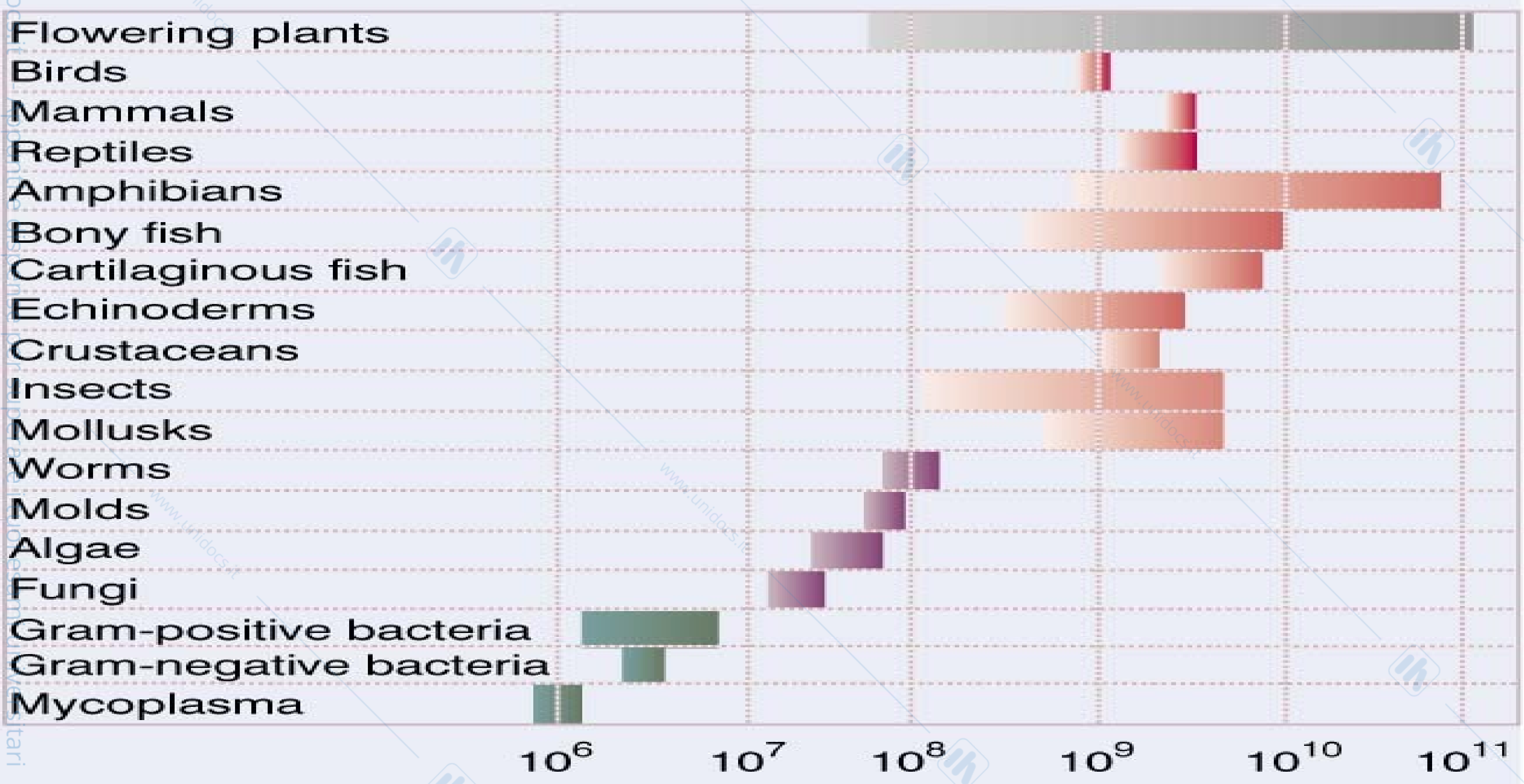


Figure 3.2 The minimum genome size found in each phylum increases from prokaryotes to mammals.

Figure 1.6 The double helix maintains a constant width because purines always face pyrimidines in the complementary A–T and G–C base pairs. The sequence in the figure is
T–A
C–G
A–T
G–C

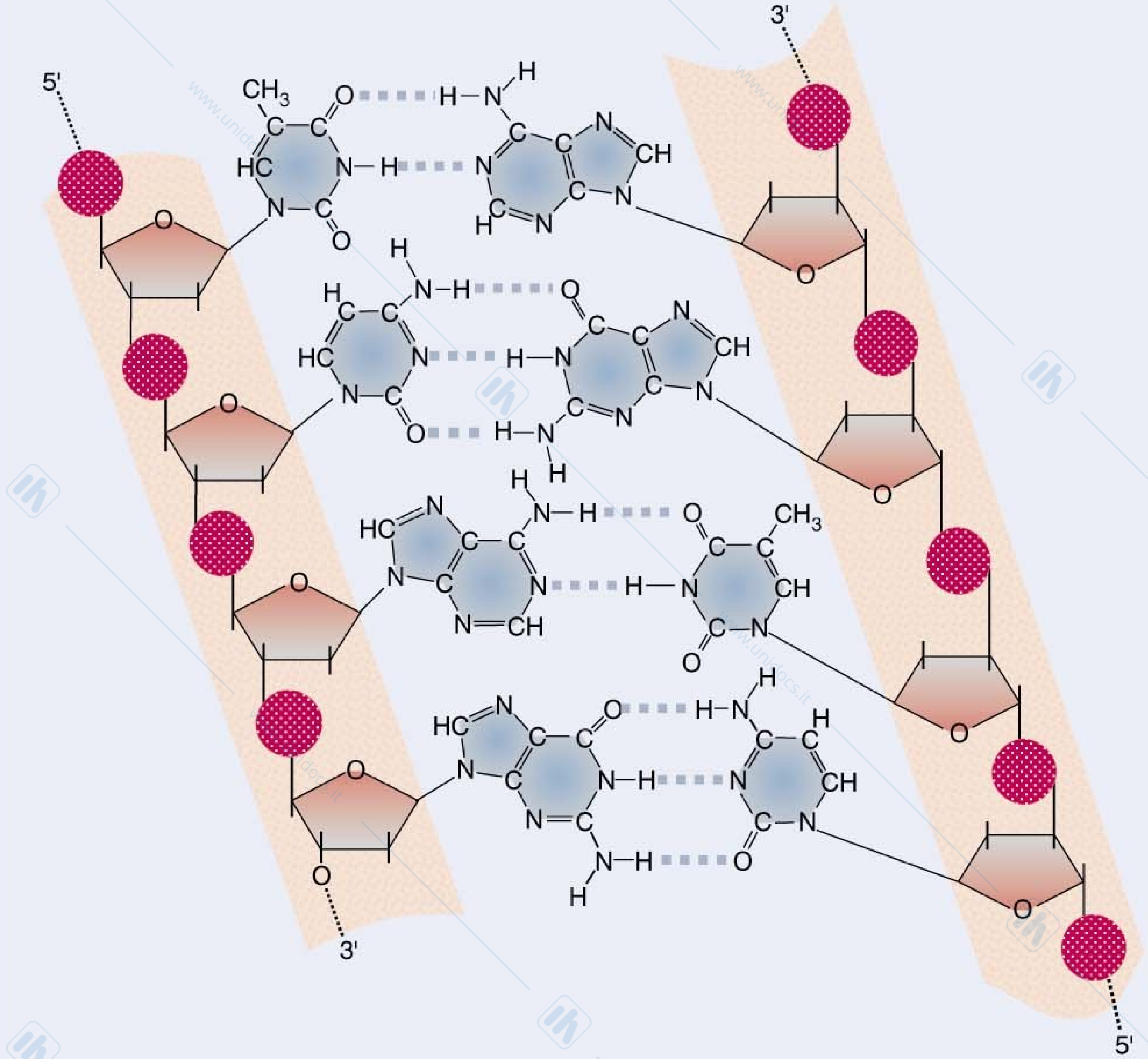
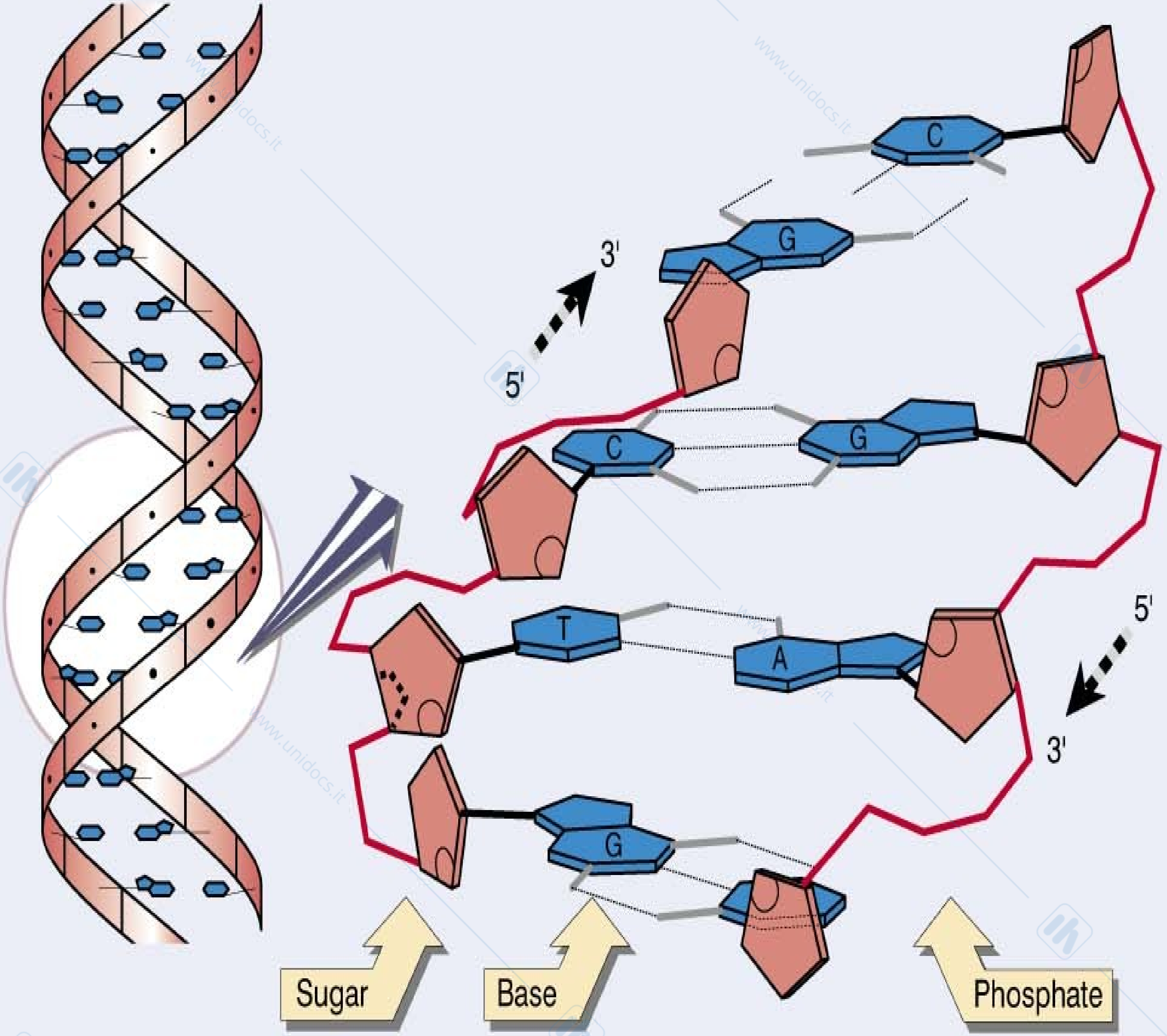
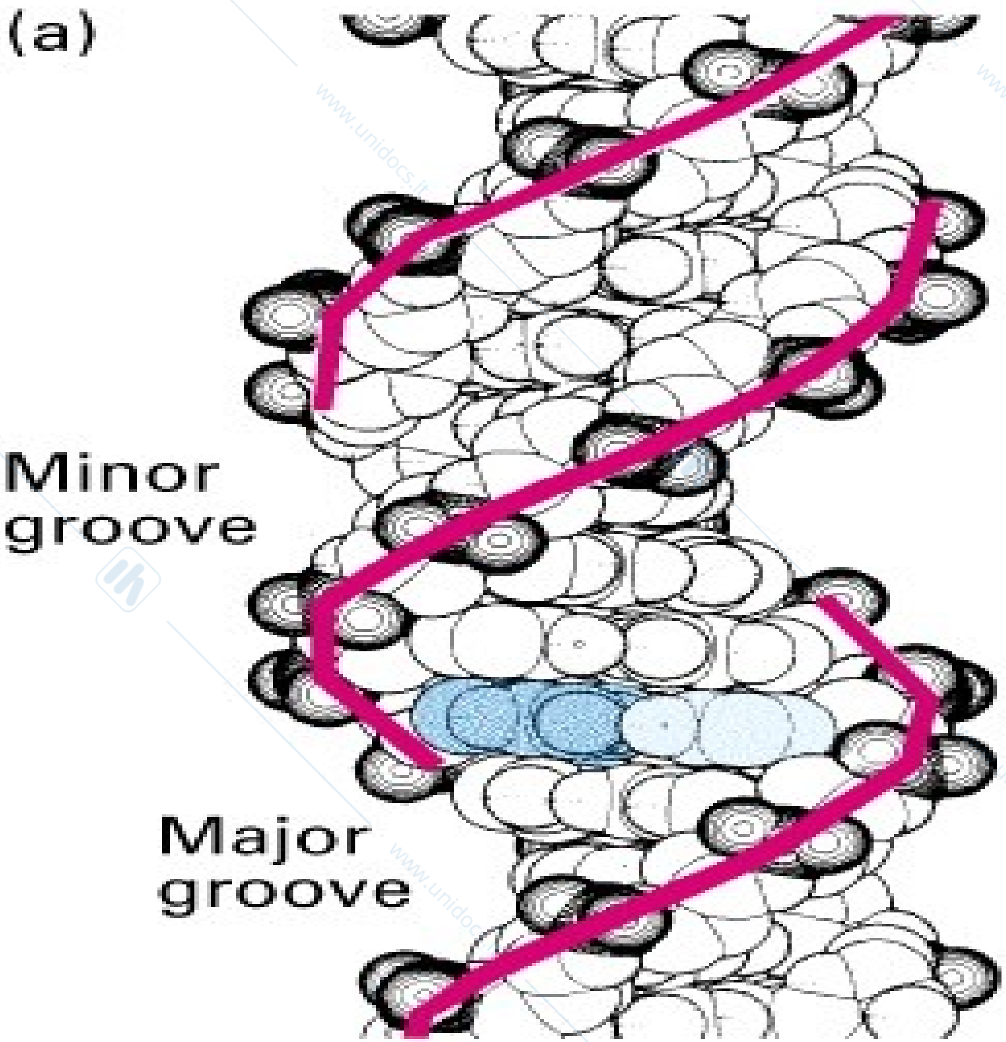
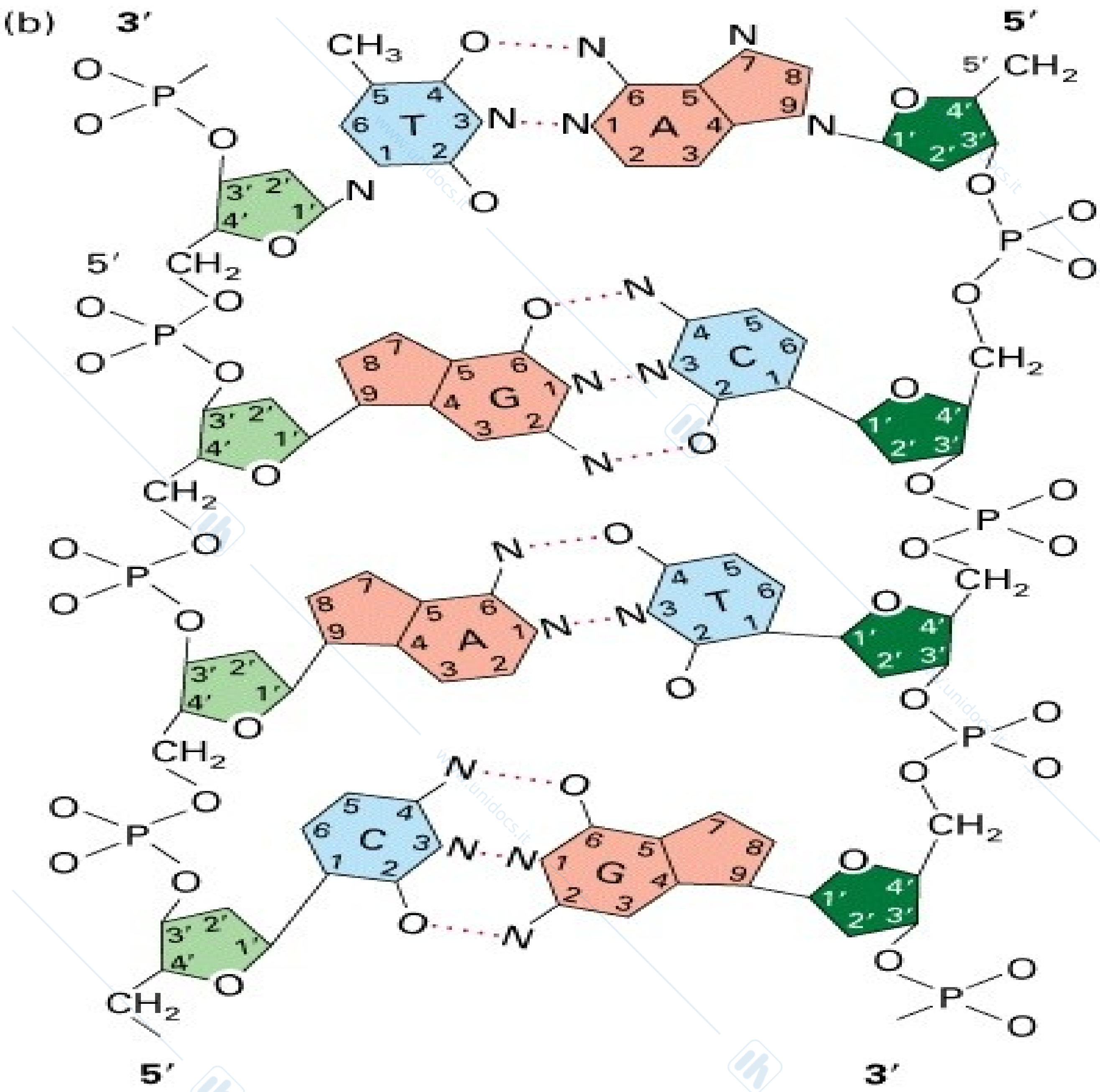


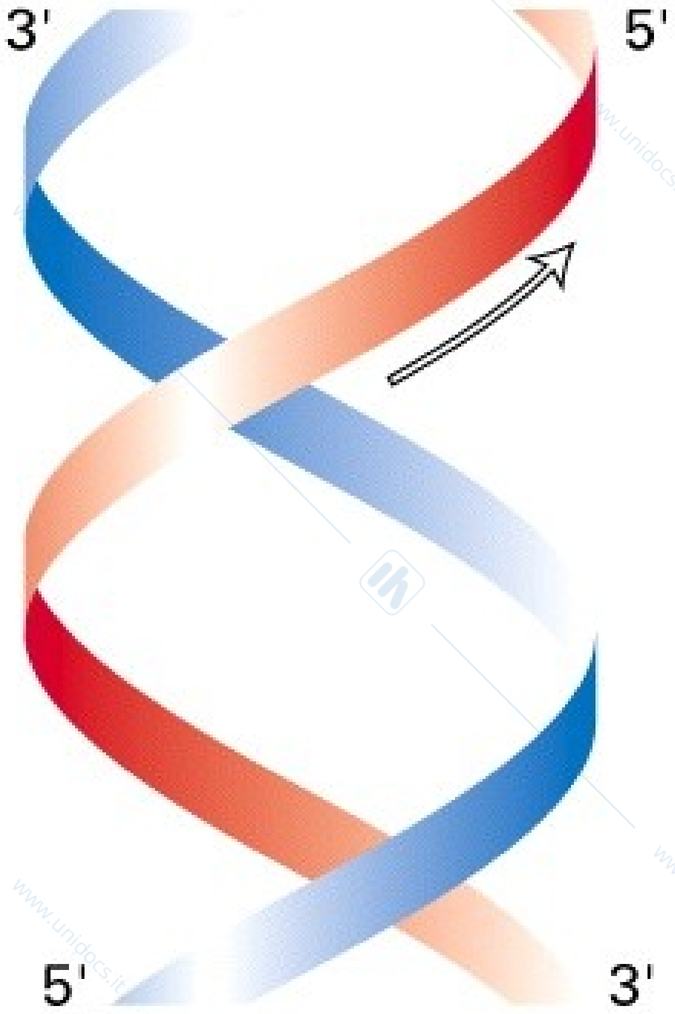
Figure 1.7 Flat base pairs lie perpendicular to the sugar-phosphate backbone.



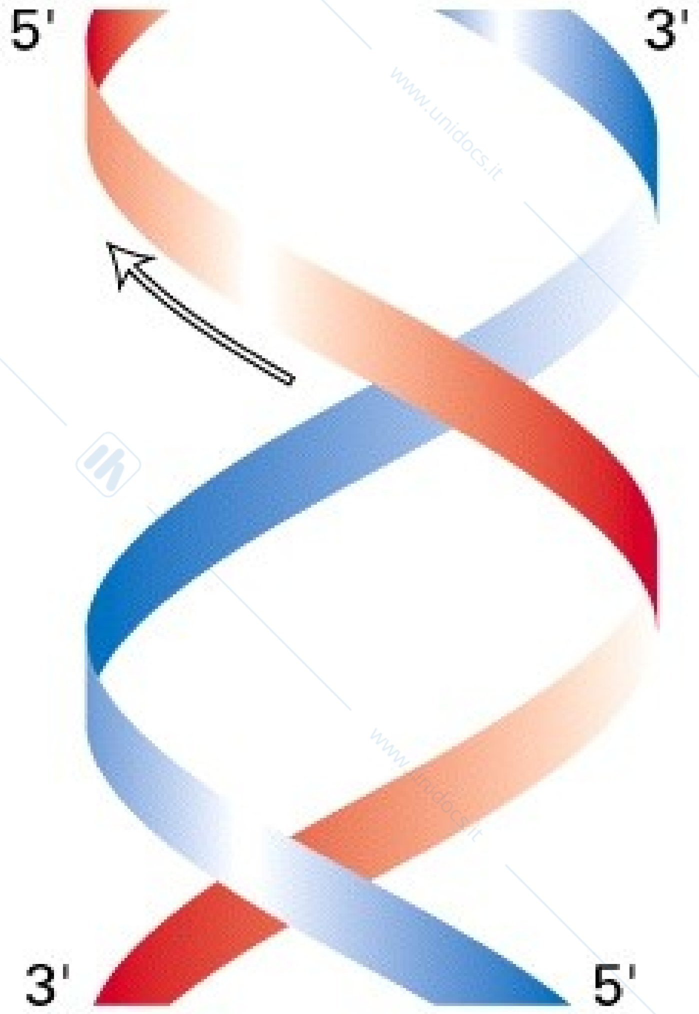


Normal B DNA


(b)
3′
5′
CH$_3$
T
A
G
C
T
A
C
G
5′ CH$_2$
CH$_2$
P
O
N
1′
2′
3′
4′
5′
5′
3′



Right-handed
double helix



Left-handed
double helix

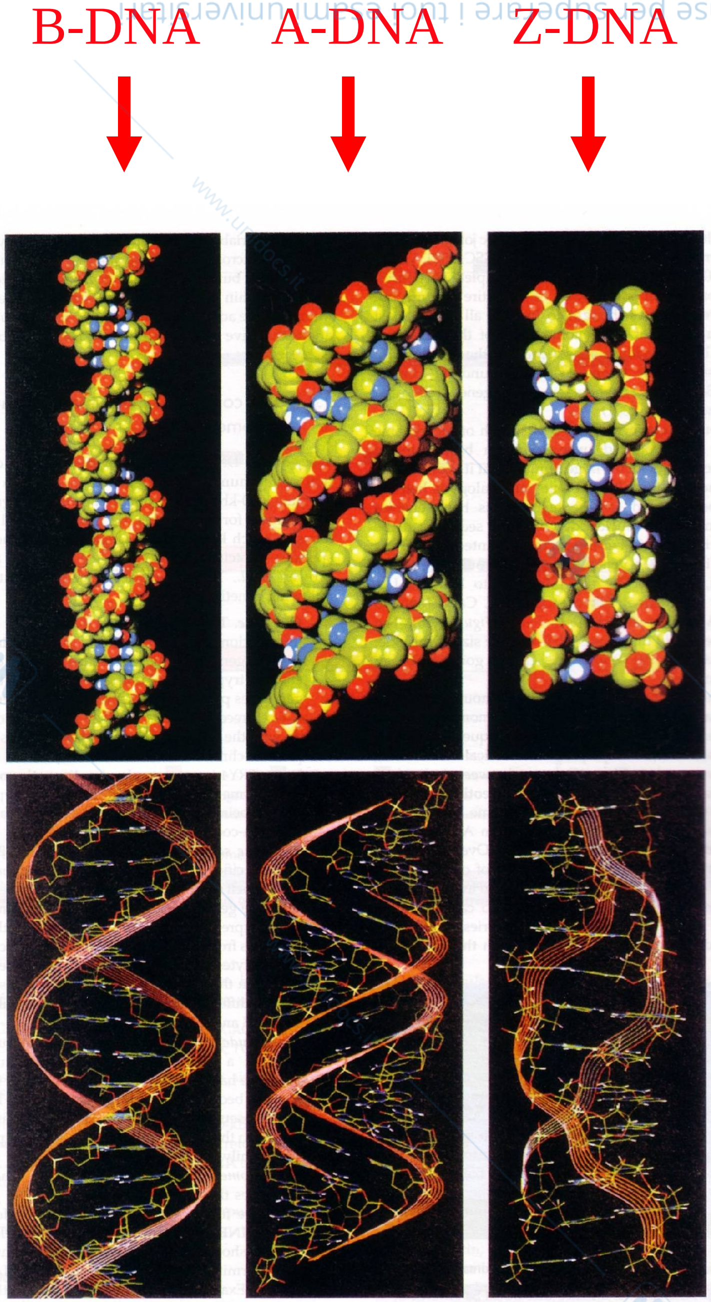
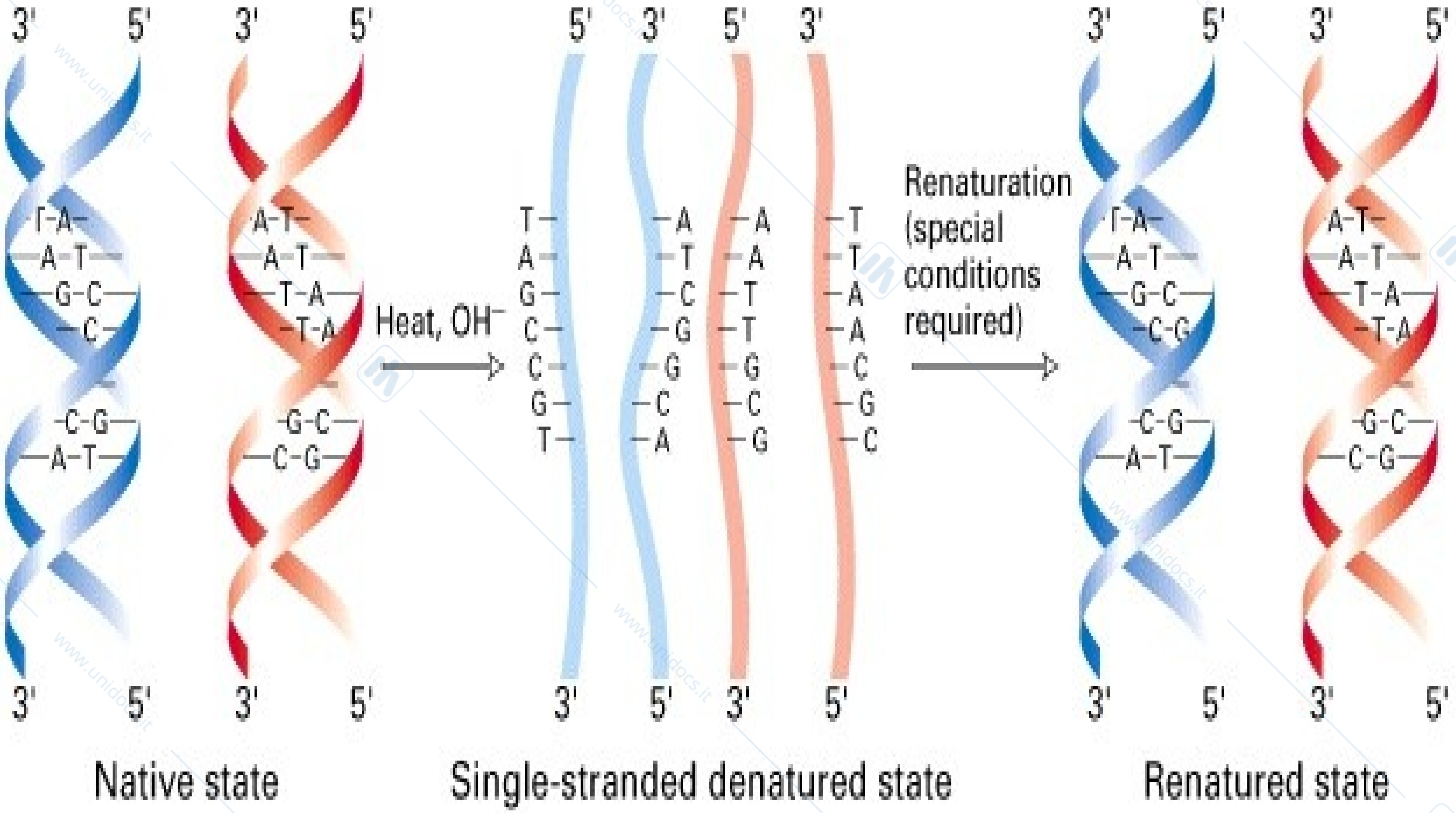


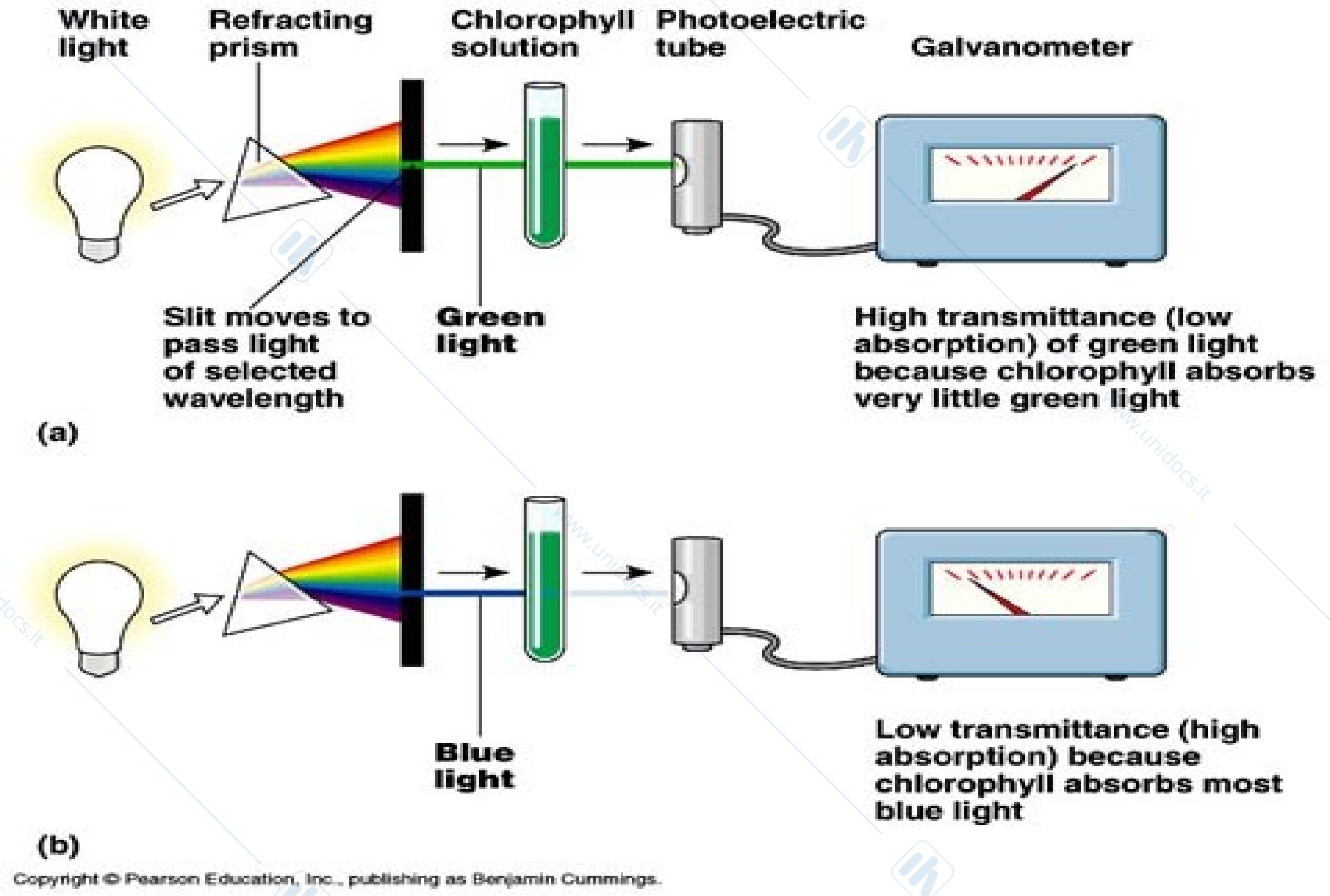
Figure 1.12 Computer-generated images of B-DNA (left), A-DNA (center) and Z-DNA (right).

CARATTERISTICHE DELLE DIVERSE CONFORMAZIONI DELLA DOPPIA ELICA DI DNA

	CONFORMAZIONE		
CARATTERISTICHE	**B-DNA**	**A-DNA**	**Z-DNA**
•Tipo di elica	Destrorsa	Destrorsa	Sinistrorsa
•Diametro dell’elica (nm)	2,37	2,55	1,84
•Innalzamento di una coppia di basi (nm)	0,34	0,29	0,37
•Lunghezza di un giro completo (=pitch)(nm)	3,4	3,2	4,5
•Numero di coppie di basi per giro completo	10	11	12
•Topologia del solco maggiore	Largo, profondo	Stretto, profondo	Piatto
•Topologia del solco minore	Stretto, superficiale	Ampio, superficiale	Stretto, profondo

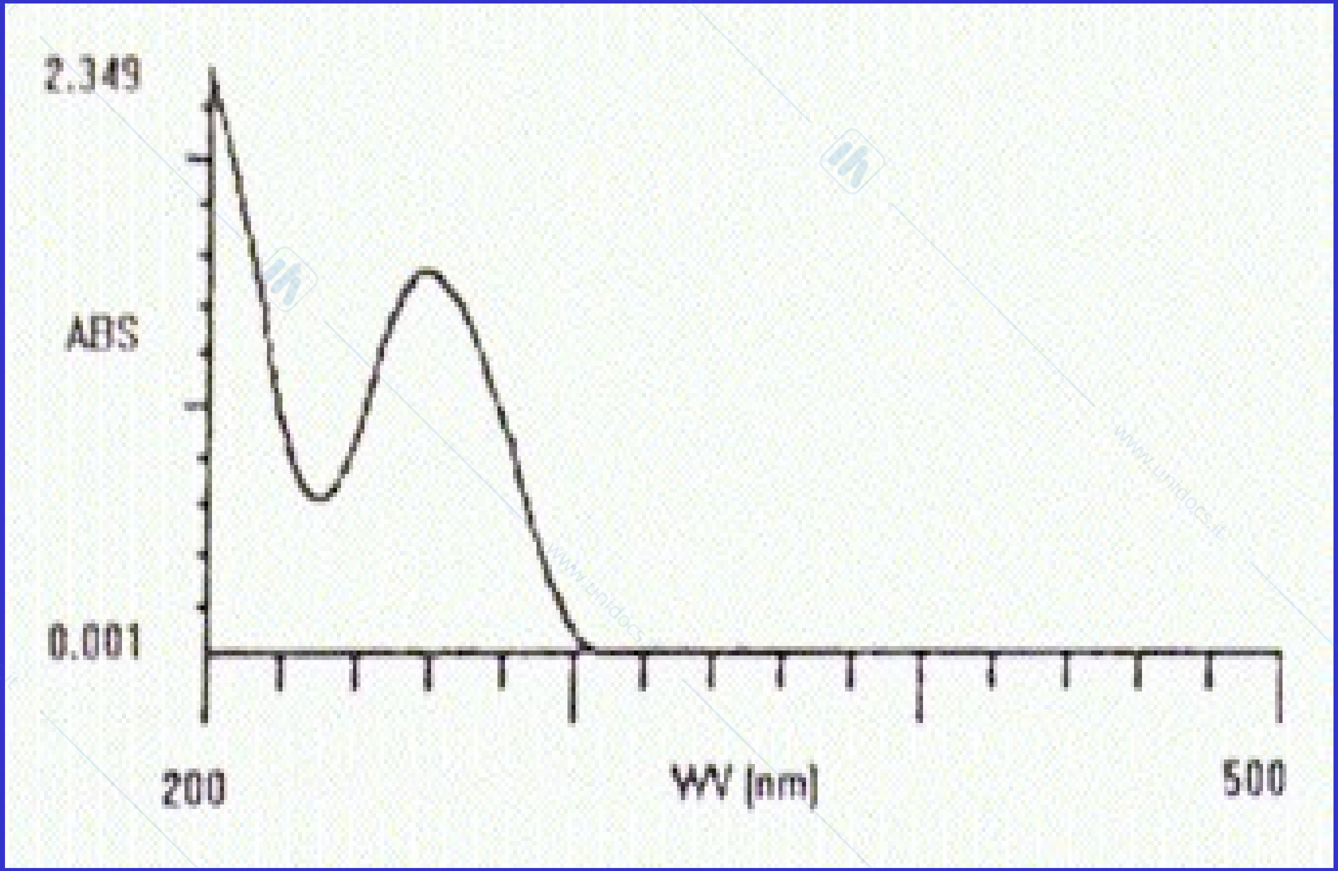

3'
5'
Heat, OH⁻
Renaturation (special conditions required)
Native state
Single-stranded denatured state
Renatured state

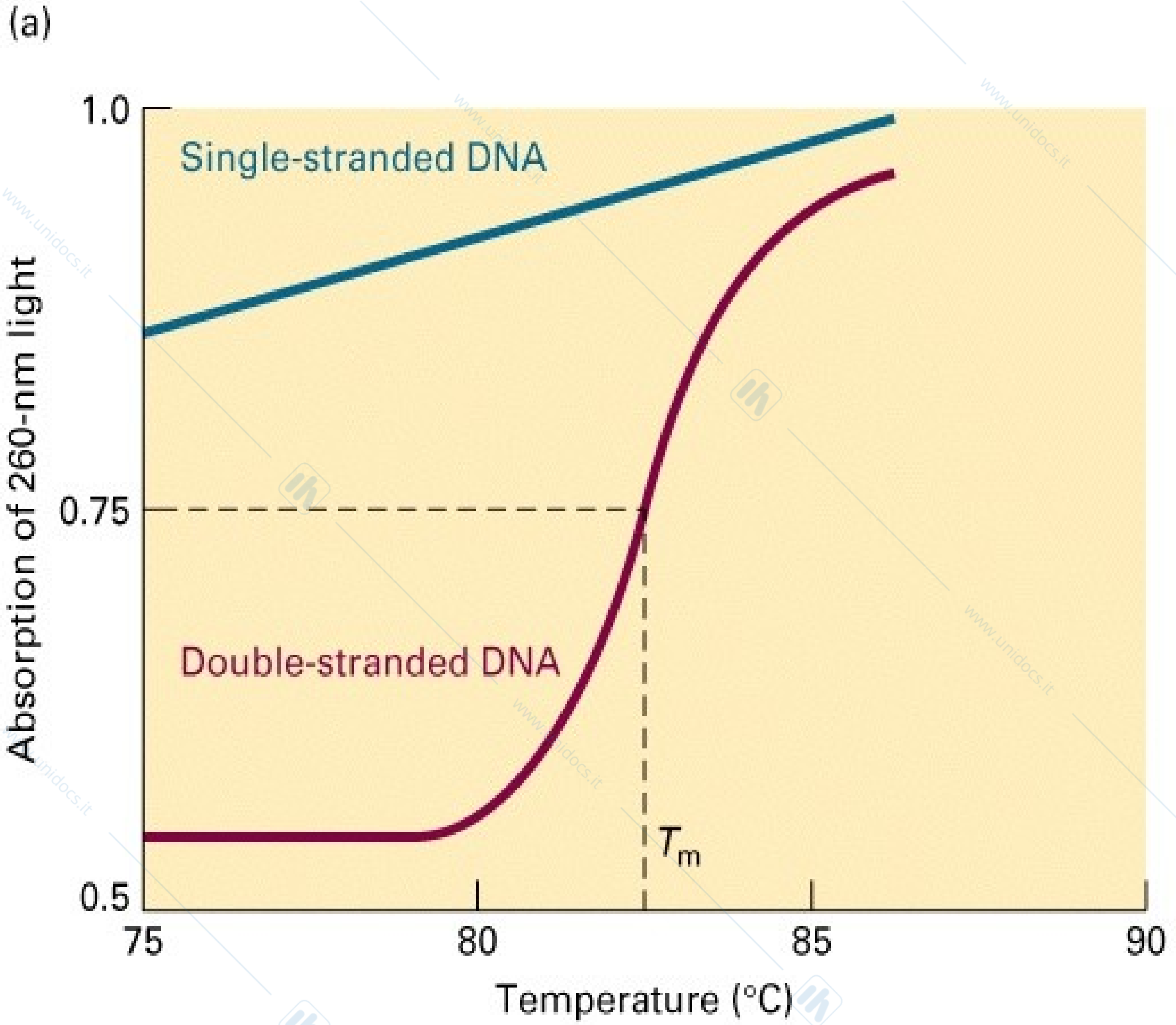
MEASURE OF LIGHT ABSORBANCE



SPECTROPHOTOMETER

DNA ABSORBANCE




(a)
Single-stranded DNA
Double-stranded DNA
Absorption of 260-nm light
1.0
0.75
0.5
75
80
85
90
Temperature (°C)
T_m

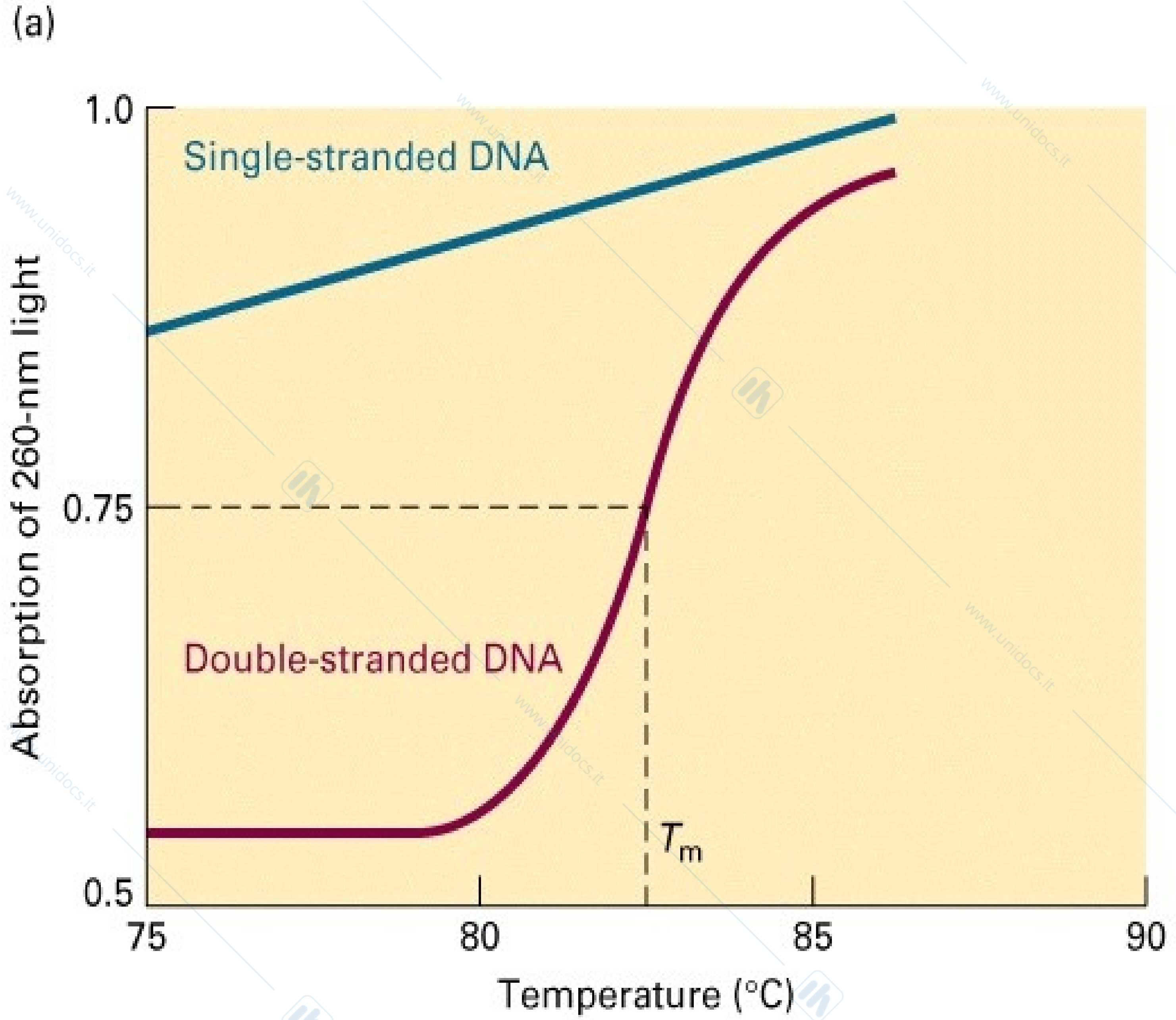

(a)
Single-stranded DNA
Double-stranded DNA
Absorption of 260-nm light
1.0
0.75
0.5
T_m
75
80
85
90
Temperature (°C)

Figure 3.4 A DNA reassociation reaction is described by the $C_0t_{1/2}$

Rate of reaction

The reaction follows the second order equation

$$\frac{dC}{dt} = -kC^2$$

C is the concentration of DNA that is single-stranded at time t
k is a reassociation rate constant.

Progress of reaction

Integrate the rate equation between the limits:
initial concentration of DNA = C_0 at time $t = 0$;
concentration remaining single stranded = C after time t

$$\frac{C}{C_0} = \frac{1}{1 + k.C_0t}$$

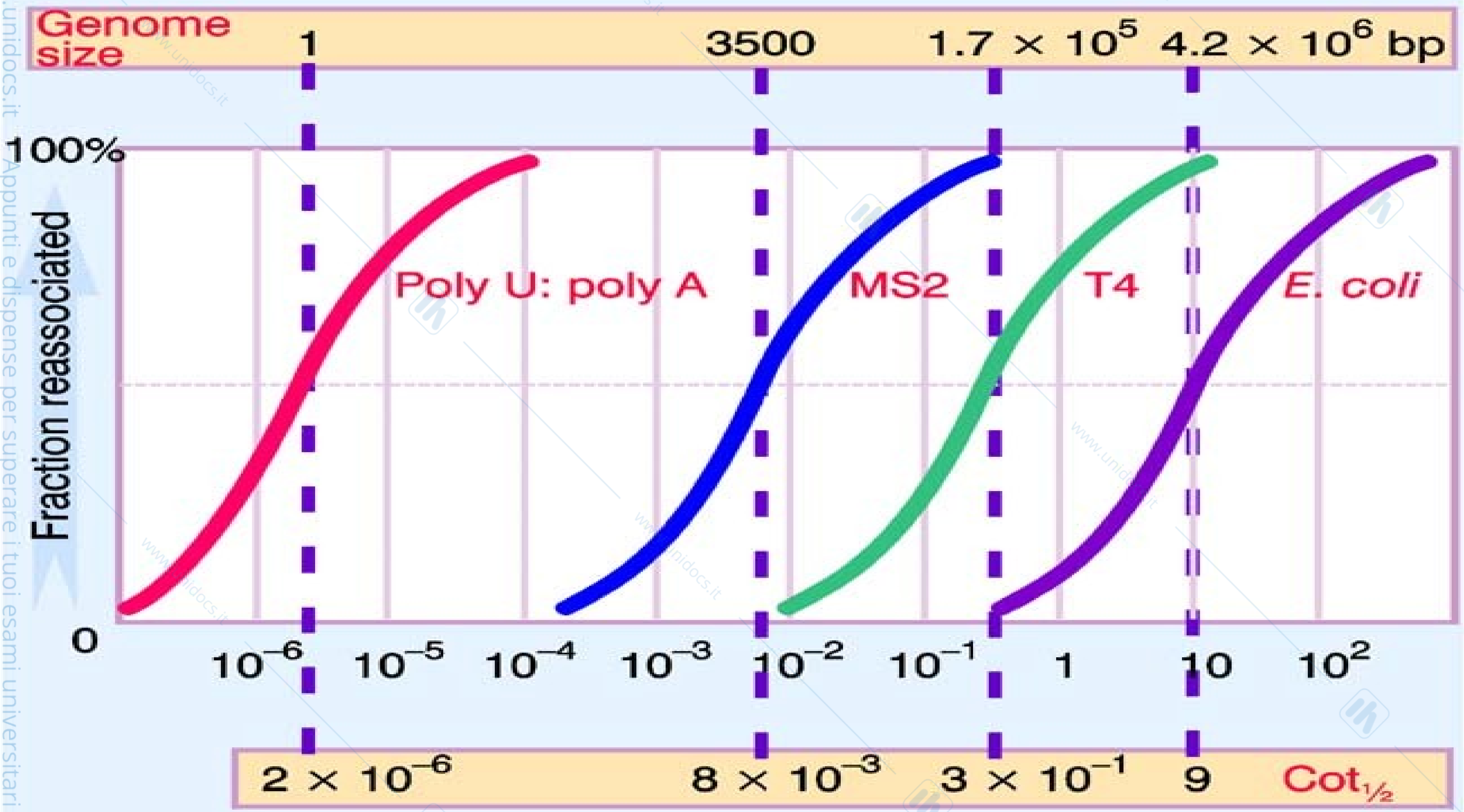
Critical parameter is $C_0t_{1/2}$

When the reaction is half complete at time $t = ½$

$$\frac{C}{C_0} = \frac{1}{2} = \frac{1}{1 + k.C_0t_{1/2}}$$

$$\text{Therefore} \quad C_0t_{1/2} = \frac{1}{k}$$

Figure 3.5 Rate of reassociation is inversely proportional to the length of the reassociating DNA.



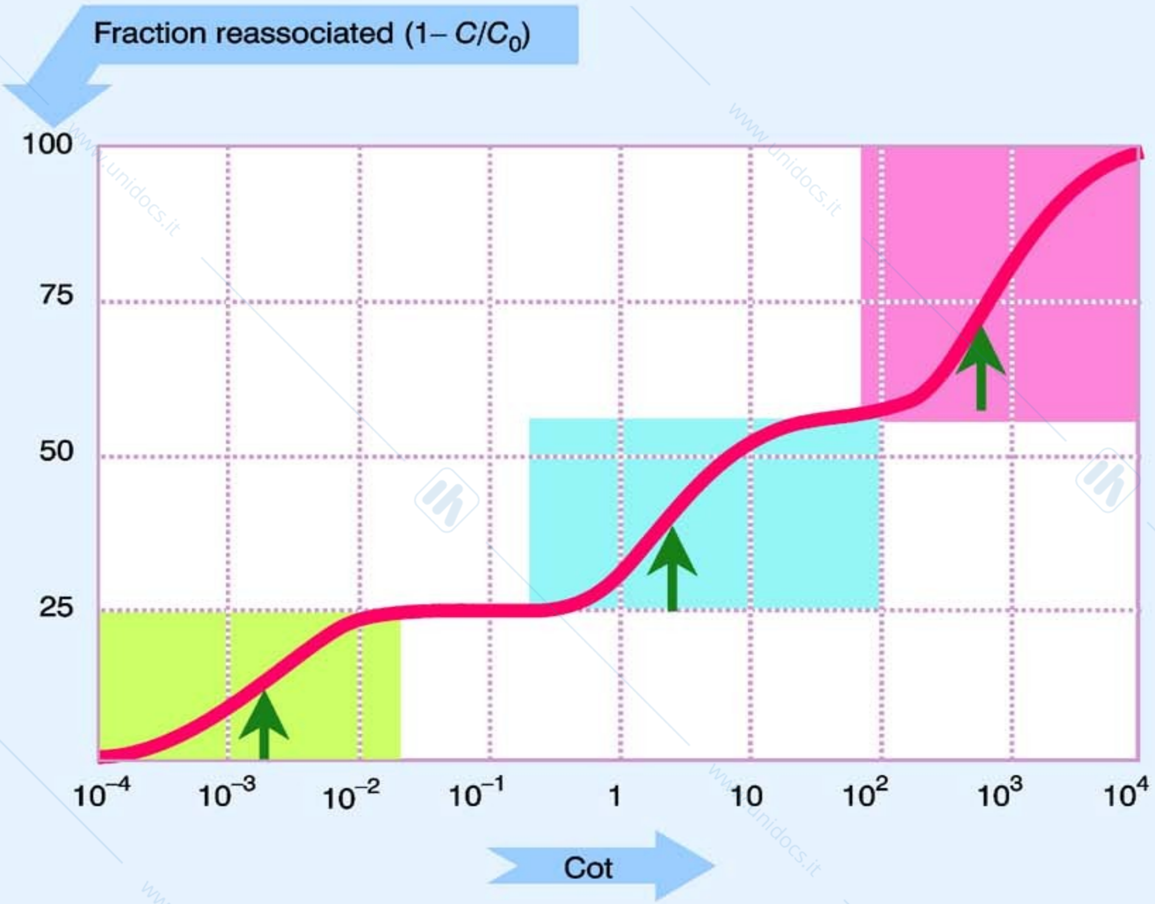
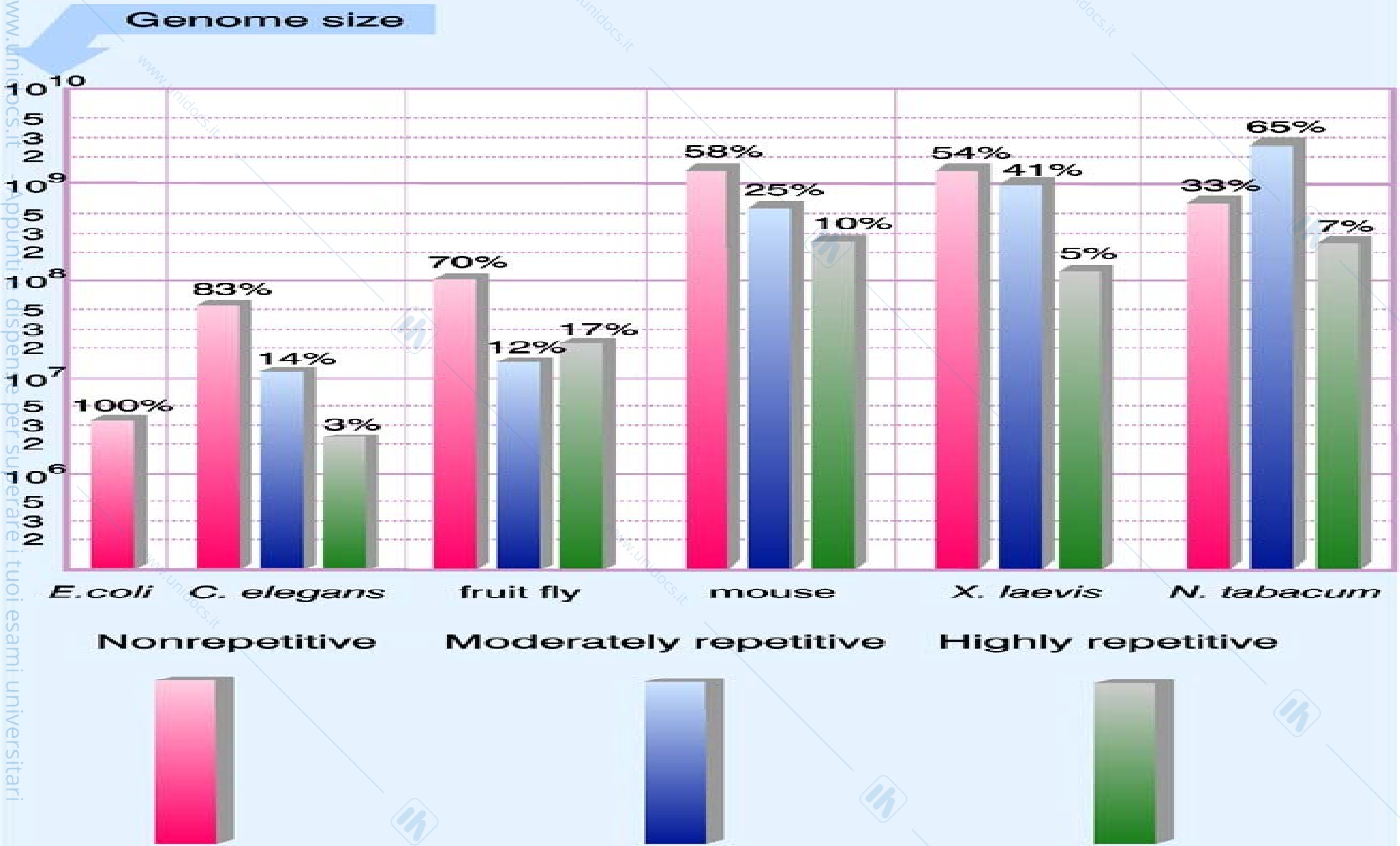
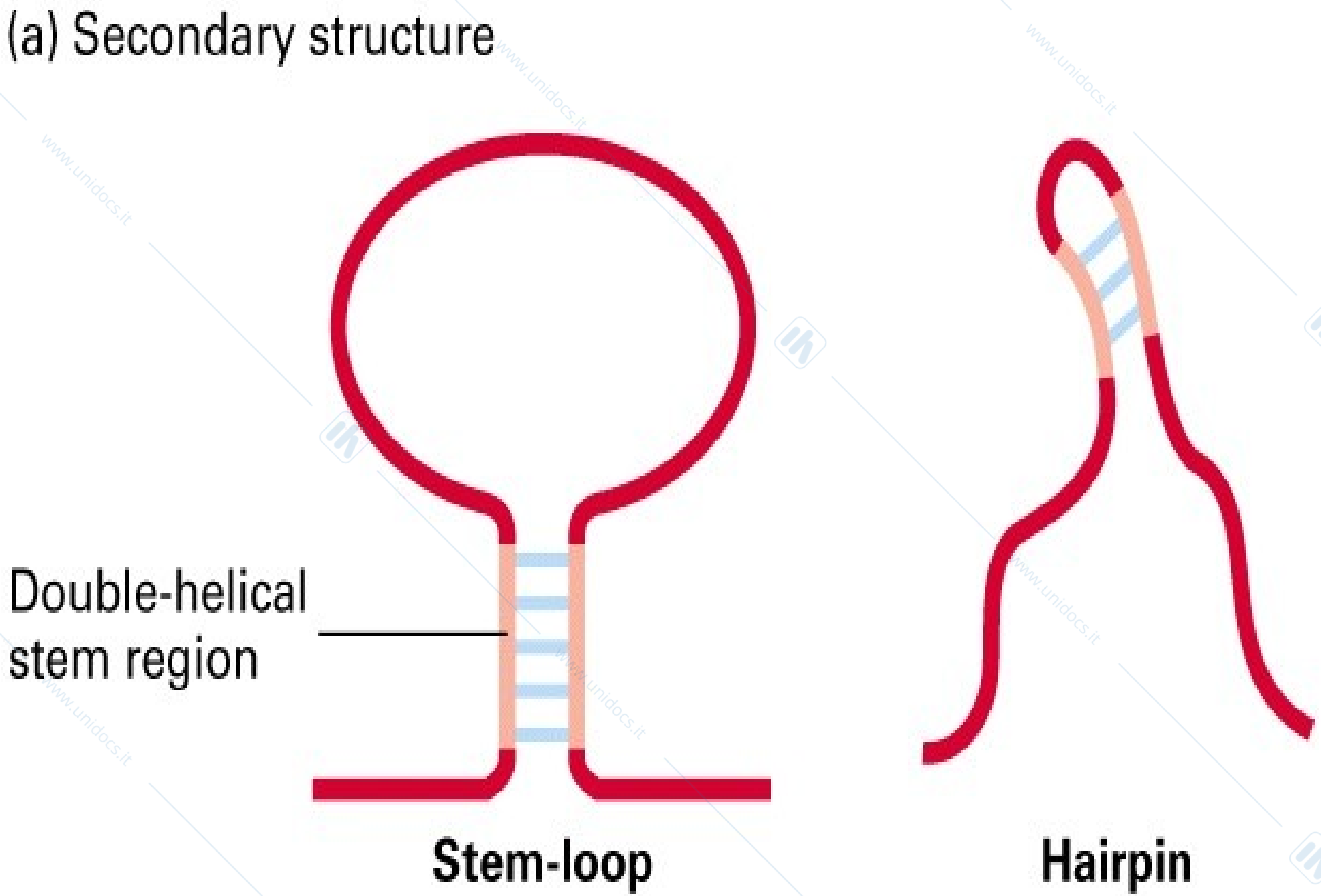


Figure 3.6 The reassociation kinetics of eukaryotic DNA show three types of component (indicated by the shaded areas). The arrows identify the $Cot_{1/2}$ values for each component.

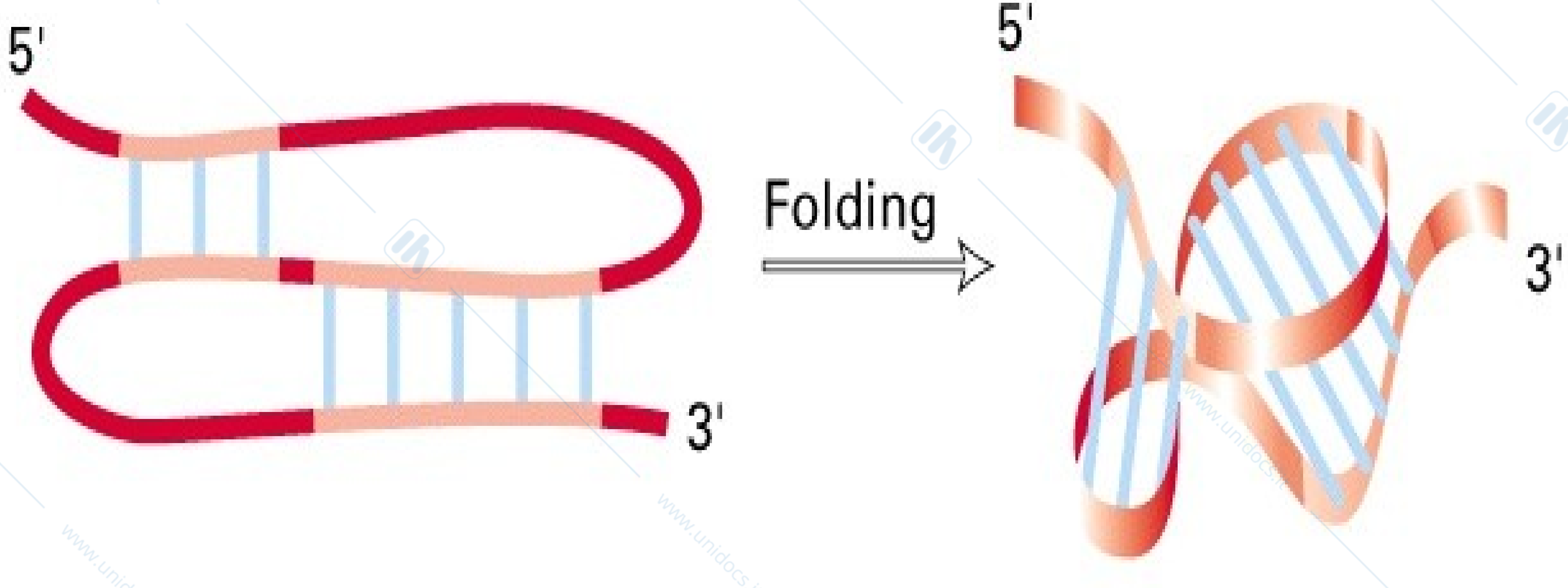
	Fast component	Intermediate component	Slow component
Percent of genome	25	30	45
$Cot_{1/2}$	0.0013	1.9	630
Complexity, bp	340	6.0×10^5	3.0×10^8
Repetition frequency	500,000	350	1

Figure 3.8 The proportions of different sequence components vary in eukaryotic genomes. The absolute content of nonrepetitive DNA increases with genome size, but reaches a plateau at ~2 × 10^9 bp.

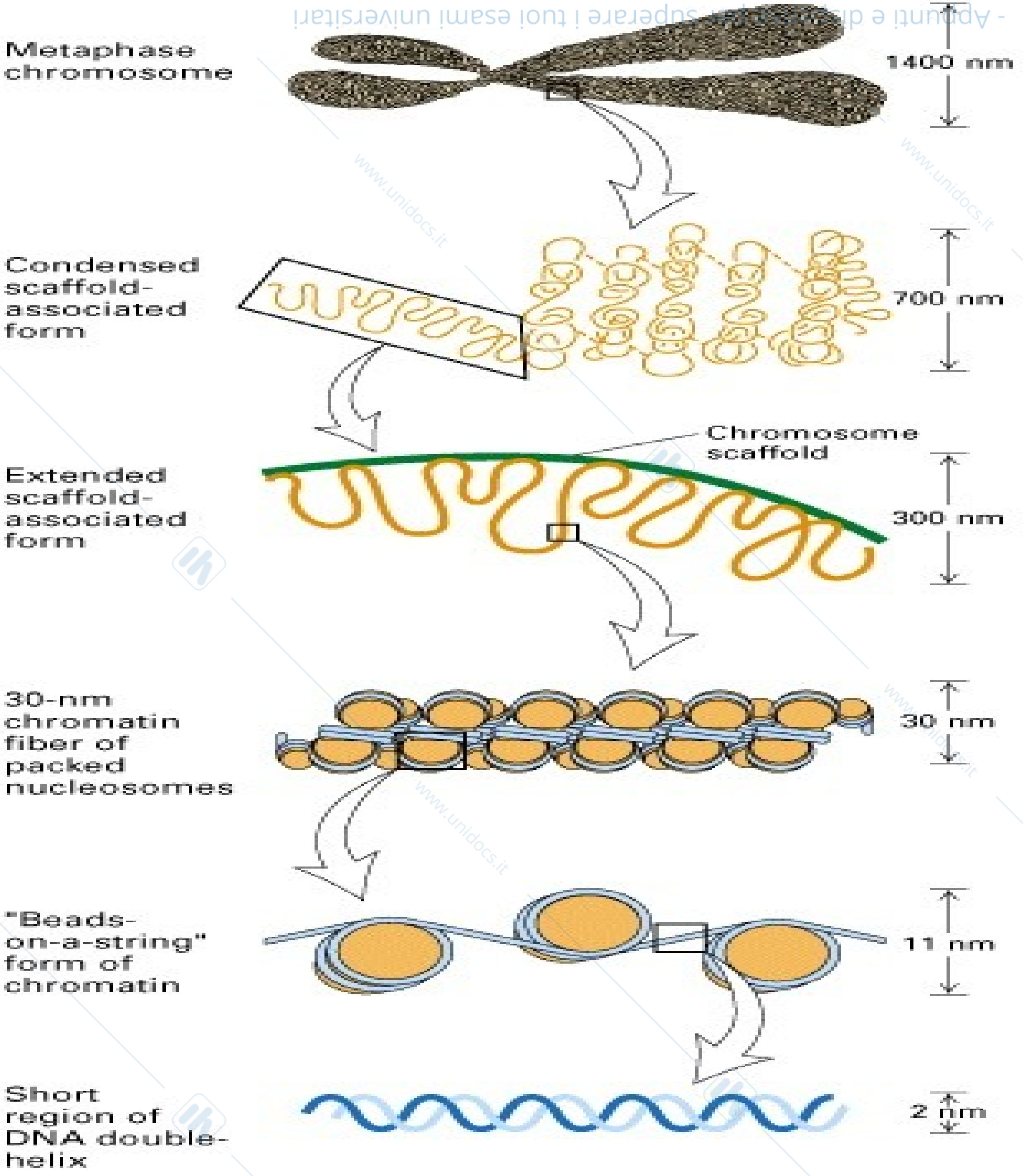



(a) Secondary structure
Double-helical stem region
Stem-loop
Hairpin

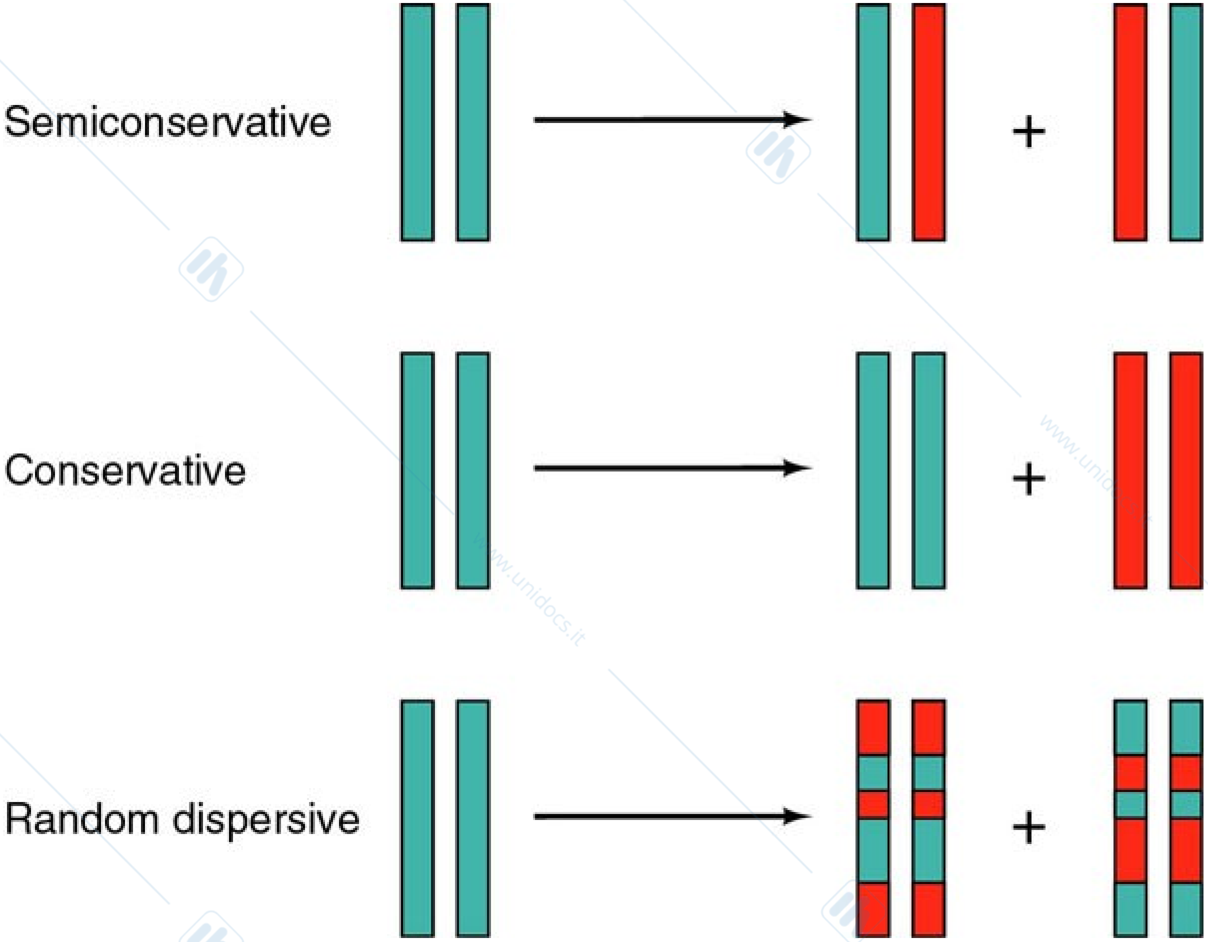
(b) Tertiary structure



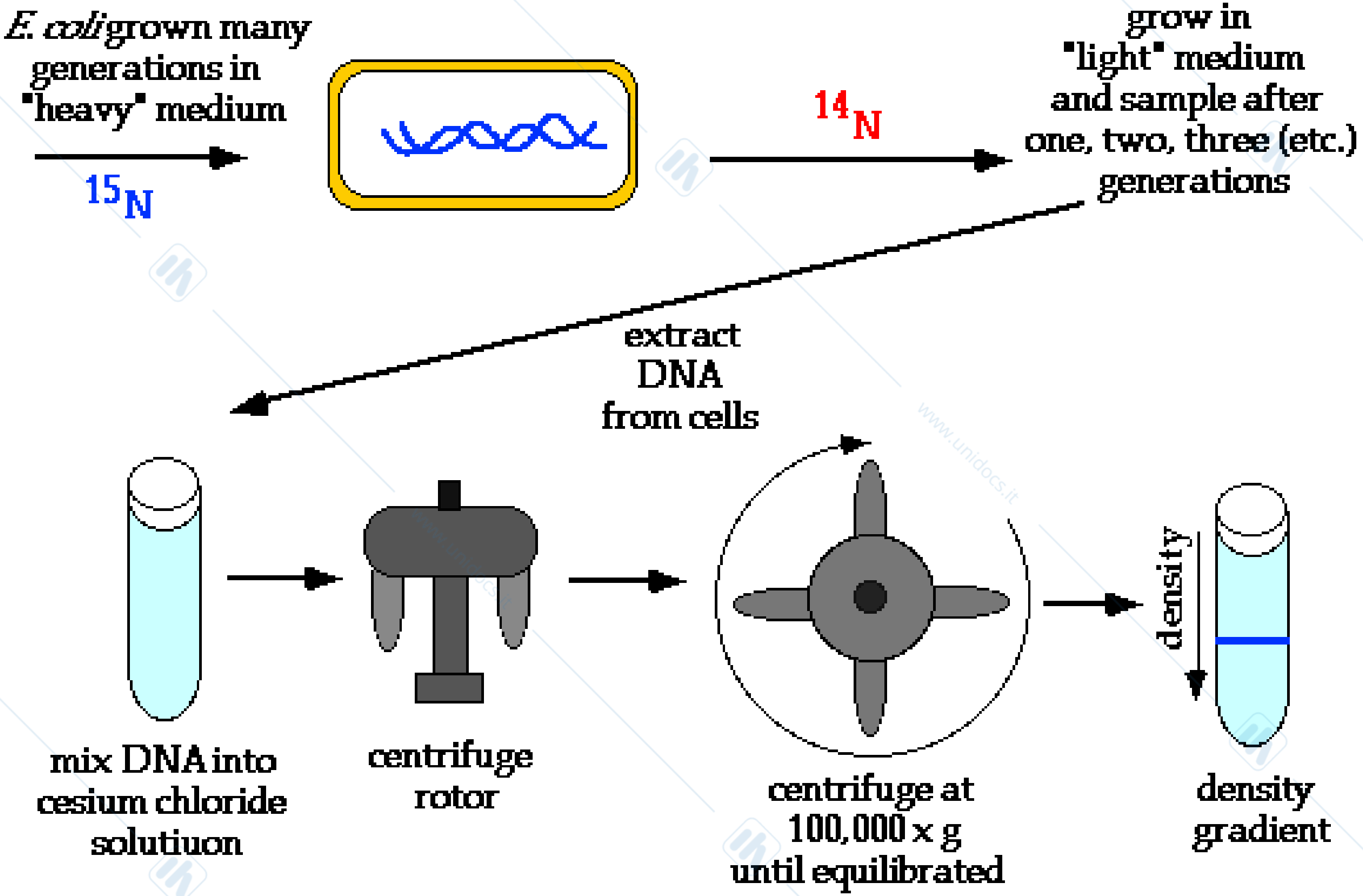
Pseudoknot


Metaphase chromosome
1400 nm
Condensed scaffold-associated form
700 nm
Chromosome scaffold
Extended scaffold-associated form
300 nm
30-nm chromatin fiber of packed nucleosomes
30 nm
"Beads-on-a-string" form of chromatin
11 nm
Short region of DNA double-helix
2 nm

Possible Mechanisms of DNA Replication



Meselson and Stahl



E. coli grown many generations in "heavy" medium

^{15}N

^{14}N

grow in "light" medium and sample after one, two, three (etc.) generations

extract DNA from cells

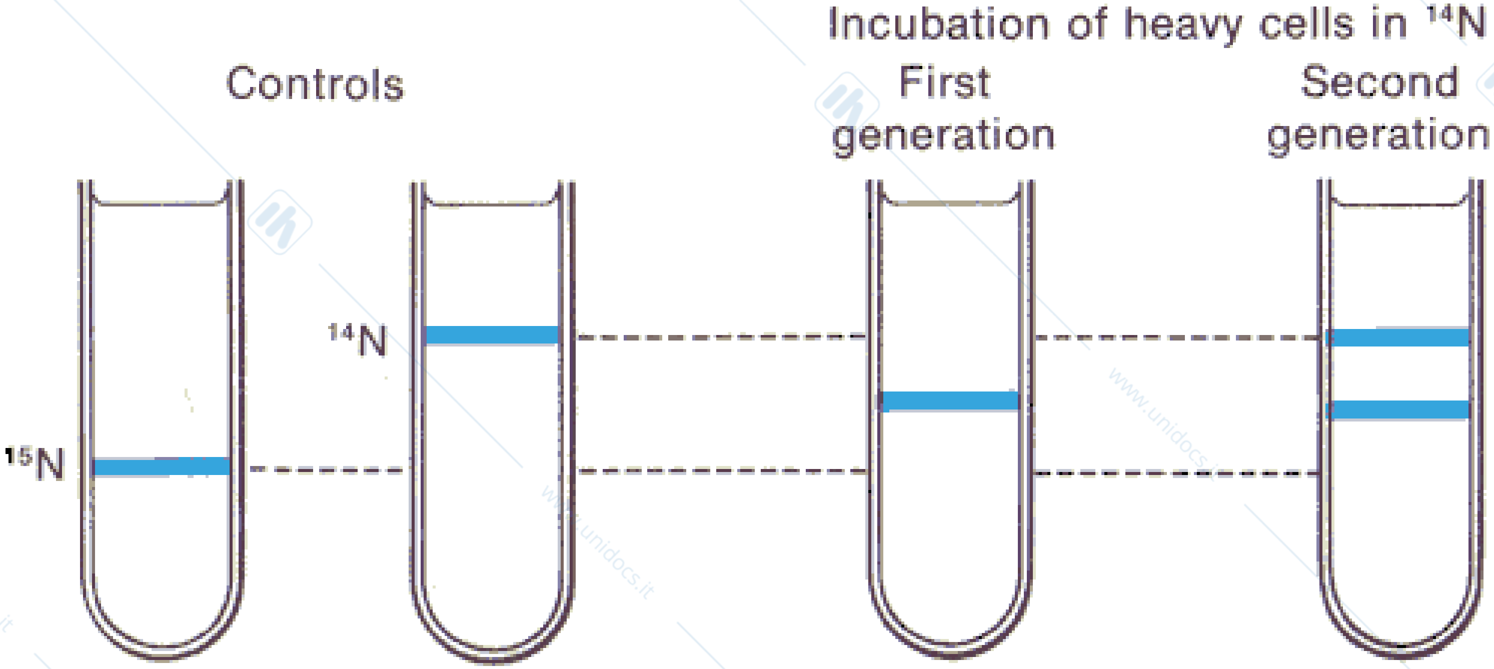
mix DNA into cesium chloride solutiuon

centrifuge rotor

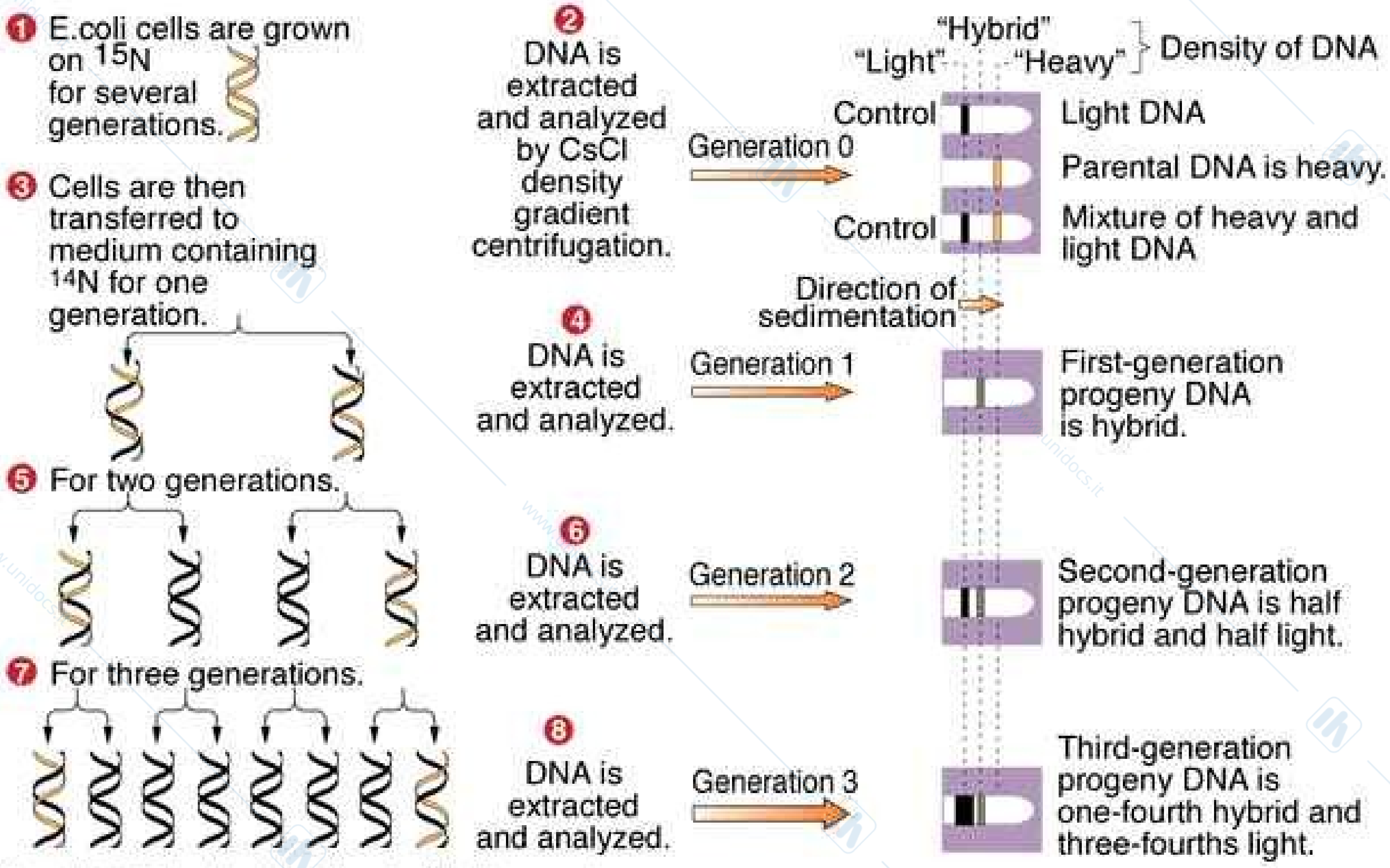
centrifuge at 100,000 x g until equilibrated

density gradient

Meselson and Stahl



Meselson and Stahl



① E.coli cells are grown on ^{15}N for several generations.

② DNA is extracted and analyzed by CsCl density gradient centrifugation.

③ Cells are then transferred to medium containing ^{14}N for one generation.

④ DNA is extracted and analyzed.

⑤ For two generations.

⑥ DNA is extracted and analyzed.

⑦ For three generations.

⑧ DNA is extracted and analyzed.

Meselson and Stahl

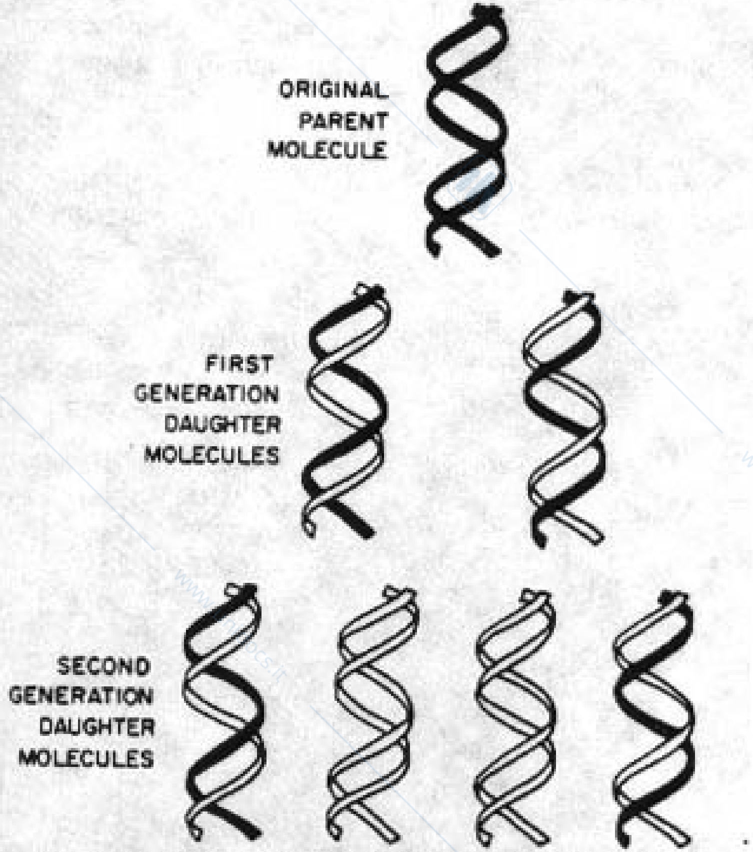
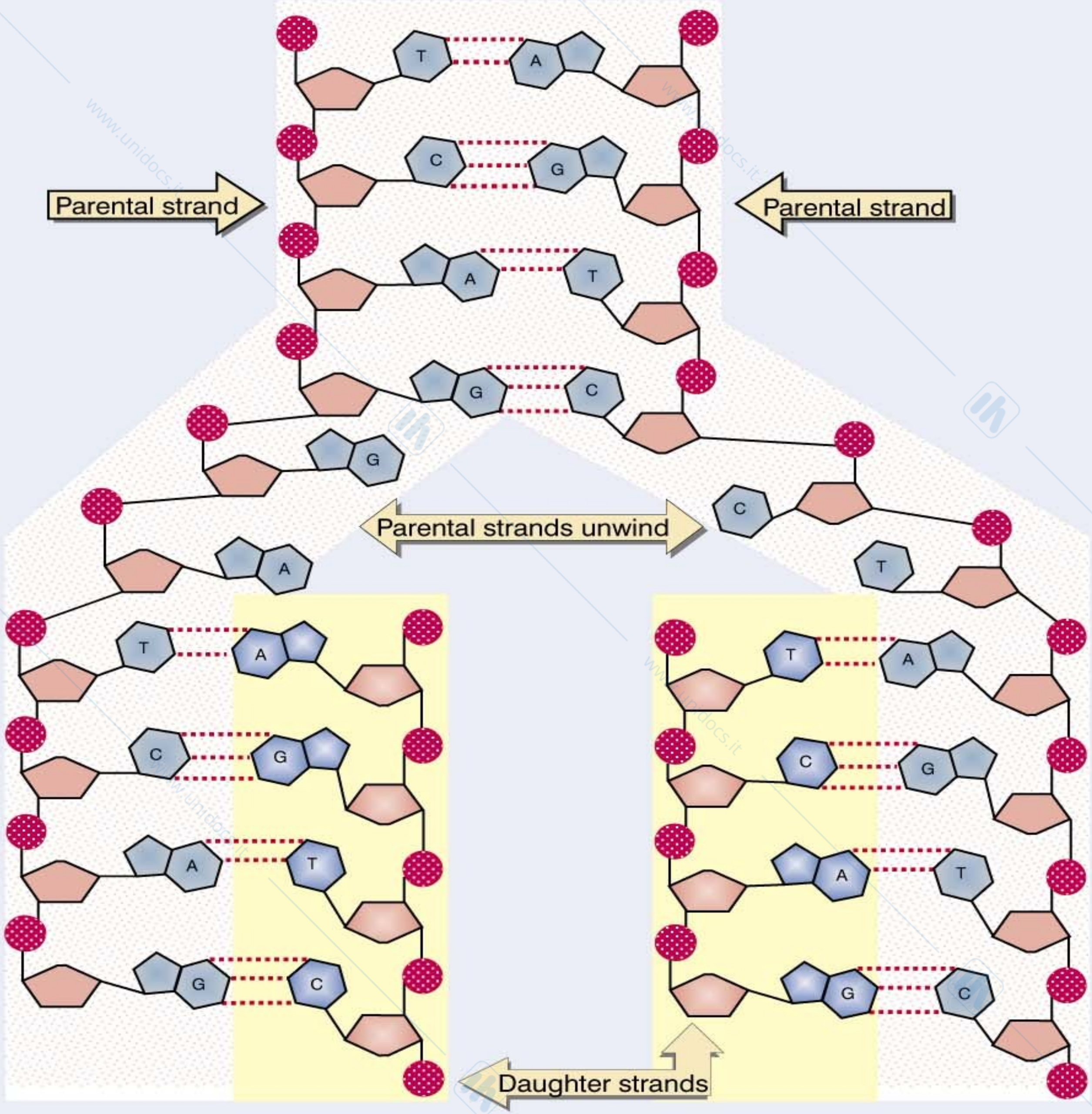
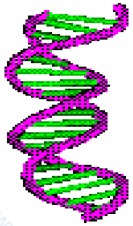


FIG. 6.—Illustration of the mechanism of DNA duplication proposed by Watson and Crick. Each daughter molecule contains one of the parental chains (*black*) paired with one new chain (*white*). Upon continued duplication, the two original parent chains remain intact, so that there will always be found two molecules each with one parental chain.

Figure 1.9 Base pairing provides the mechanism for replicating DNA.





Replication Machinery

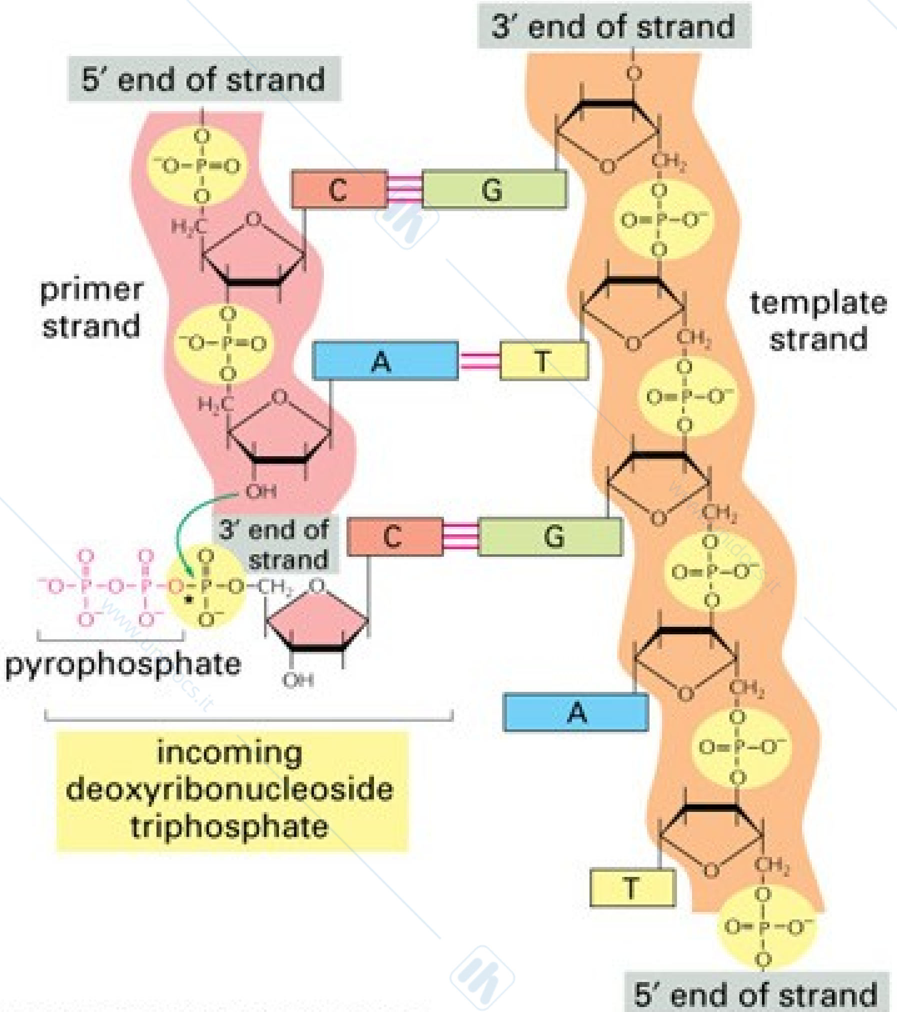
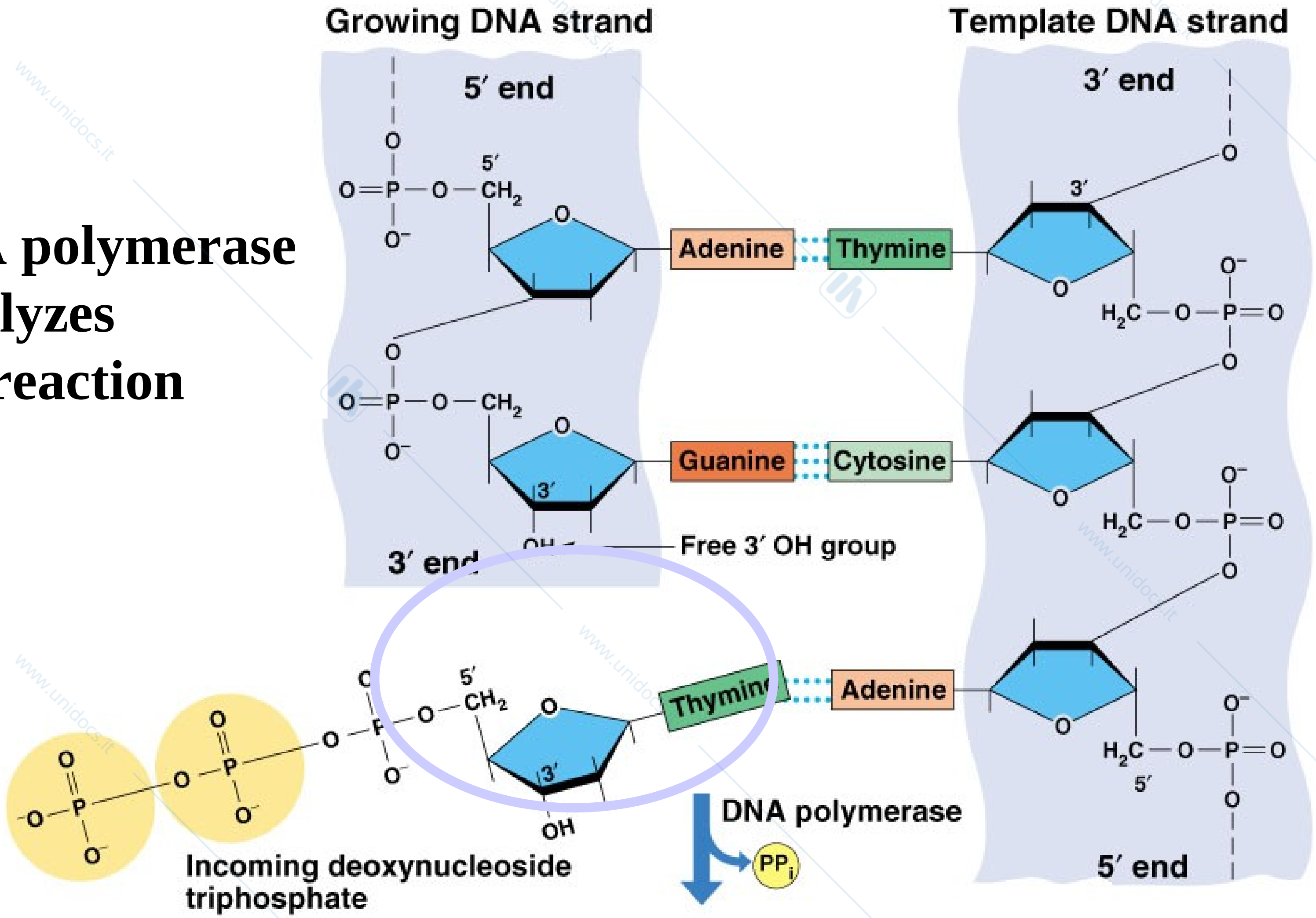


Figure 6-10 Essential Cell Biology, 2/e. (© 2004 Garland Science)

DNA Replication

DNA polymerase catalyzes this reaction



DNA Replication Proteins

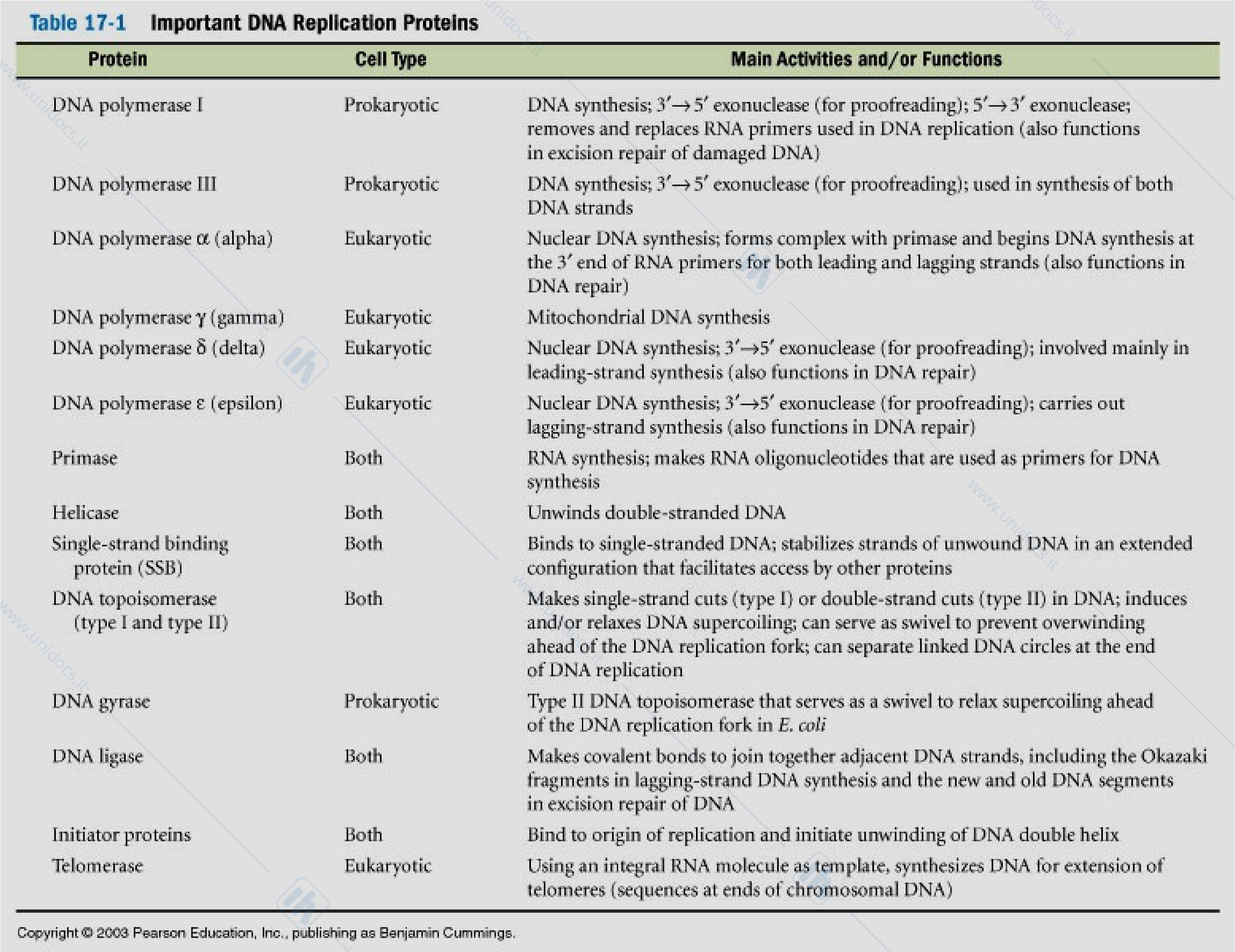


Table 17-1 Important DNA Replication Proteins

Protein	Cell Type	Main Activities and/or Functions
DNA polymerase I	Prokaryotic	DNA synthesis; 3′→5′ exonuclease (for proofreading); 5′→3′ exonuclease; removes and replaces RNA primers used in DNA replication (also functions in excision repair of damaged DNA)
DNA polymerase III	Prokaryotic	DNA synthesis; 3′→5′ exonuclease (for proofreading); used in synthesis of both DNA strands
DNA polymerase α (alpha)	Eukaryotic	Nuclear DNA synthesis; forms complex with primase and begins DNA synthesis at the 3′ end of RNA primers for both leading and lagging strands (also functions in DNA repair)
DNA polymerase γ (gamma)	Eukaryotic	Mitochondrial DNA synthesis
DNA polymerase δ (delta)	Eukaryotic	Nuclear DNA synthesis; 3′→5′ exonuclease (for proofreading); involved mainly in leading-strand synthesis (also functions in DNA repair)
DNA polymerase ε (epsilon)	Eukaryotic	Nuclear DNA synthesis; 3′→5′ exonuclease (for proofreading); carries out lagging-strand synthesis (also functions in DNA repair)
Primase	Both	RNA synthesis; makes RNA oligonucleotides that are used as primers for DNA synthesis
Helicase	Both	Unwinds double-stranded DNA
Single-strand binding protein (SSB)	Both	Binds to single-stranded DNA; stabilizes strands of unwound DNA in an extended configuration that facilitates access by other proteins
DNA topoisomerase (type I and type II)	Both	Makes single-strand cuts (type I) or double-strand cuts (type II) in DNA; induces and/or relaxes DNA supercoiling; can serve as swivel to prevent overwinding ahead of the DNA replication fork; can separate linked DNA circles at the end of DNA replication
DNA gyrase	Prokaryotic	Type II DNA topoisomerase that serves as a swivel to relax supercoiling ahead of the DNA replication fork in *E. coli*
DNA ligase	Both	Makes covalent bonds to join together adjacent DNA strands, including the Okazaki fragments in lagging-strand DNA synthesis and the new and old DNA segments in excision repair of DNA
Initiator proteins	Both	Bind to origin of replication and initiate unwinding of DNA double helix
Telomerase	Eukaryotic	Using an integral RNA molecule as template, synthesizes DNA for extension of telomeres (sequences at ends of chromosomal DNA)

Figure 12.1 Replicated DNA is seen as a replication eye flanked by nonreplicated DNA.

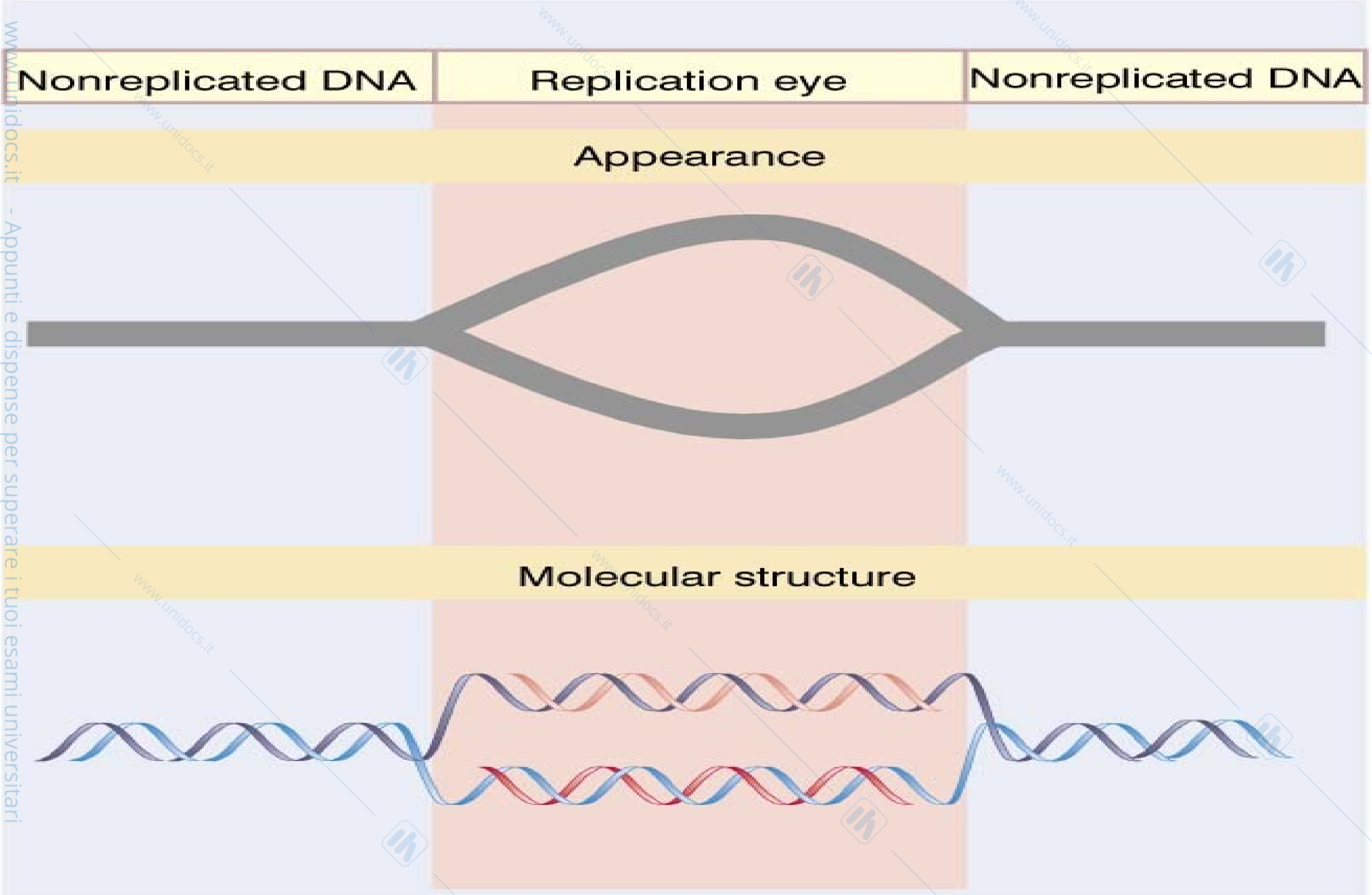
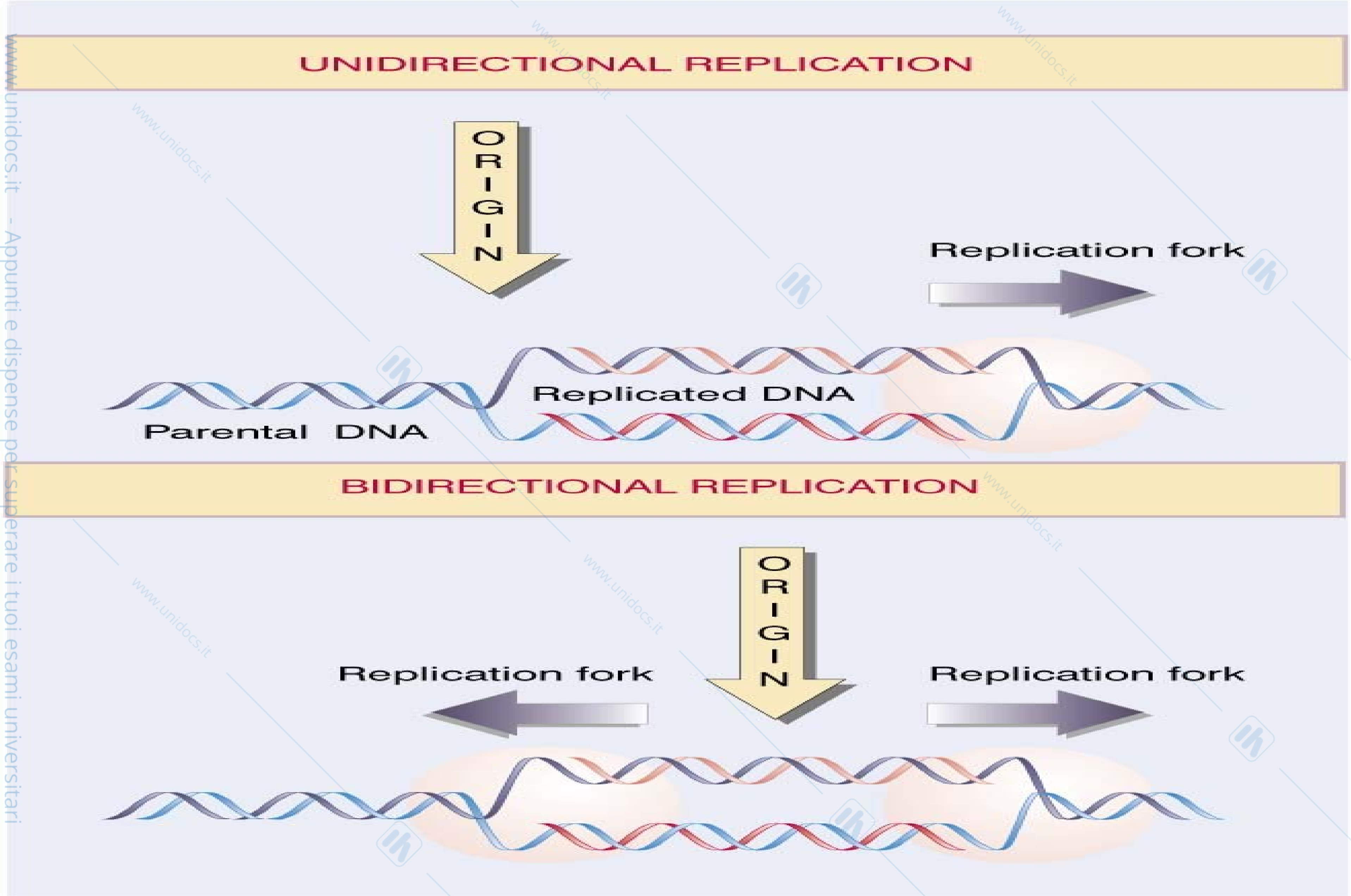
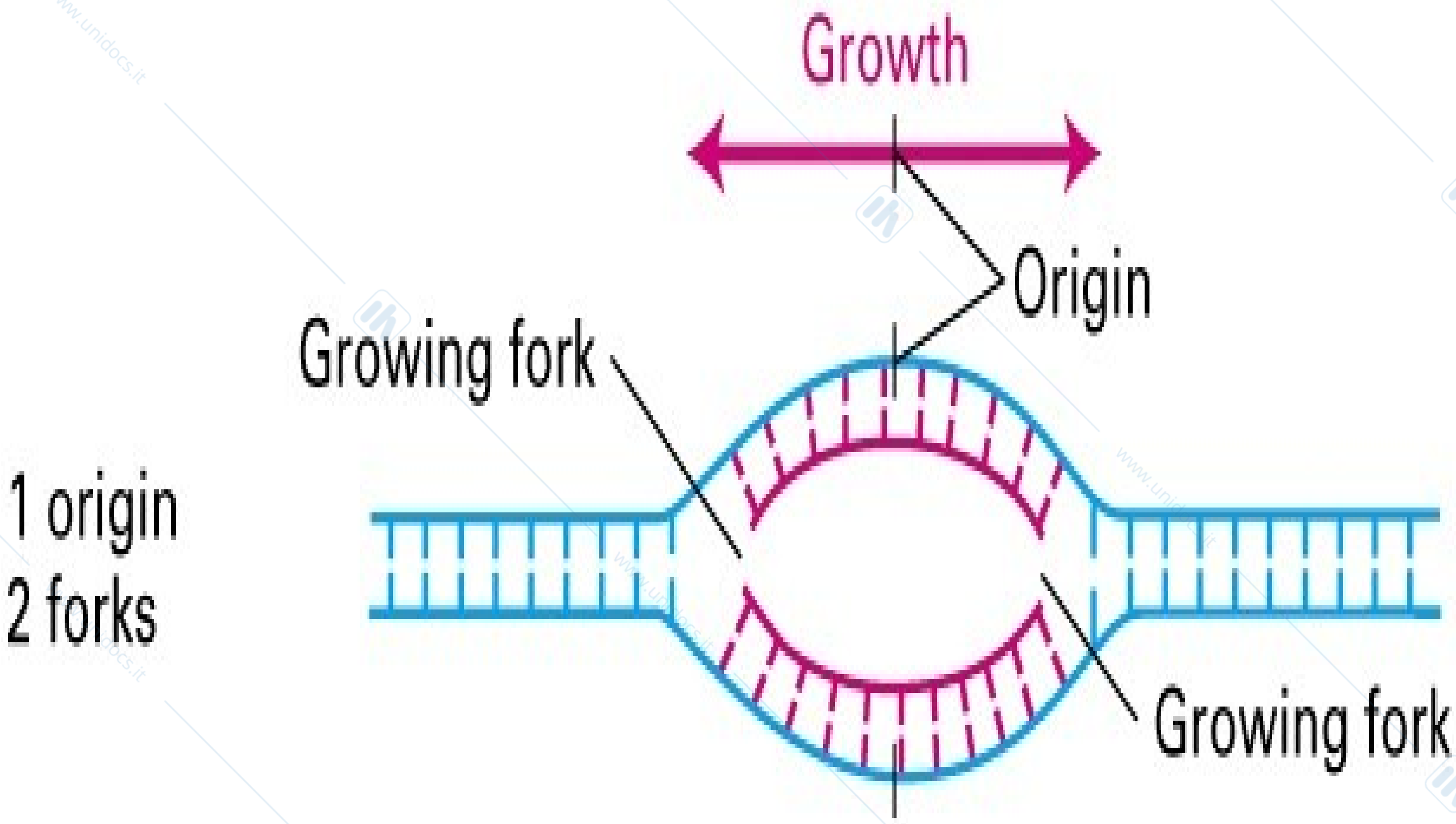
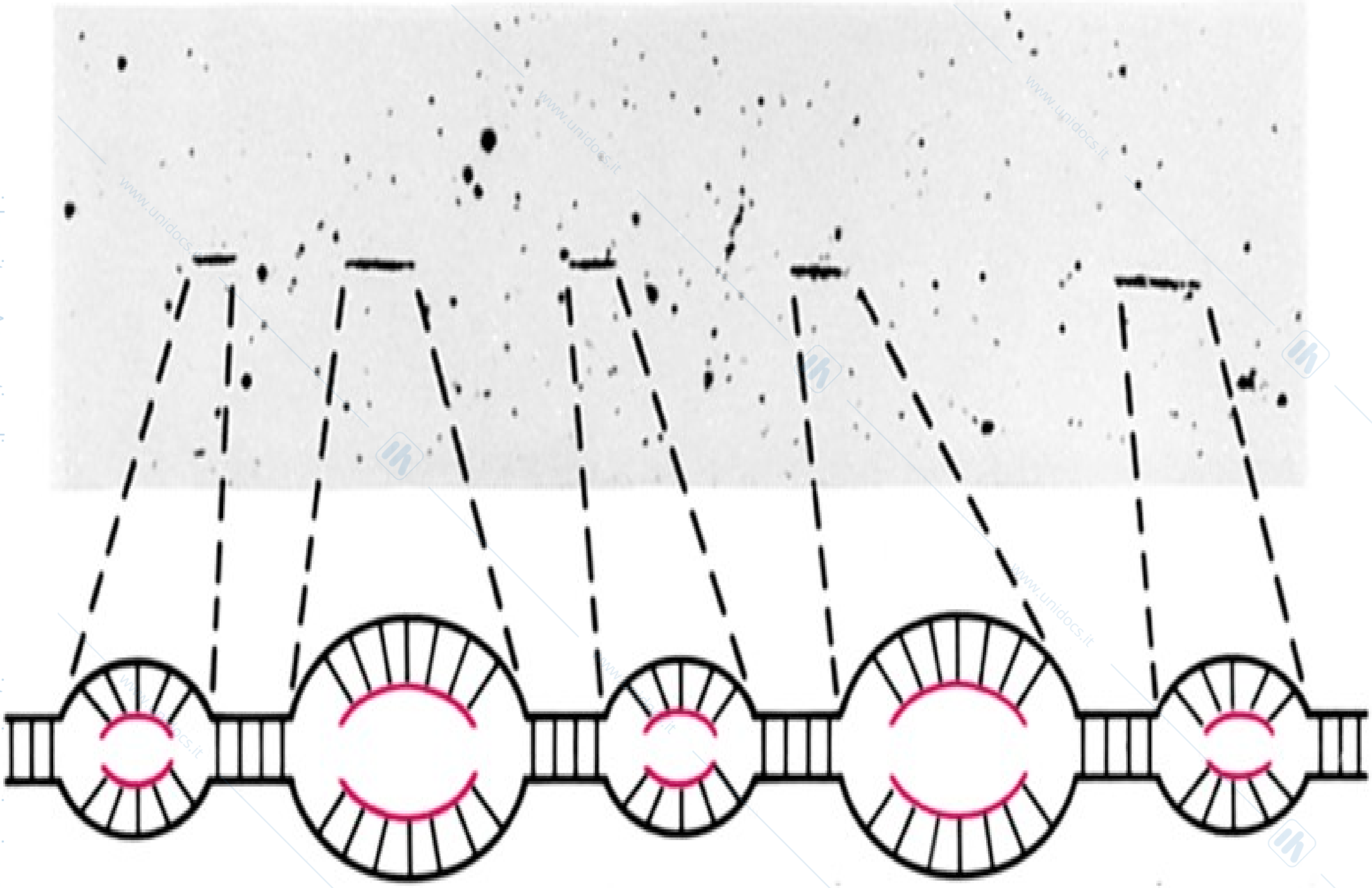


Figure 12.2 Replicons may be unidirectional or bidirectional, depending on whether one or two replication forks are formed at the origin.

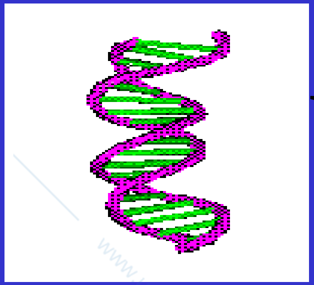


(c) Bidirectional growth of both strands from one origin



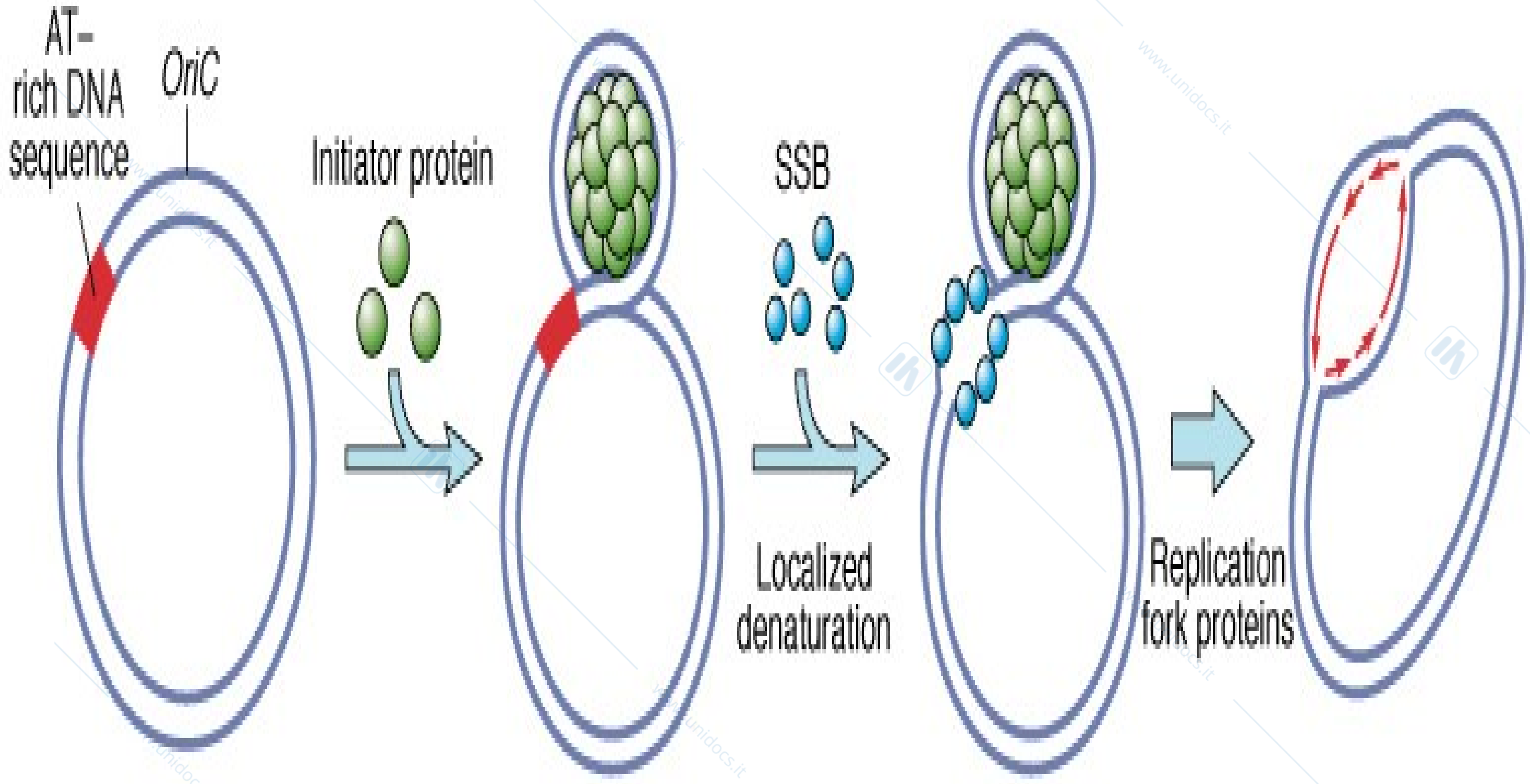


Micrograph courtesy of Joel Huberman.

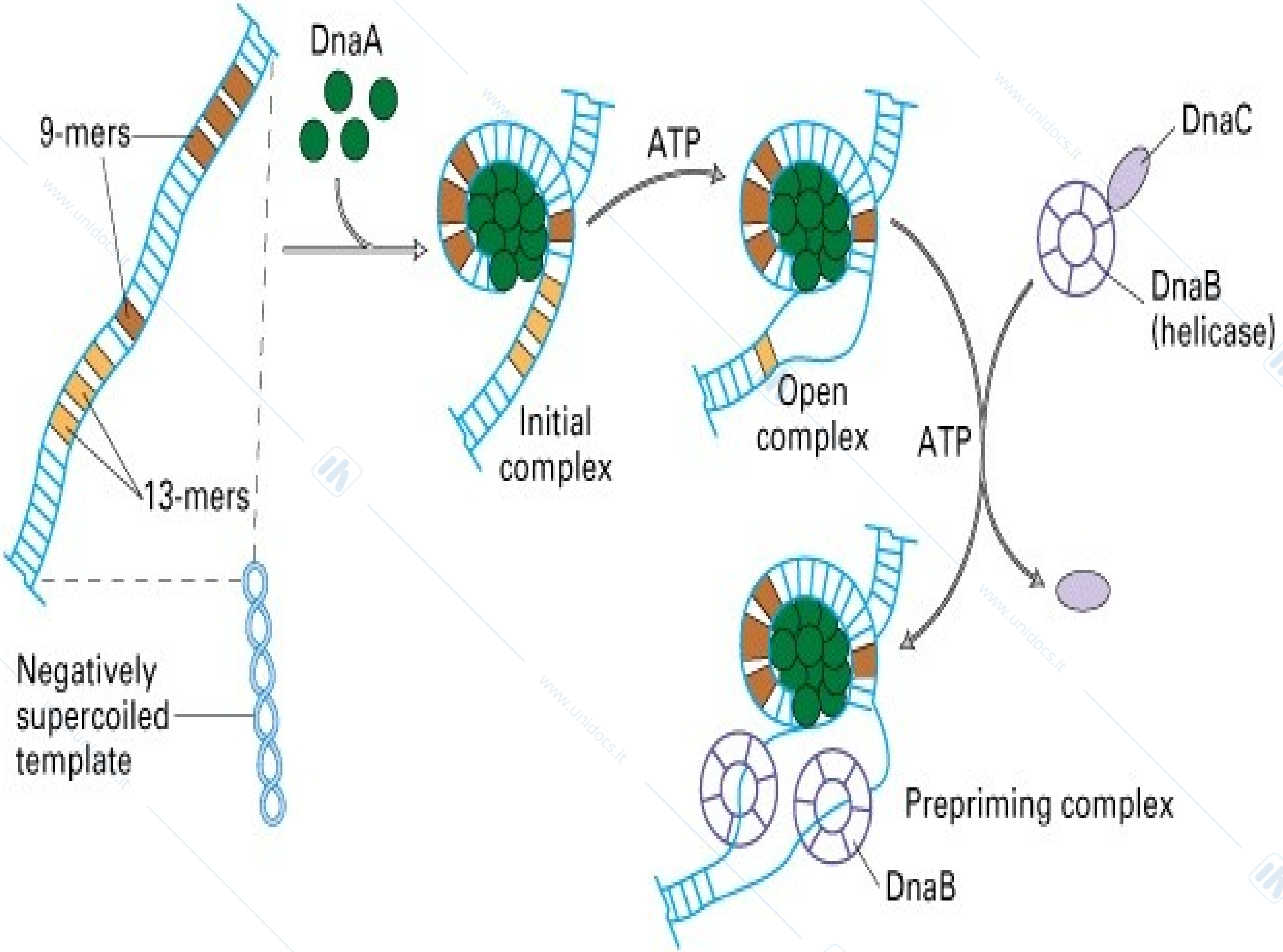


uantitative Parameters (after Mathews and vanHolde)

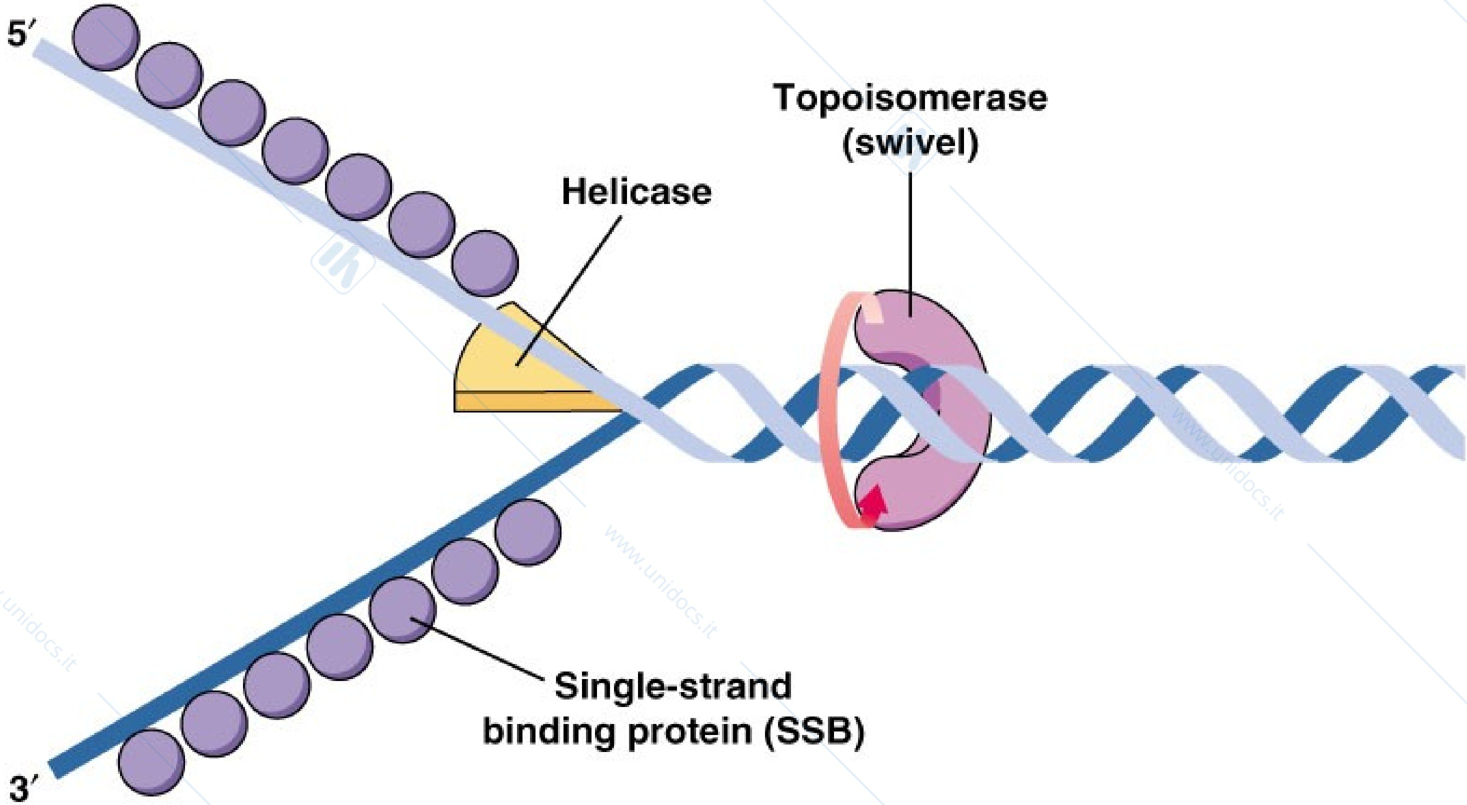
Feature	Prokarotic (*E. coli*)	Eukaryotic (Hela cells in culture)
DNA content (nucleotide pairs per cell)	**3.9×10^{6}**	**$\sim 10^{9}$**
DNA replication rate (nucleotides/sec/rep. fork)	**850**	**60-90**
Number of replication origins/cell	**1**	**10^{3} - 10^{4}**
Hours for complete genome replication	**0.67**	**8**

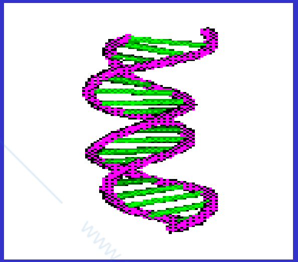


After K. J. Marians, reproduced with permission from the *Annual Review of Biochemistry*, volume 61, © 1992, by Annual Reviews Inc.

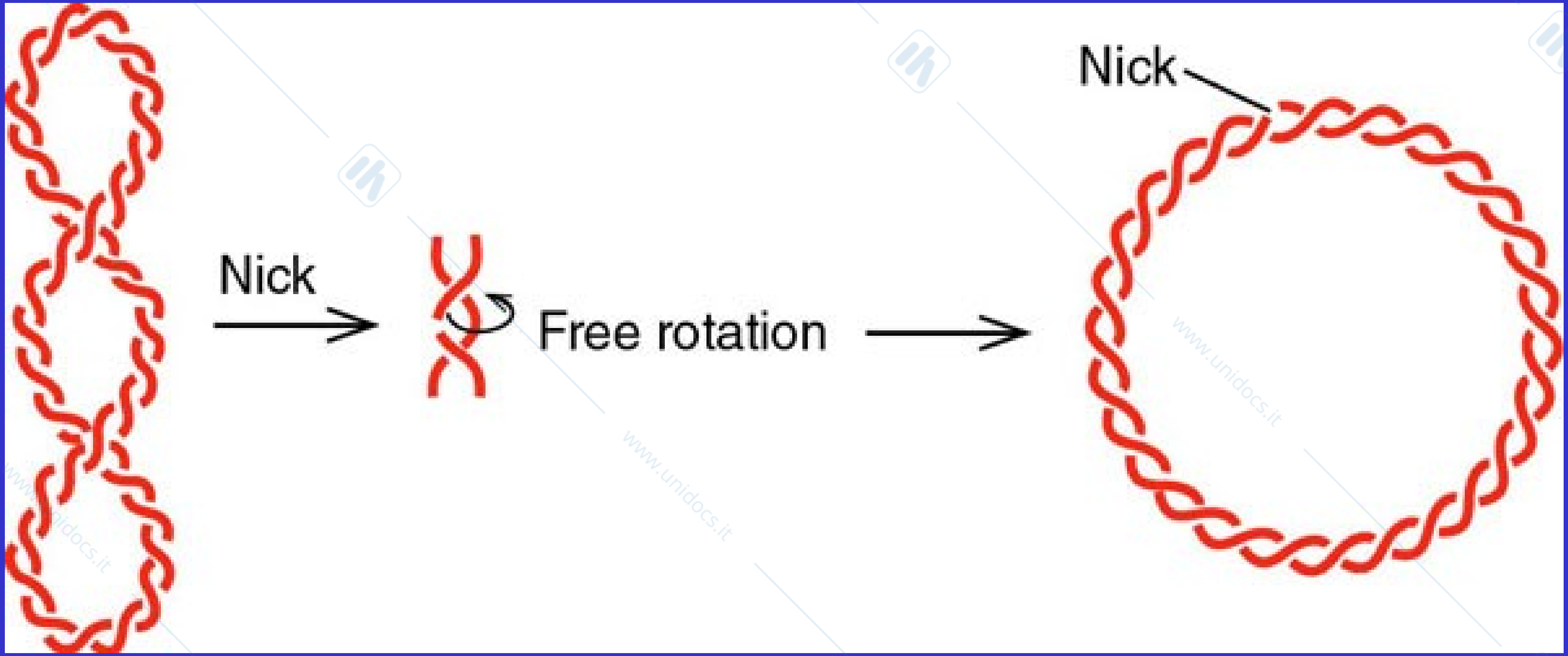

DnaA
9-mers
13-mers
Negatively supercoiled template
ATP
Initial complex
Open complex
DnaC
DnaB (helicase)
ATP
Prepriming complex
DnaB

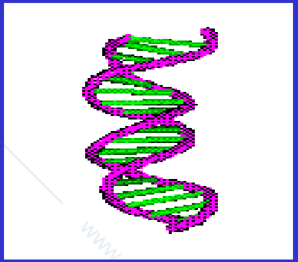
Unwinding of DNA at Replication Fork



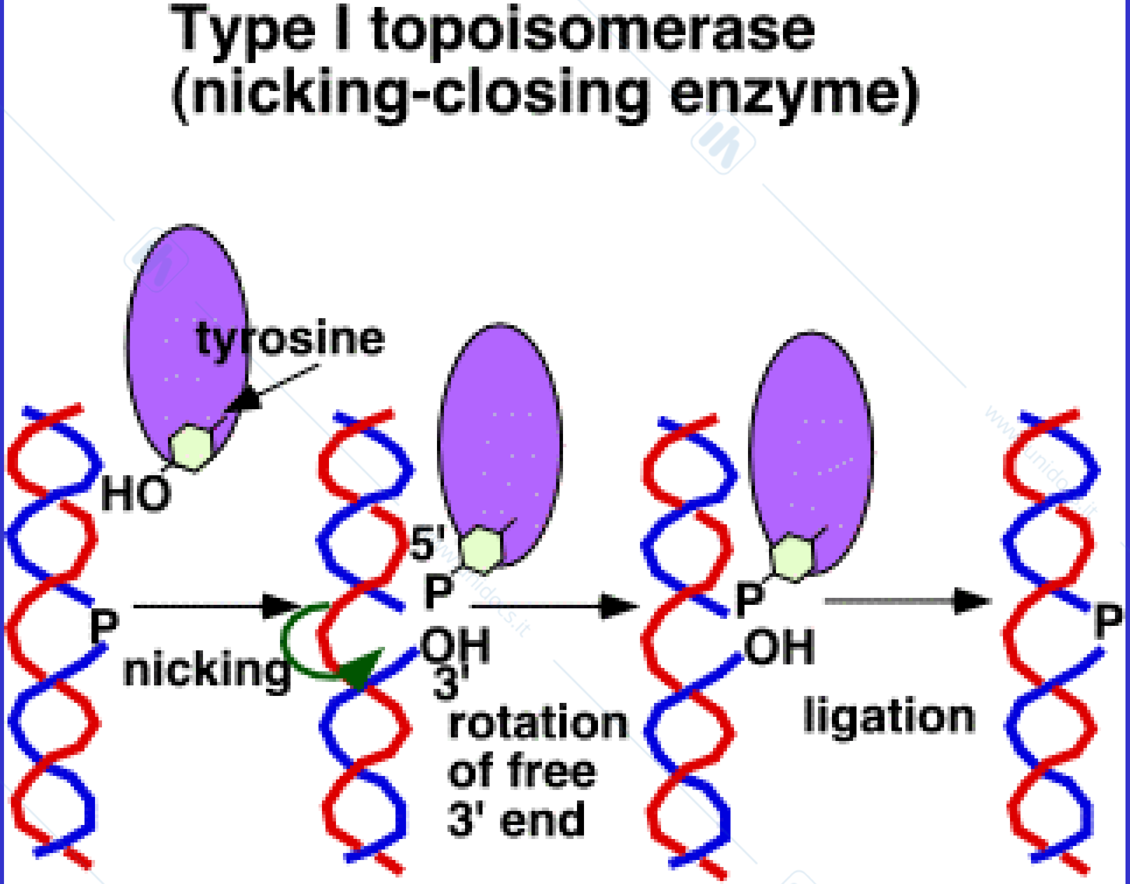


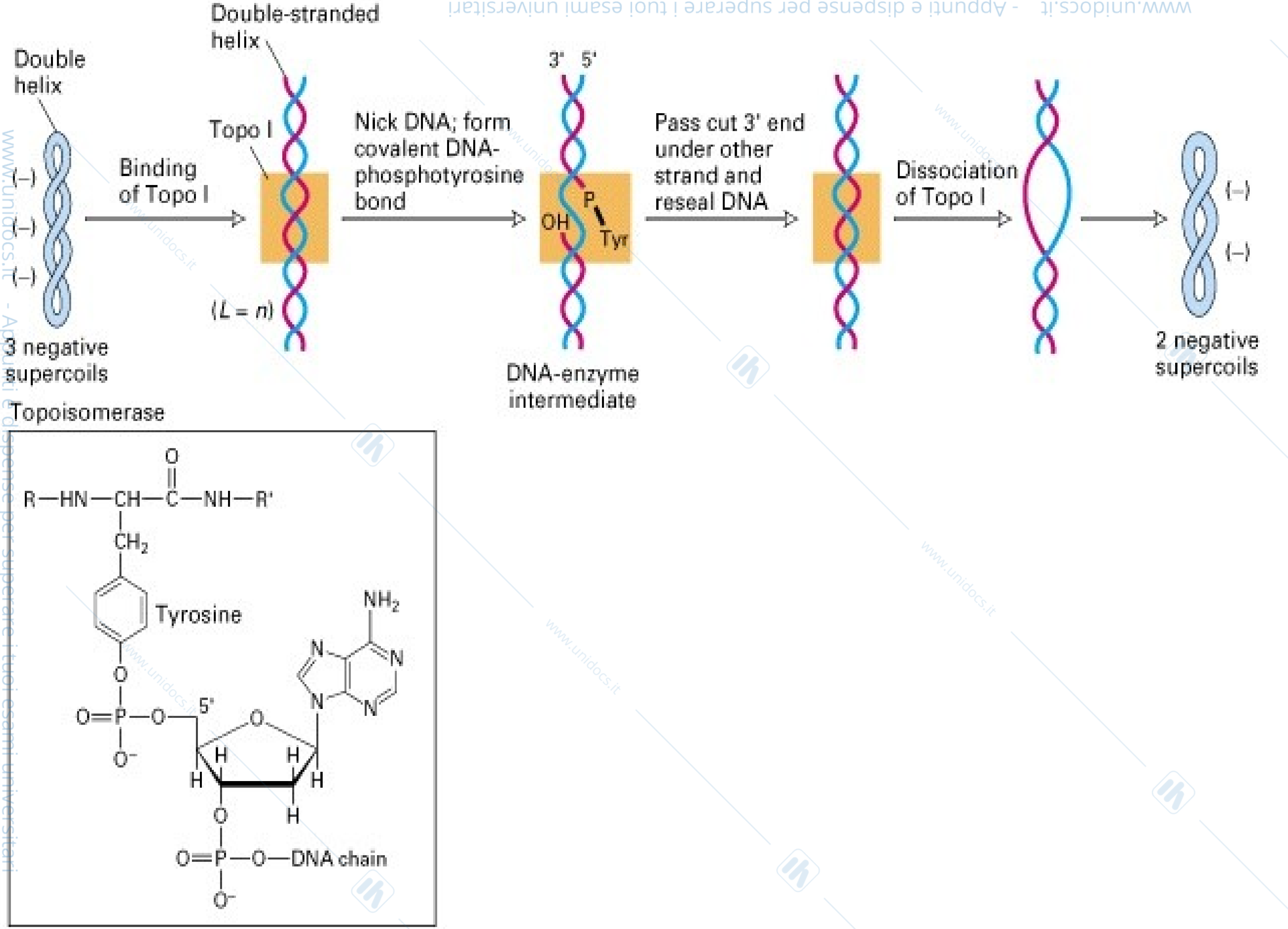
Topoisomerase I



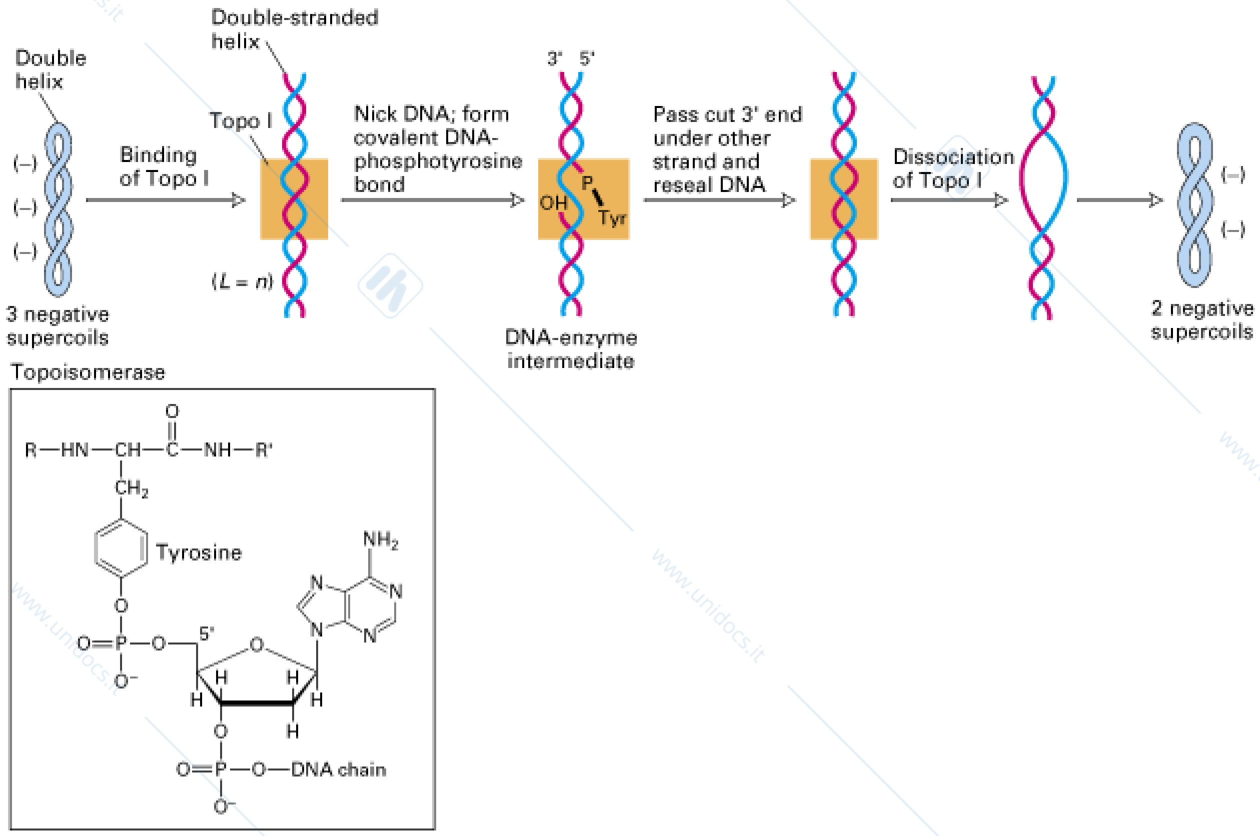


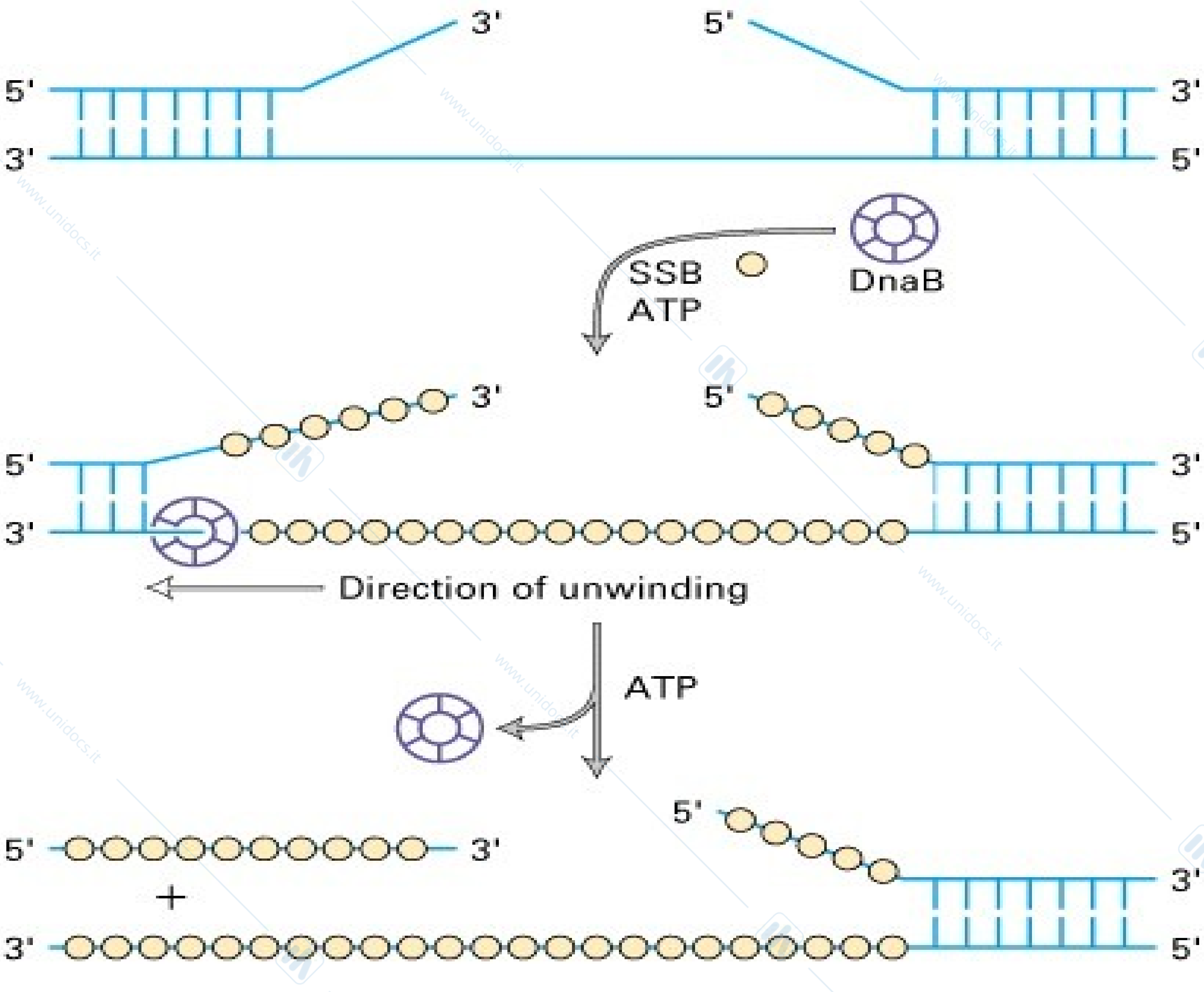
Topoisomerase I

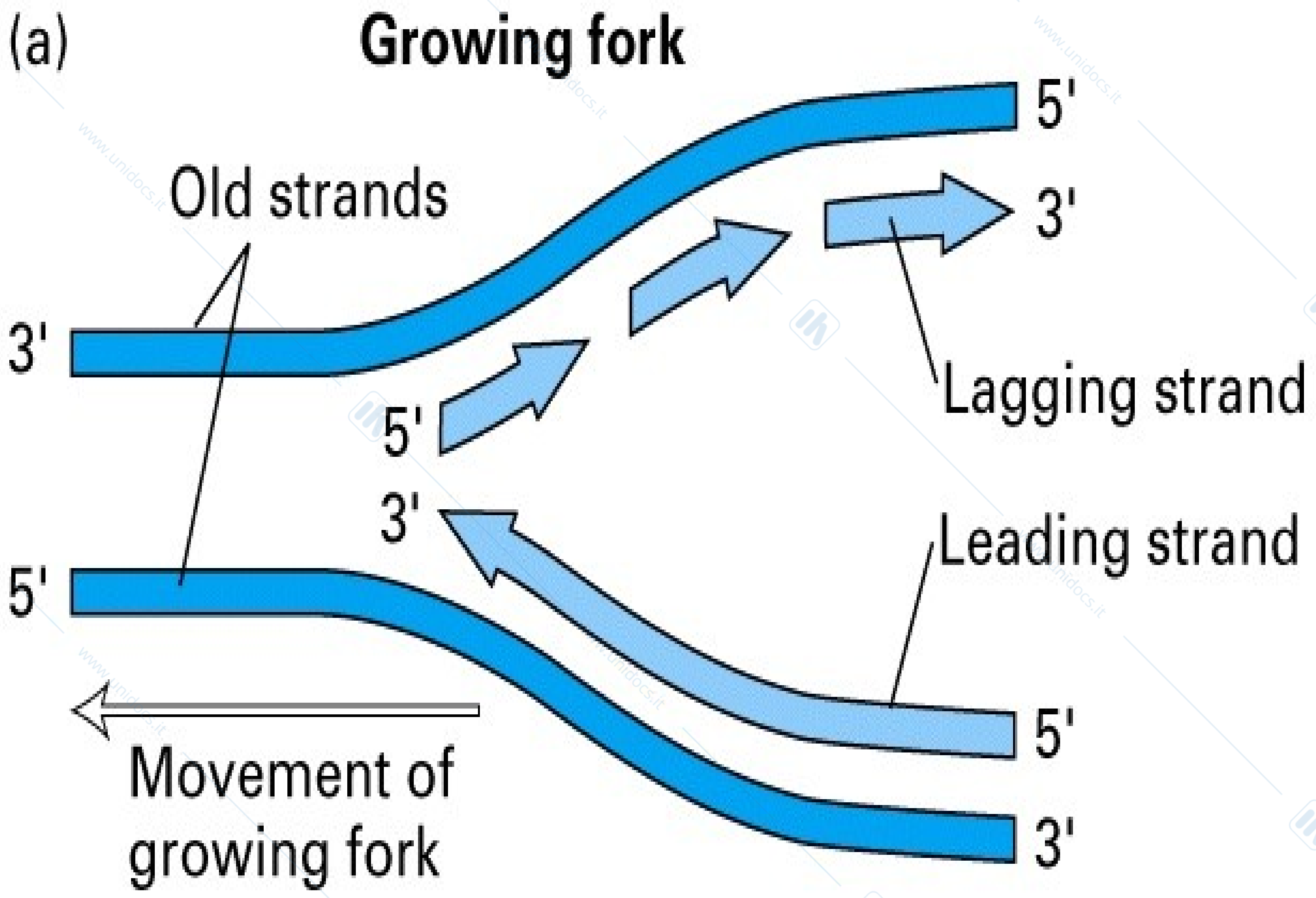


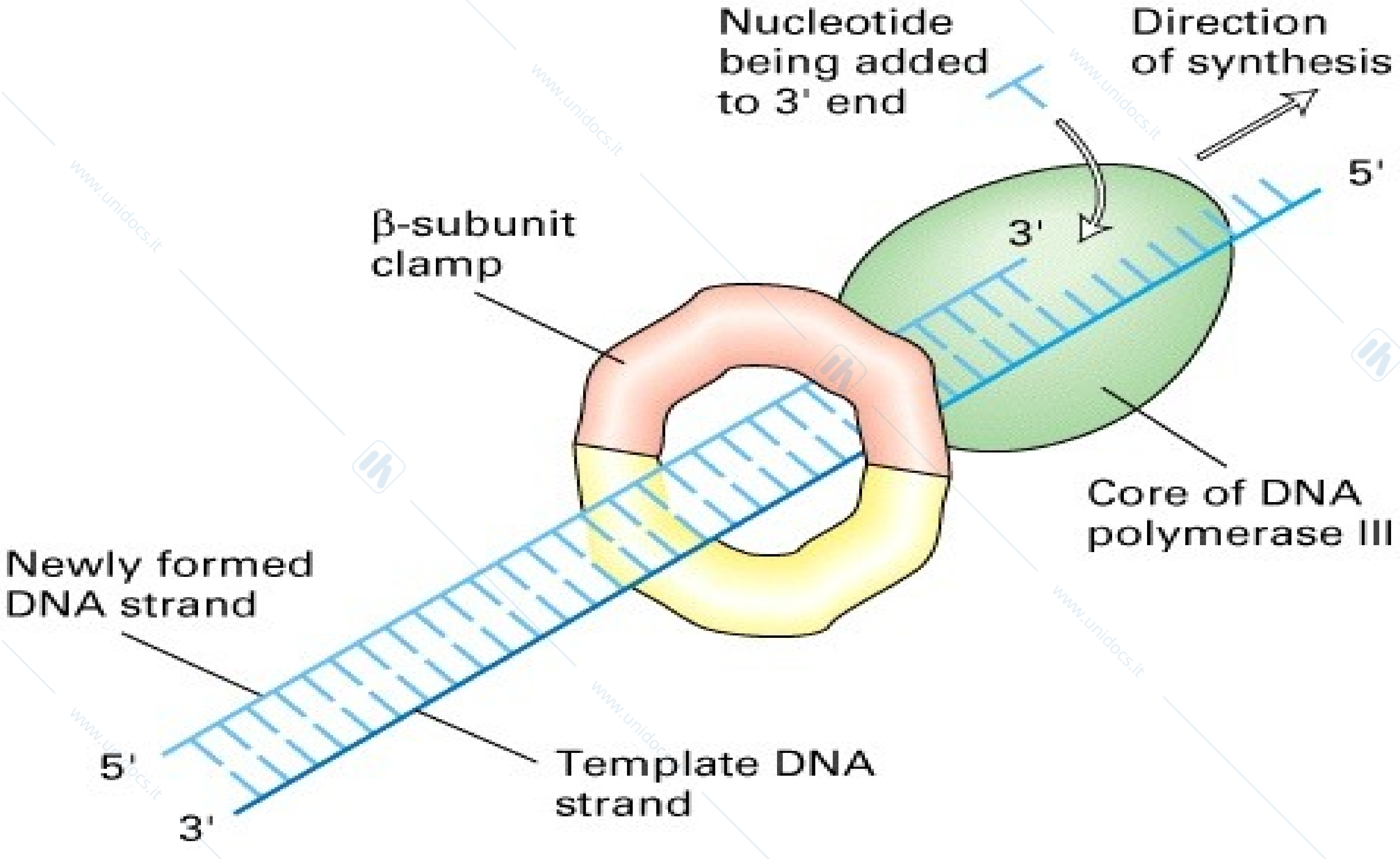

Double helix
(–)
(–)
(–)
3 negative supercoils
Binding of Topo I
Double-stranded helix
Topo I
(L = n)
Nick DNA; form covalent DNA-phosphotyrosine bond
3'
5'
OH
P
Tyr
DNA-enzyme intermediate
Pass cut 3' end under other strand and reseal DNA
Dissociation of Topo I
(–)
(–)
2 negative supercoils
Topoisomerase
R—HN—CH—C—NH—R'
O
CH2
Tyrosine
O
O=P—O
O⁻
5'
O
H
H
H
H
H
O
O=P—O—DNA chain
O⁻
NH2
N
N
N
N

Topsoisomerase I

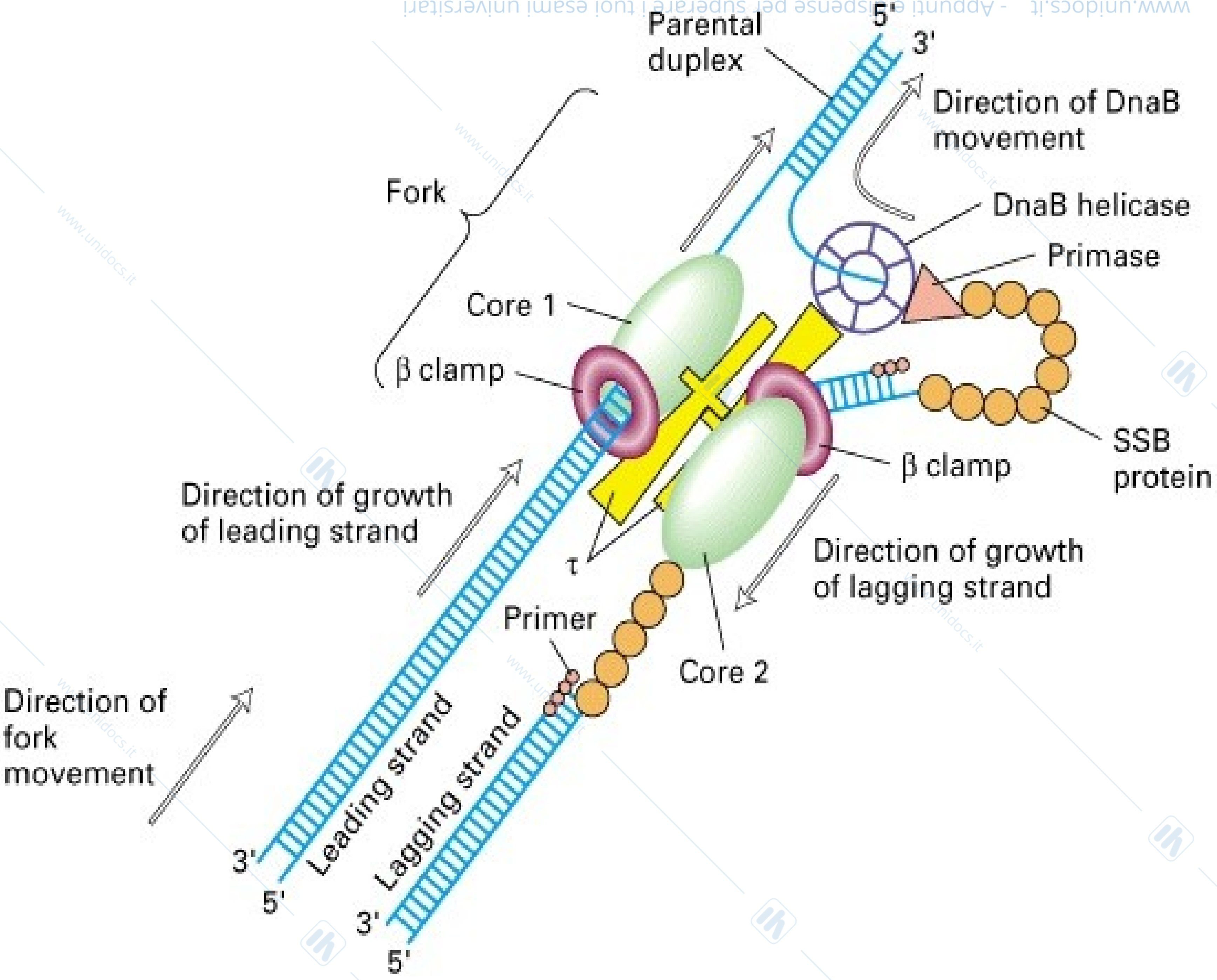


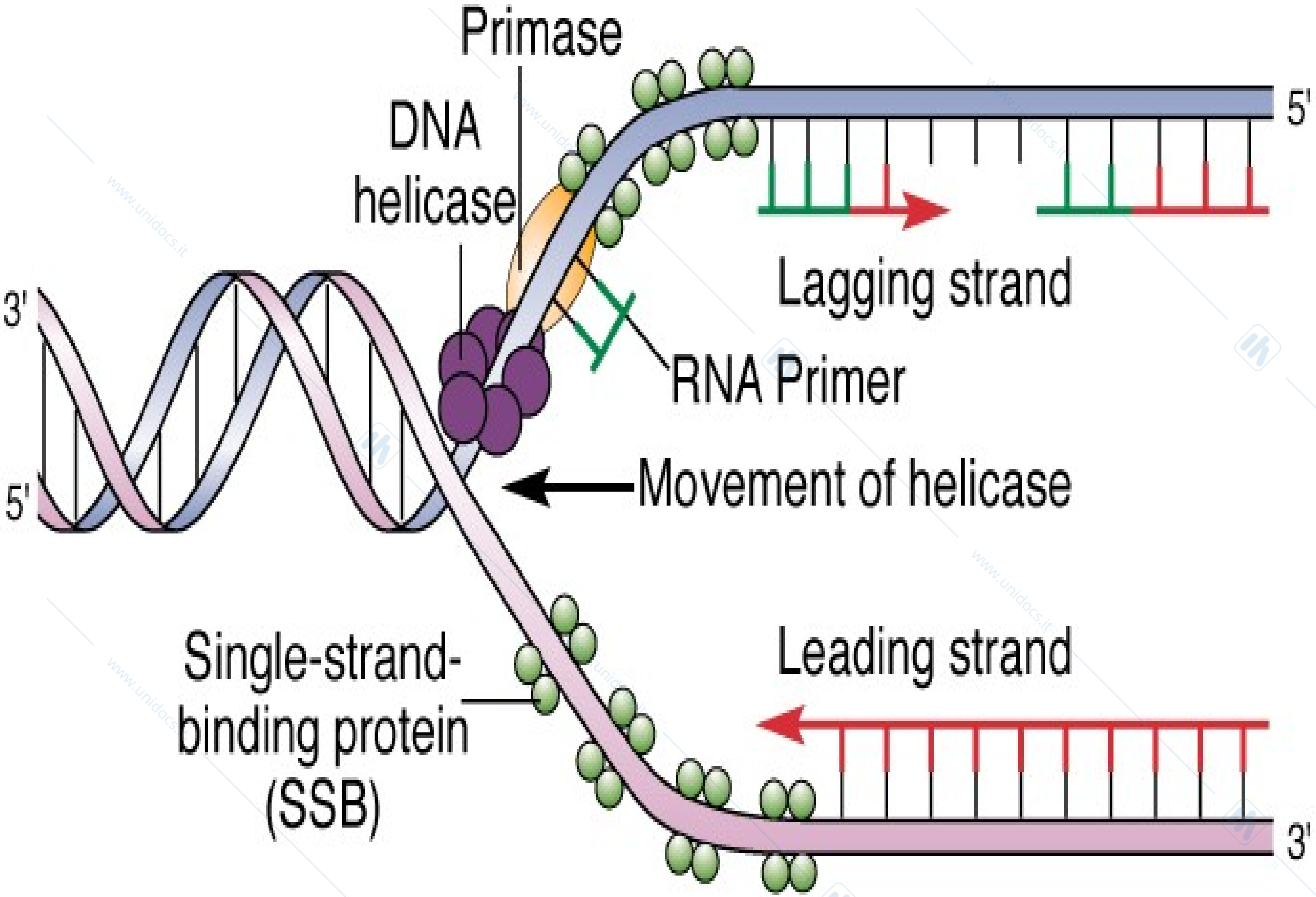

5'
3'
3'
5'
3'
5'
SSB
ATP
DnaB
5'
3'
3'
5'
3'
5'
Direction of unwinding
ATP
5'
3'
5'
3'
+
3'
5'

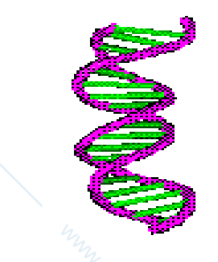

(a)
Growing fork
Old strands
5'
3'
3'
5'
5'
3'
Lagging strand
Leading strand
5'
3'
Movement of growing fork


Nucleotide being added to 3' end
Direction of synthesis
5'
3'
β-subunit clamp
Core of DNA polymerase III
Newly formed DNA strand
5'
3'
Template DNA strand

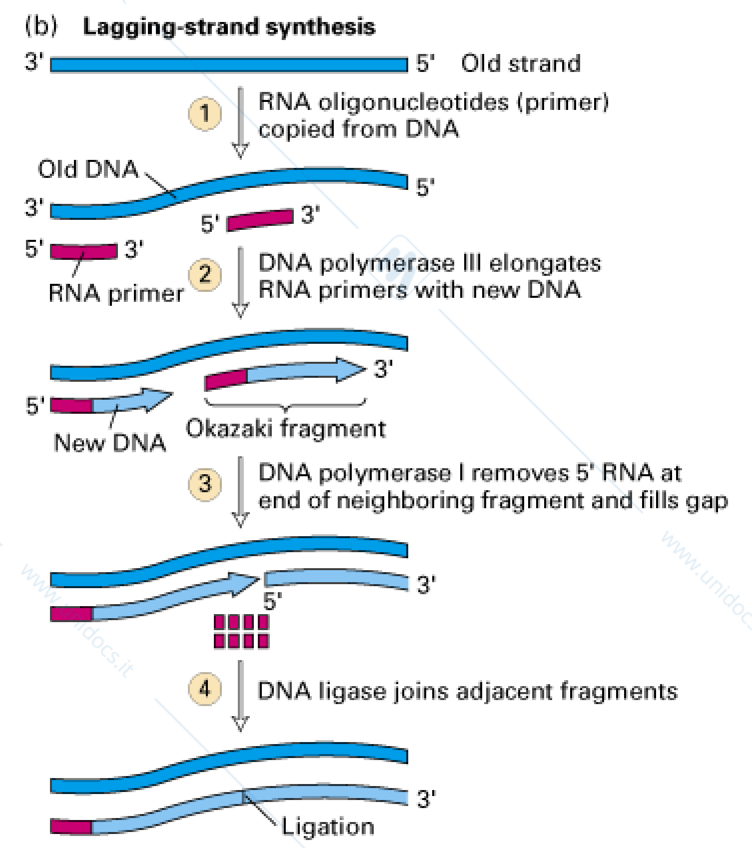
(b)

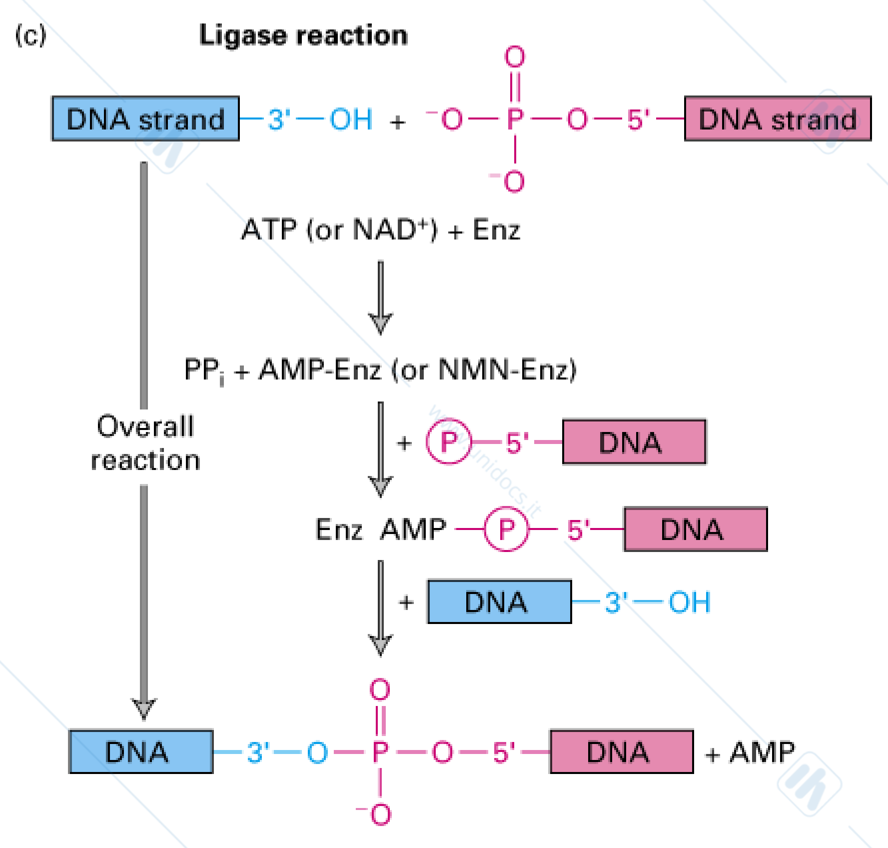

Parental duplex
5'
3'
Direction of DnaB movement
Fork
DnaB helicase
Primase
Core 1
β clamp
SSB protein
β clamp
Direction of growth of leading strand
τ
Direction of growth of lagging strand
Primer
Core 2
Direction of fork movement
3'
5'
Leading strand
Lagging strand
3'
5'

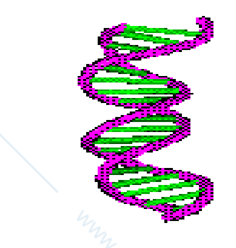

Primase
DNA helicase
5'
Lagging strand
3'
RNA Primer
5'
Movement of helicase
Single-strand-binding protein (SSB)
Leading strand
3'



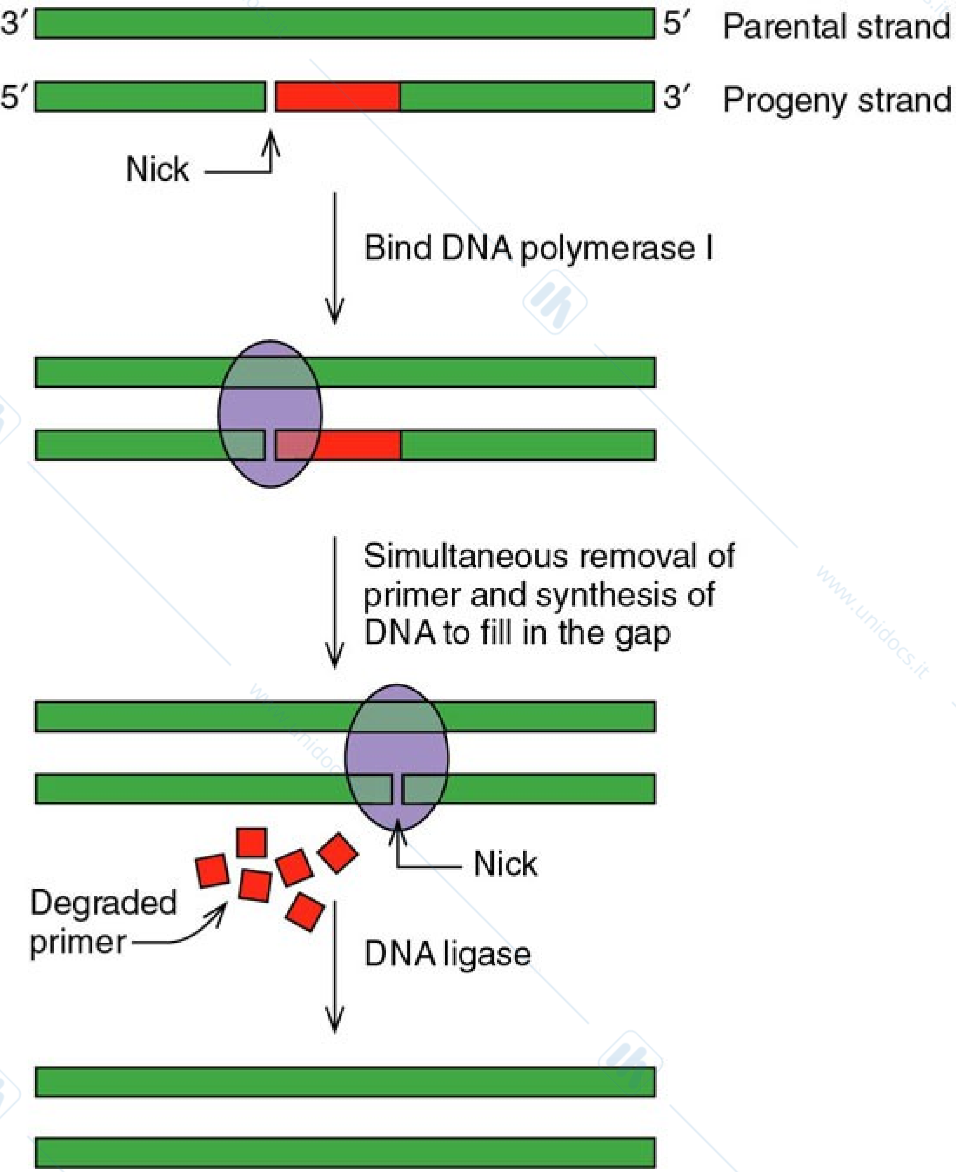
Lagging Strand Synthesis

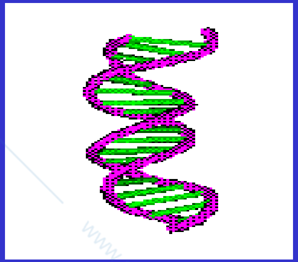




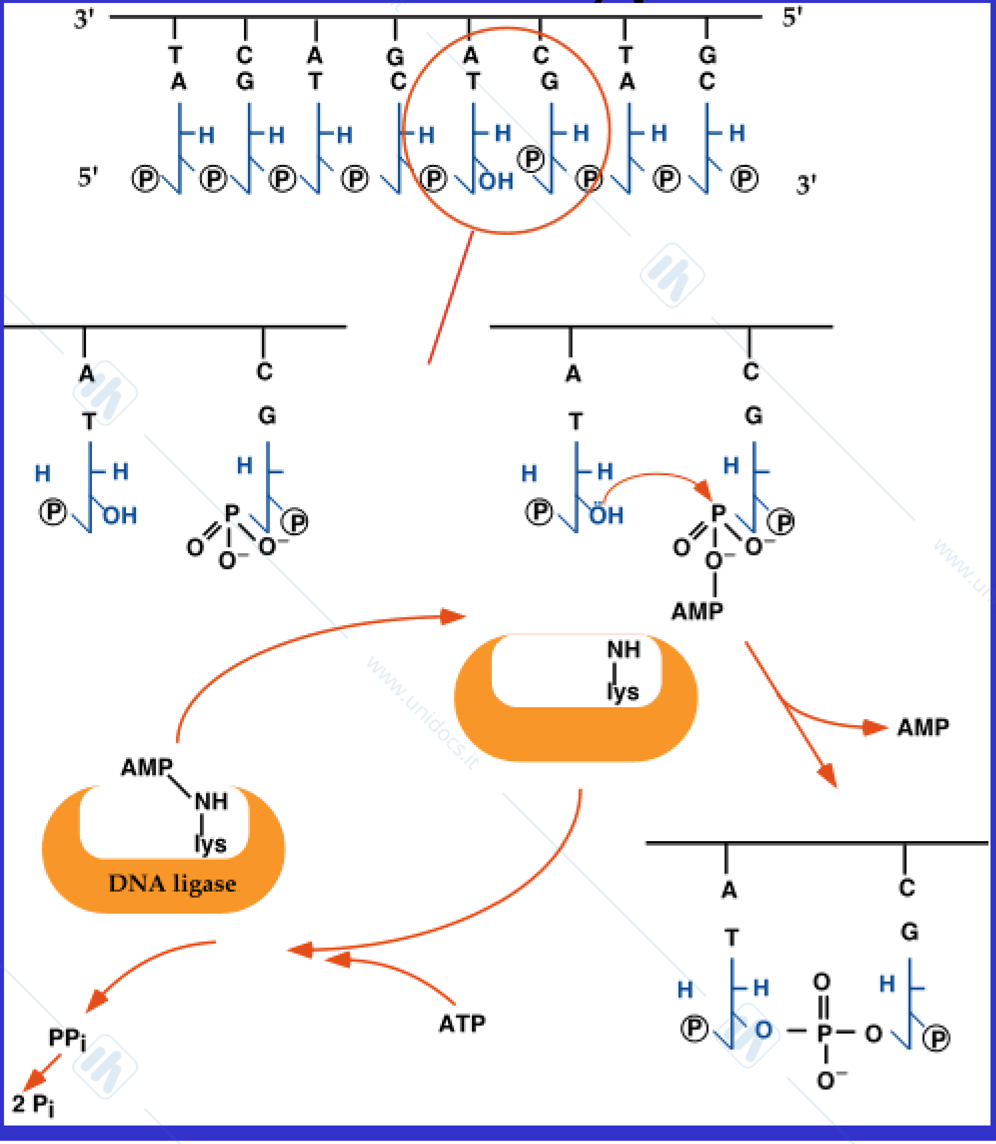


Replacement of RNA Primers

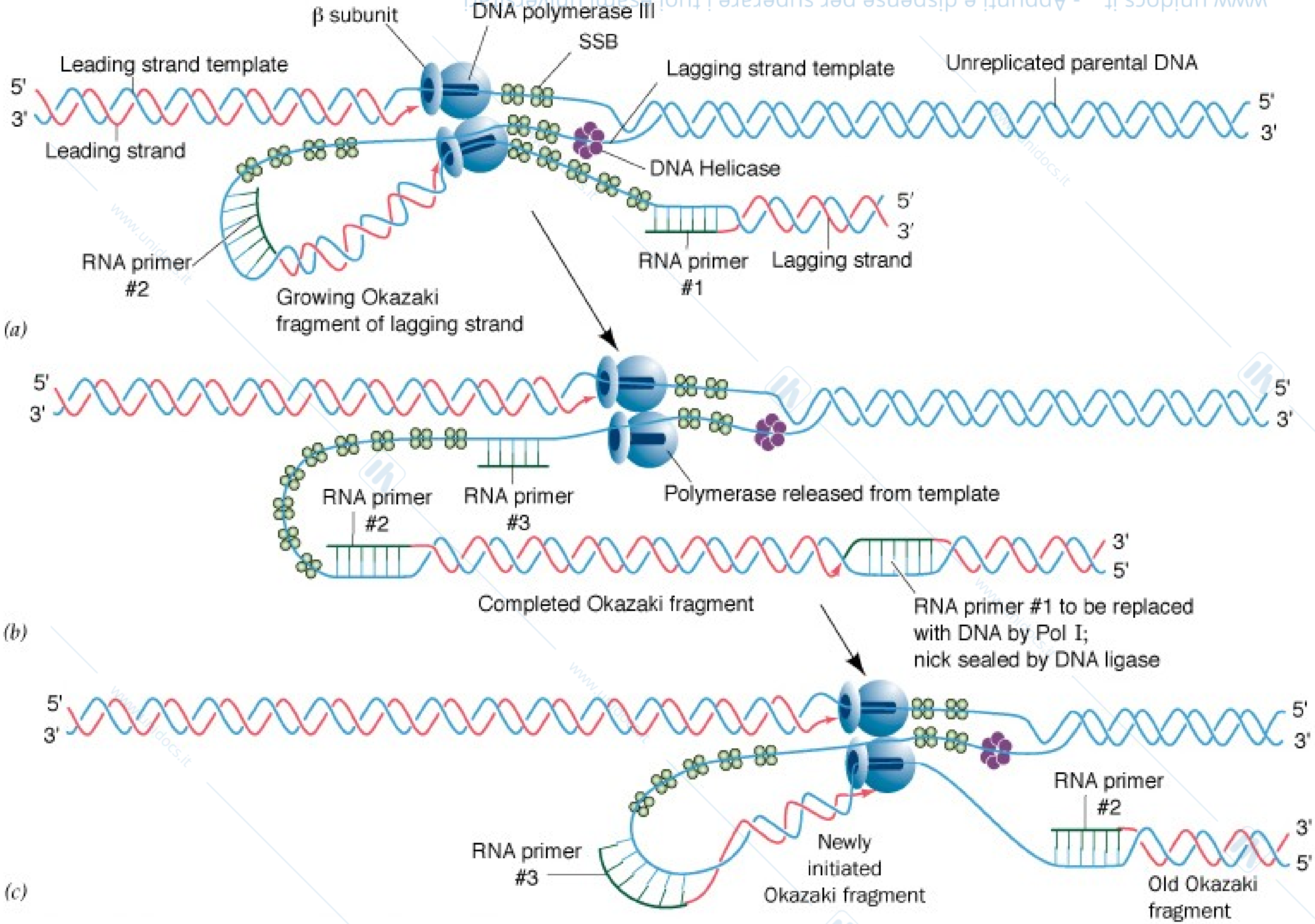




DNA Ligase



3'
5'
T A
C G
A T
G C
A T
C G
T A
G C
H
P
OH
5'
3'
A
T
C
G
H
P
OH
P
O
O⁻
AMP
NH
lys
DNA ligase
ATP
PPi
2 Pi
O
P — O
O⁻



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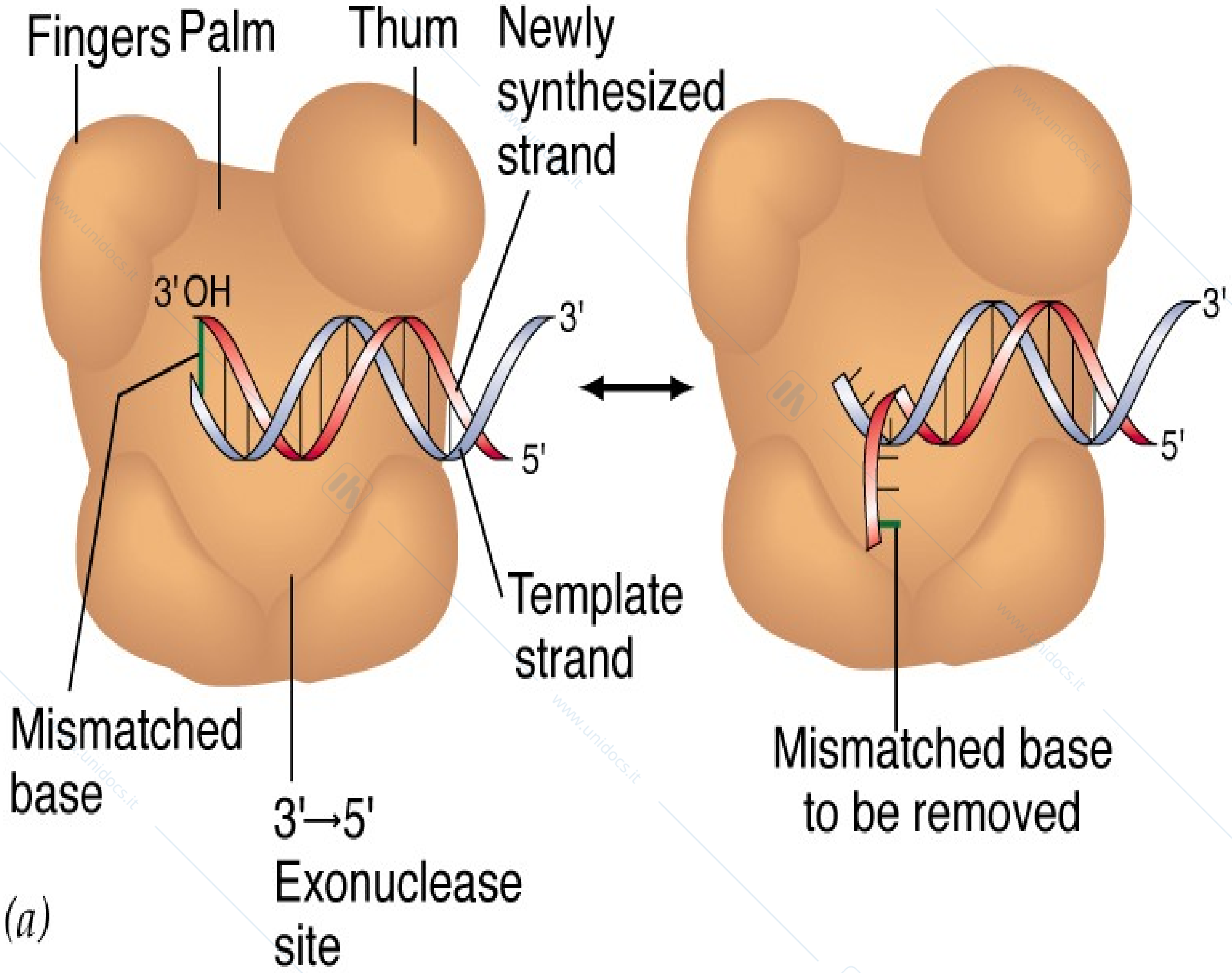

Fingers
Palm
Thum
Newly synthesized strand
3'OH
3'
5'
Mismatched base
3'→5' Exonuclease site
Template strand
(a)
3'
5'
Mismatched base to be removed

DNA Proofreading and "Editing"

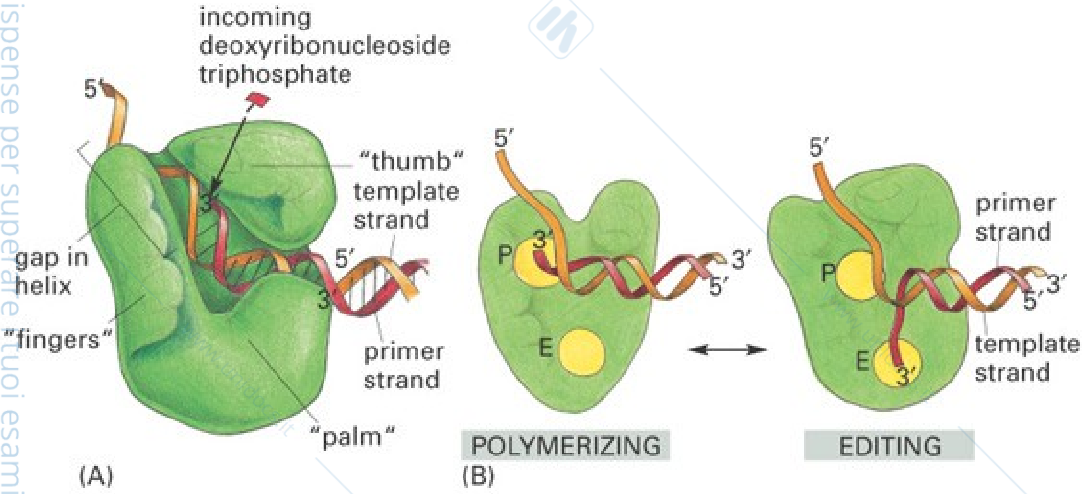
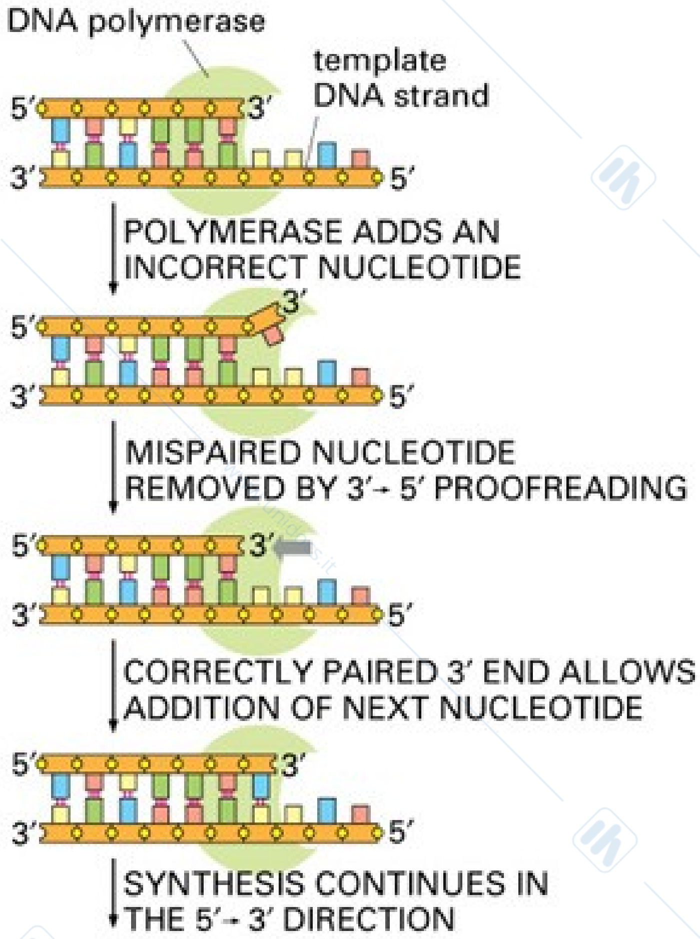


Figure 6-14 Essential Cell Biology, 2/e. (© 2004 Garland Science)



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DNA Proofreading and "Editing"

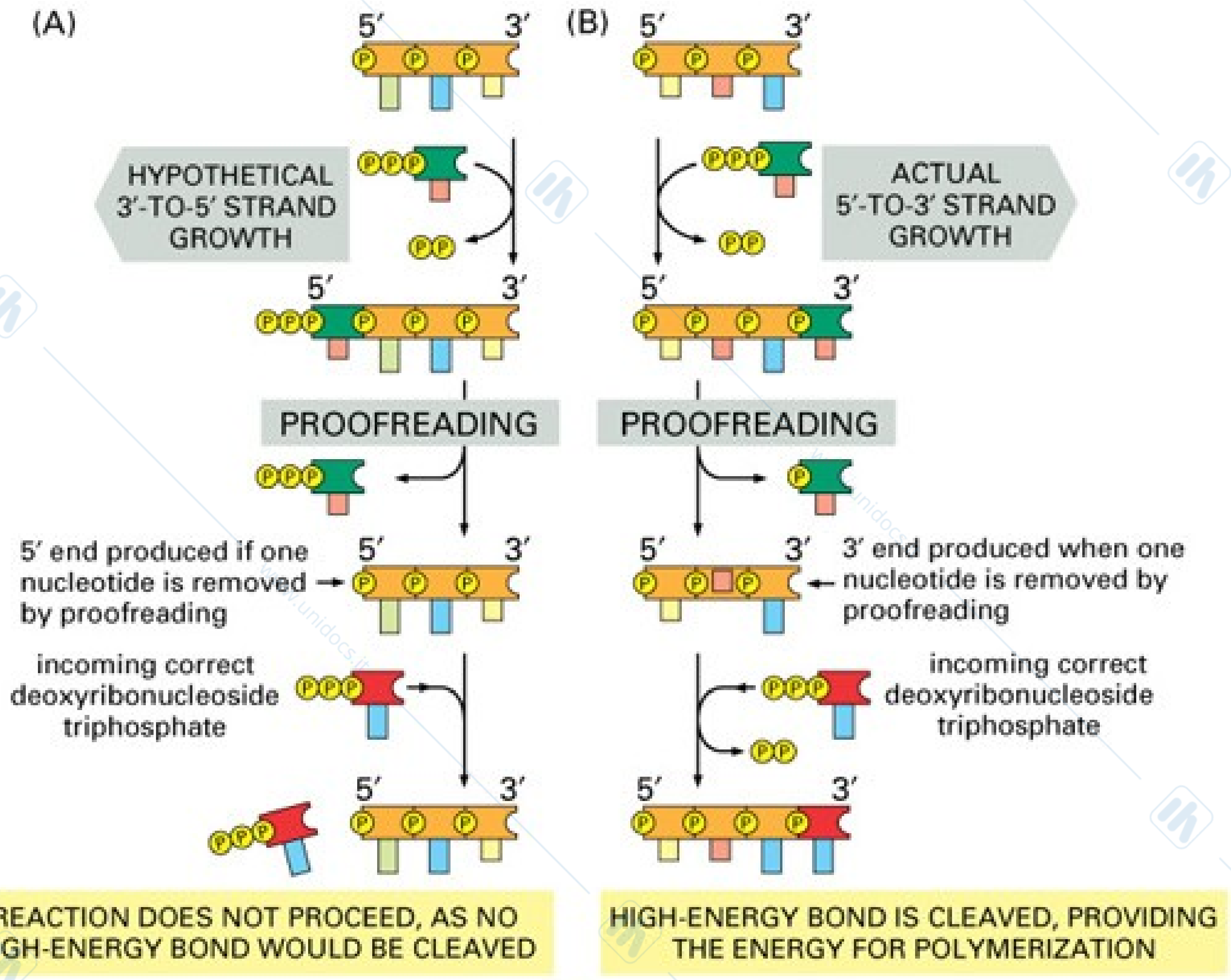

Figure 6-15 **Essential Cell Biology, 2/e. (© 2004 Garland Science)**

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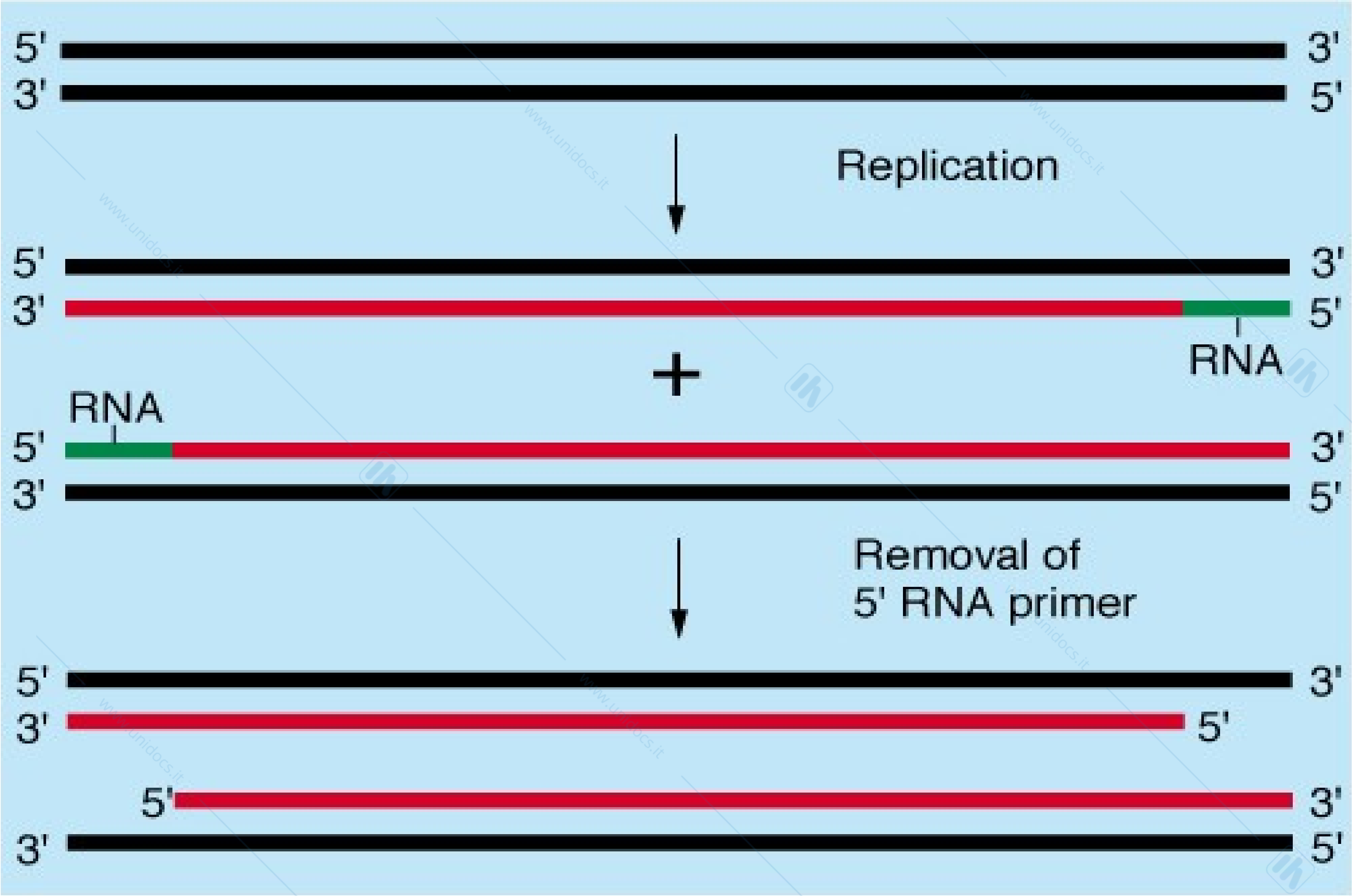
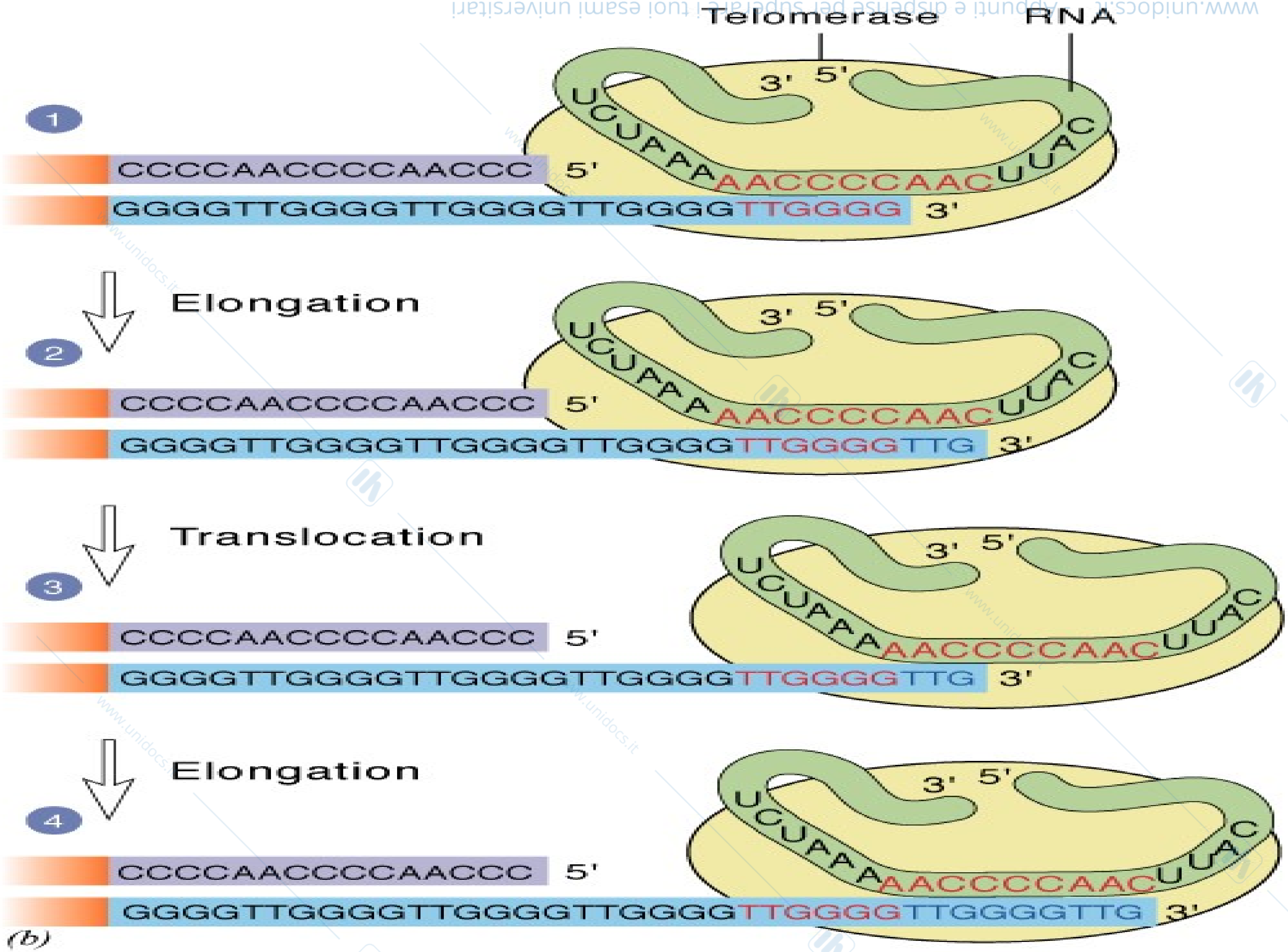

5'
3'
3'
5'
Replication
5'
3'
3'
5'
RNA
+
RNA
5'
3'
3'
5'
Removal of
5' RNA primer
5'
3'
3'
5'
5'
3'
3'
5'

(a)



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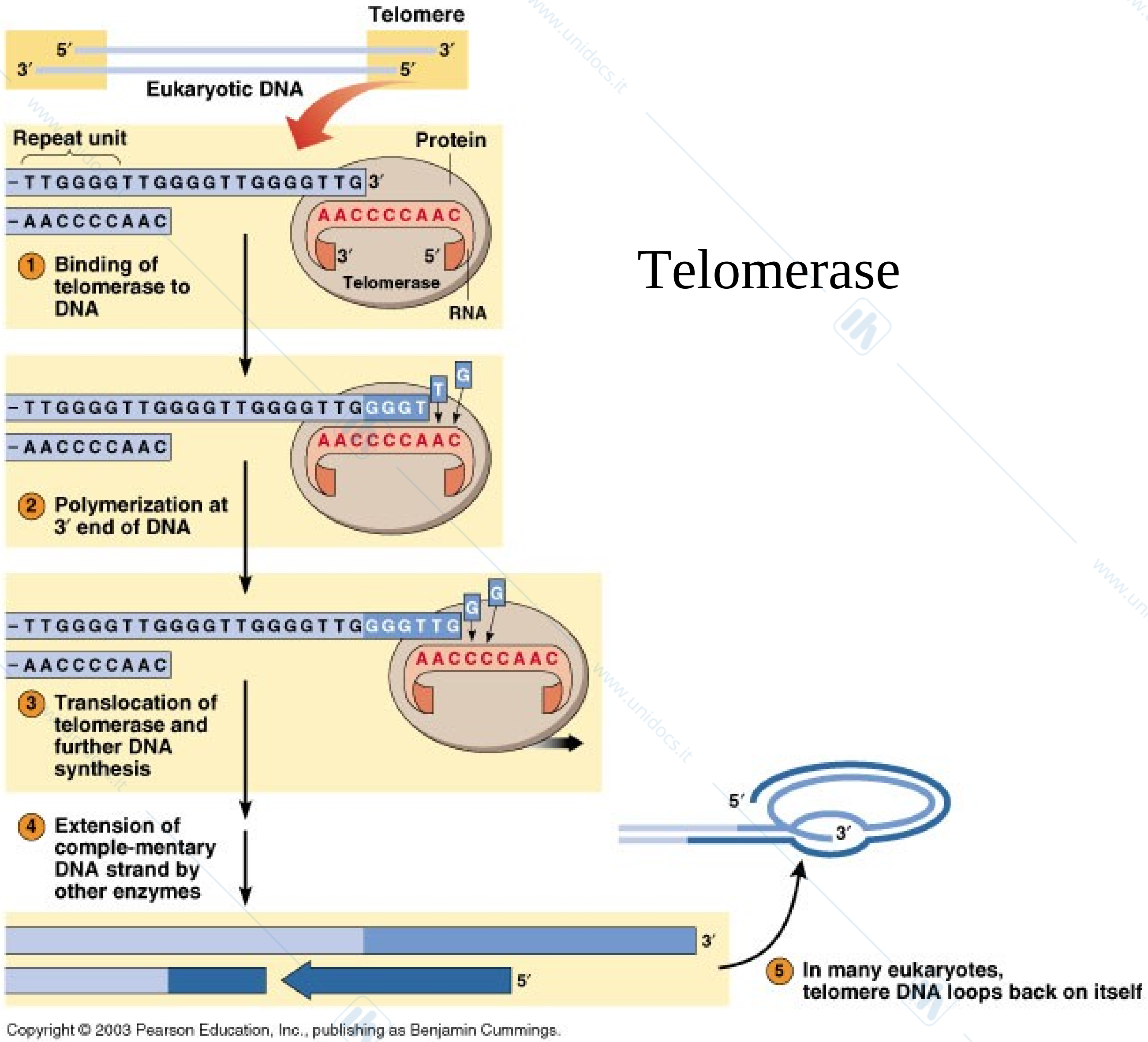
End Replication Problem



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Telomerase

Figure 12.11 The D loop maintains an opening in mammalian mitochondrial DNA, which has separate origins for the replication of each strand.