

NUKLEOVÉ KYSELINY

MISCHER – **nuklein** (1868 – 1869)

Složení :

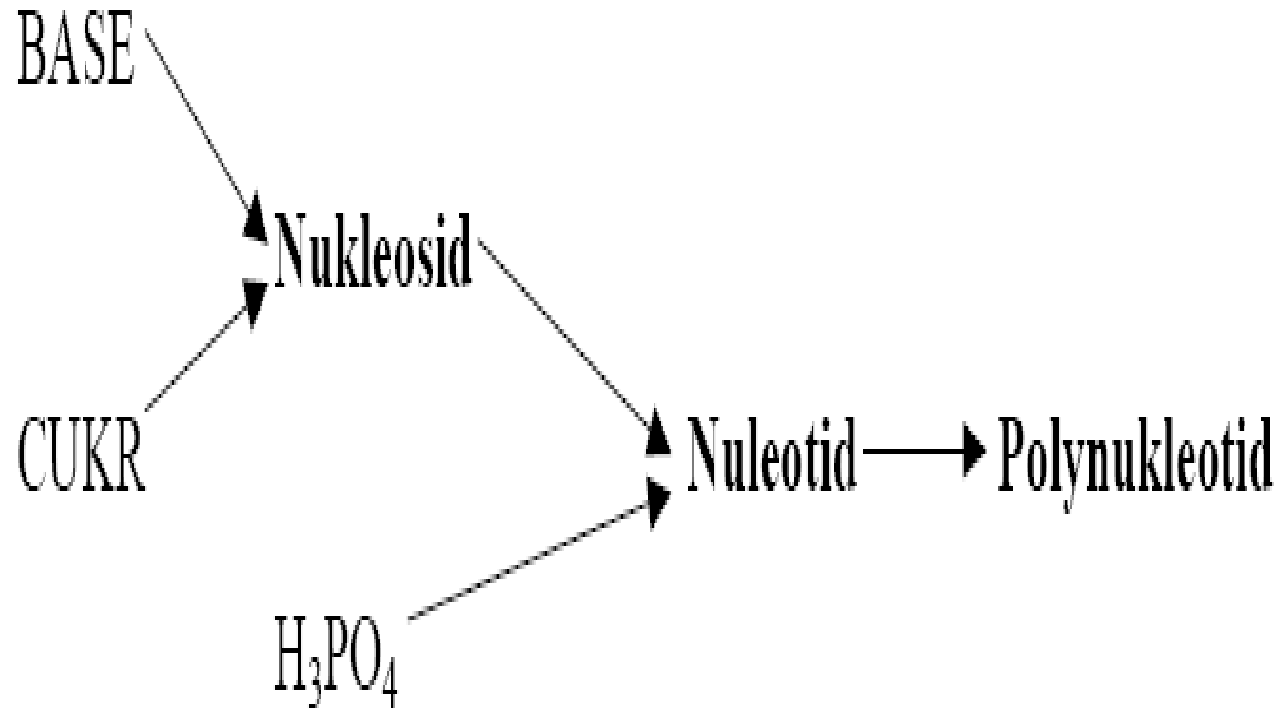
- Dusíkaté báze – purinové, pyrimidinové
- Sacharid – ribosa, deoxyribosa
- H_3PO_4

Funkce :

DNA – nositel genetické informace

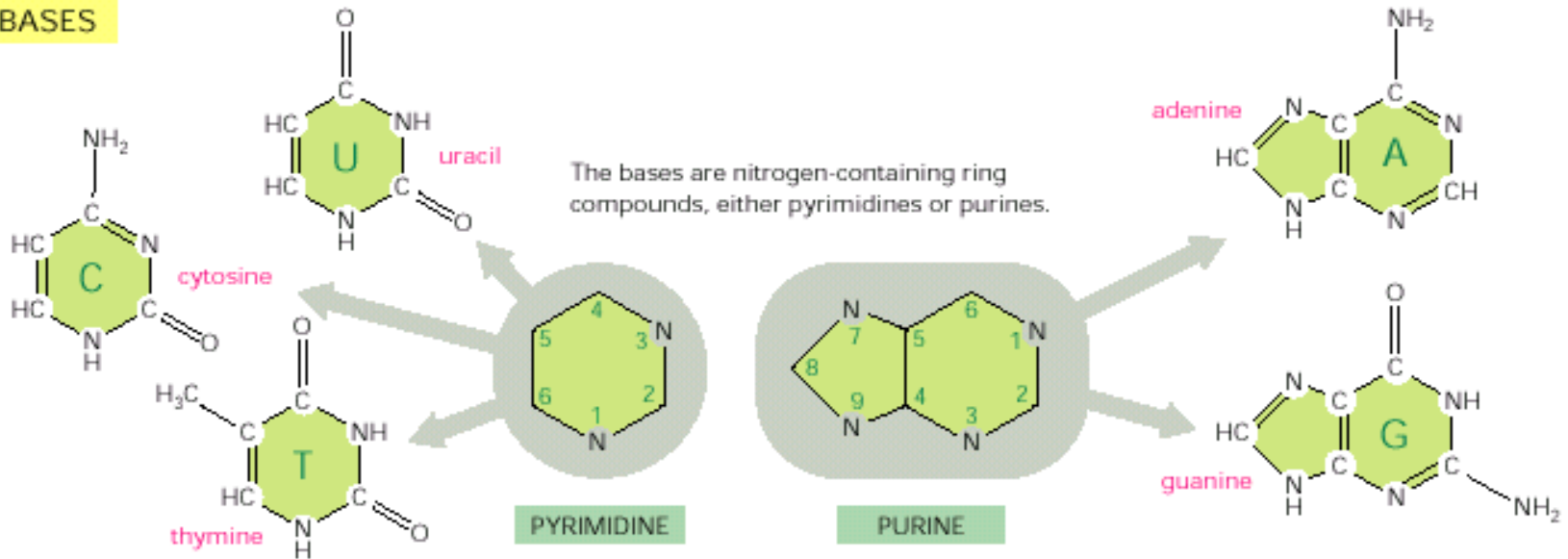
- Viry
- Prokaryonta –cytoplazma
- Eukaryonta – jádro, mitochondrie, chloroplasty

RNA – realizace genetické informace (u RNA virů i nositel genetické informace)

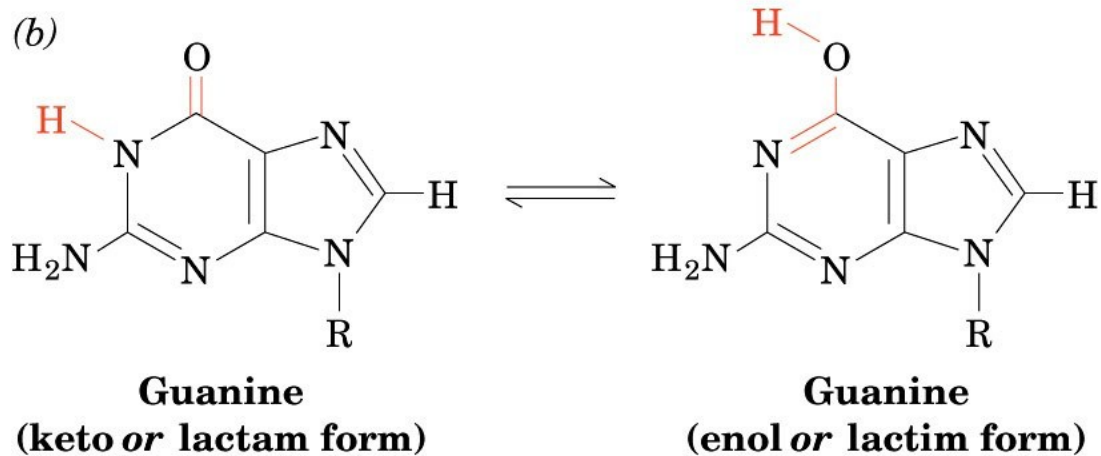
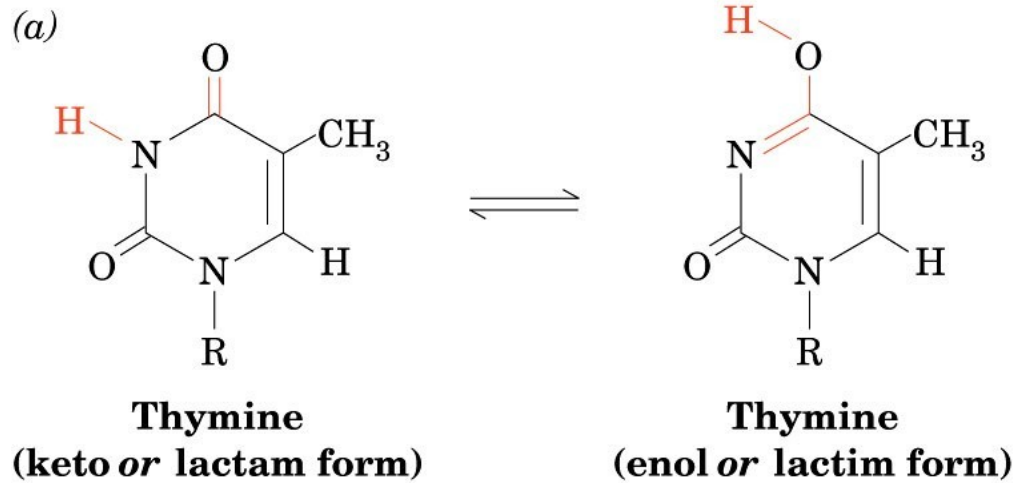


Báze

BASES



Báze -tautomerie

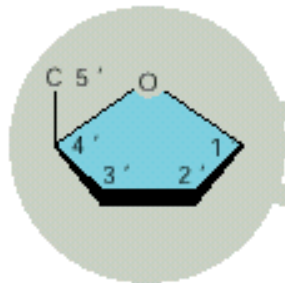


Monosacharidy

SUGARS

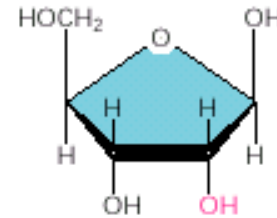
PENTOSE

a five-carbon sugar

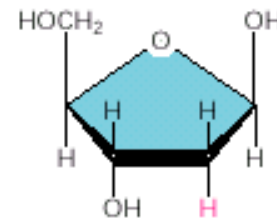


two kinds are used

Each numbered carbon on the sugar of a nucleotide is followed by a prime mark; therefore, one speaks of the "5-prime carbon," etc.



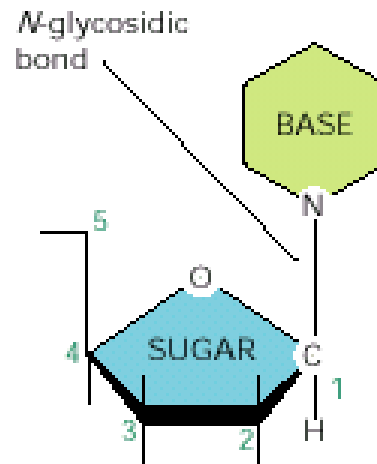
β -D-ribose
used in ribonucleic acid



β -D-2-deoxyribose
used in deoxyribonucleic acid

Nukleosid

BASIC SUGAR LINKAGE

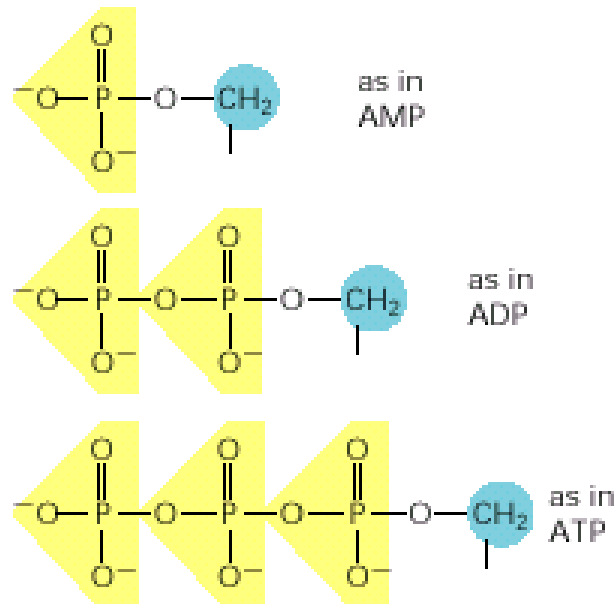


The base is linked to the same carbon (C1) used in sugar-sugar bonds.

Kyselina fosforečná

PHOSPHATES

The phosphates are normally joined to the C5 hydroxyl of the ribose or deoxyribose sugar (designated 5'). Mono-, di-, and triphosphates are common.

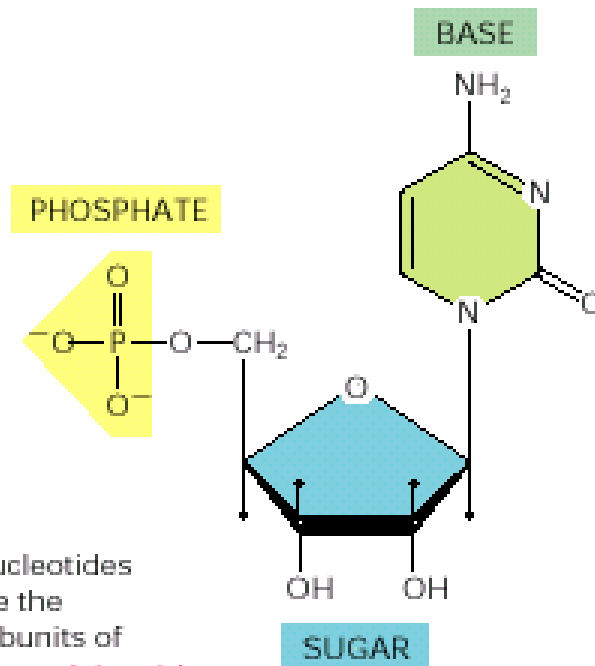


The phosphate makes a nucleotide negatively charged.

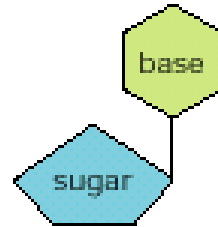
Nukleotid

NUCLEOTIDES

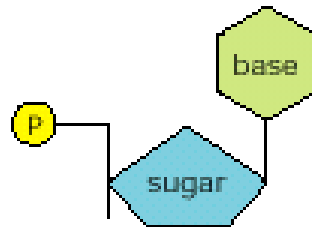
A nucleotide consists of a nitrogen-containing base, a five-carbon sugar, and one or more phosphate groups.



Názvosloví



BASE + SUGAR = NUCLEOSIDE



BASE + SUGAR + PHOSPHATE = NUCLEOTIDE

BASE	NUCLEOSIDE	ABBR.
adenine	adenosine	A
guanine	guanosine	G
cytosine	cytidine	C
uracil	uridine	U
thymine	thymidine	T

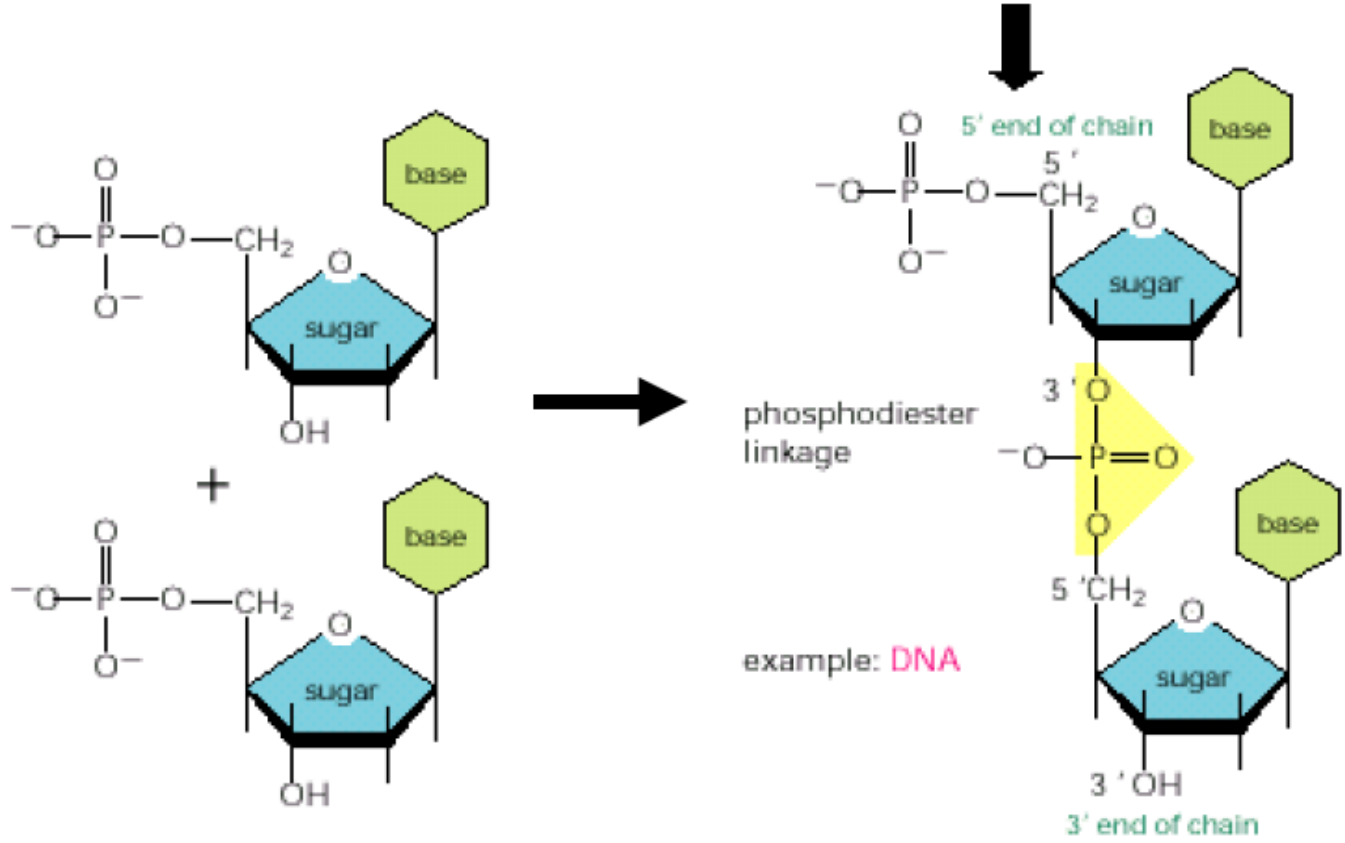
Nucleotides are abbreviated by three capital letters. Some examples follow:

AMP = adenosine monophosphate
dAMP = deoxyadenosine monophosphate
UDP = uridine diphosphate
ATP = adenosine triphosphate

Funkce nukleotidů

- přenašeče energie (ATP, GTP, ligasy, hydrolasy)
- fosforylační činidla (ATP - kinasy)
- aktivátory meziproductů biosyntéz - UDP glukosa
- součásti kofaktorů - NAD(P), FAD, PAPS,
- využití v terapii - antivirotika(AIDS, herpes) – AZT
- **stavební složky nukleových kyselin**

Polynukleotid – nukleová kyselina



Struktura a funkce DNA

A,T,G,C + deoxyribosa

Primární struktura – sekvence basí

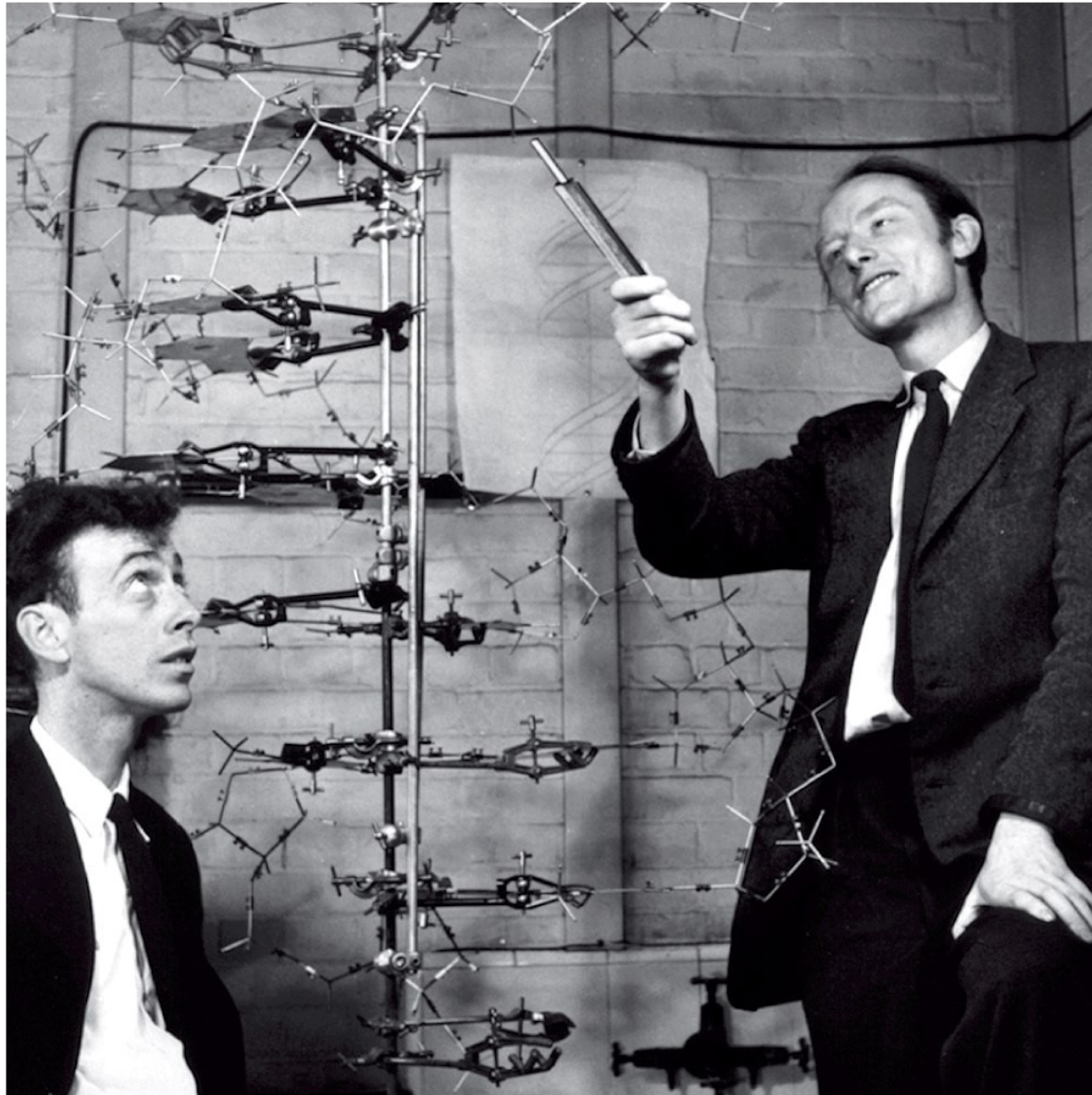
Sekundární struktura – Watson, Crick (1953) – dvojšroubovice

- Chragaffovy pravidla – poměr basí v DNA

$A+G=T+C$ $A=T$ $G=C$ $A+C=G+T$

- Donohue – báze v tautomerních ketoformách

- Franklinová – RTG difrakční analýza



Unnumbered figure pg 9 Concepts in Biochemistry, 3/e

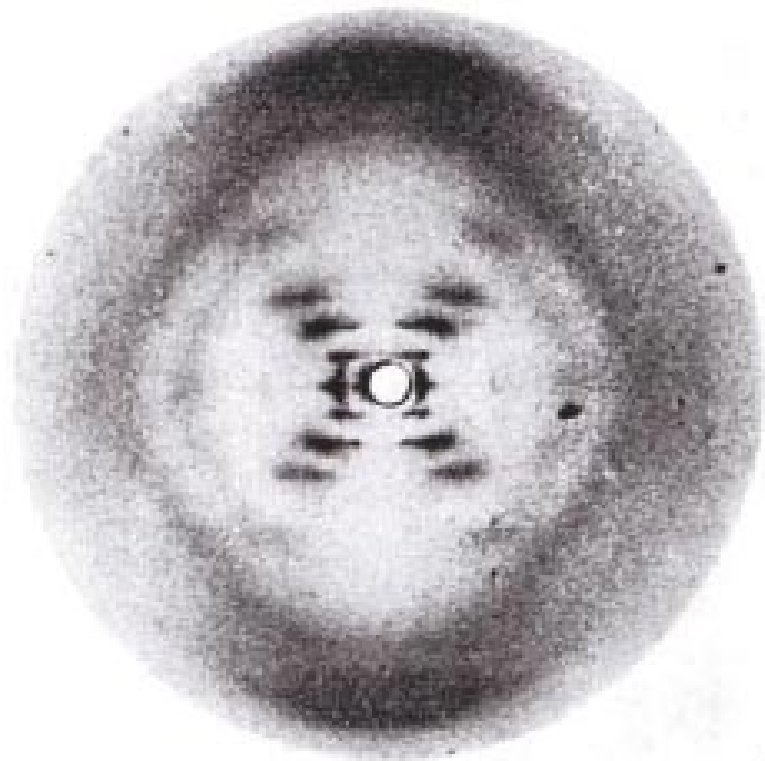
Chargaffovy pravidla

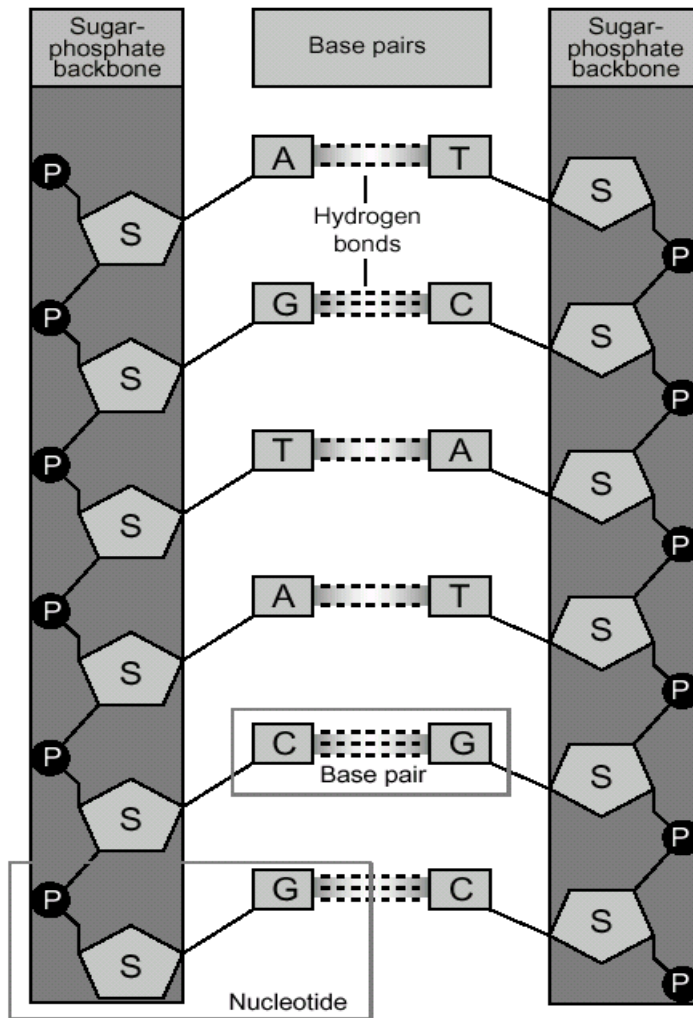
$$A+G=T+C \quad A=T \quad G=C$$

$$A+C=G+T$$

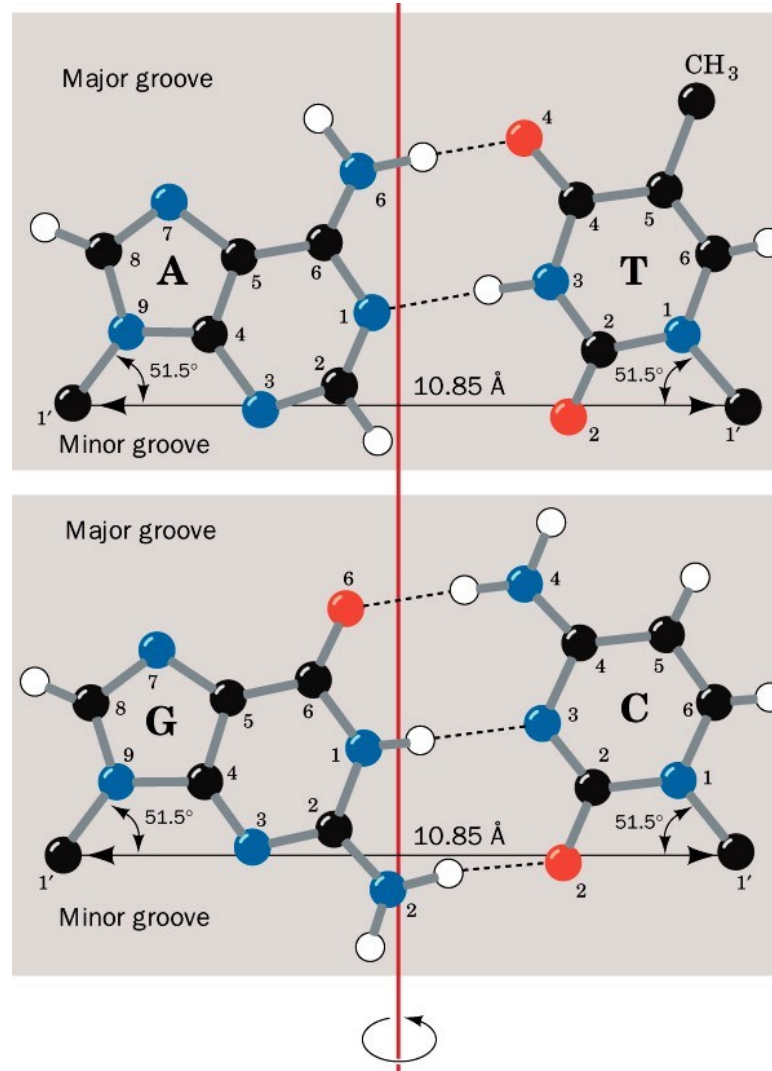
Zastoupení basí v DNA (molární %)

<u>Organismus</u>	<u>A</u>	<u>T</u>	<u>G</u>	<u>C</u>
Člověk	30.9	29.4	19.9	19.8
Kuře	28.8	29.2	20.5	21.5
Kobylka luční	29.3	29.3	20.5	20.7
Pšenice	27.3	27.1	22.7	22.8
Kvasinky	31.3	32.9	18.7	17.1
E. coli	24.7	23.6	26.0	25.7

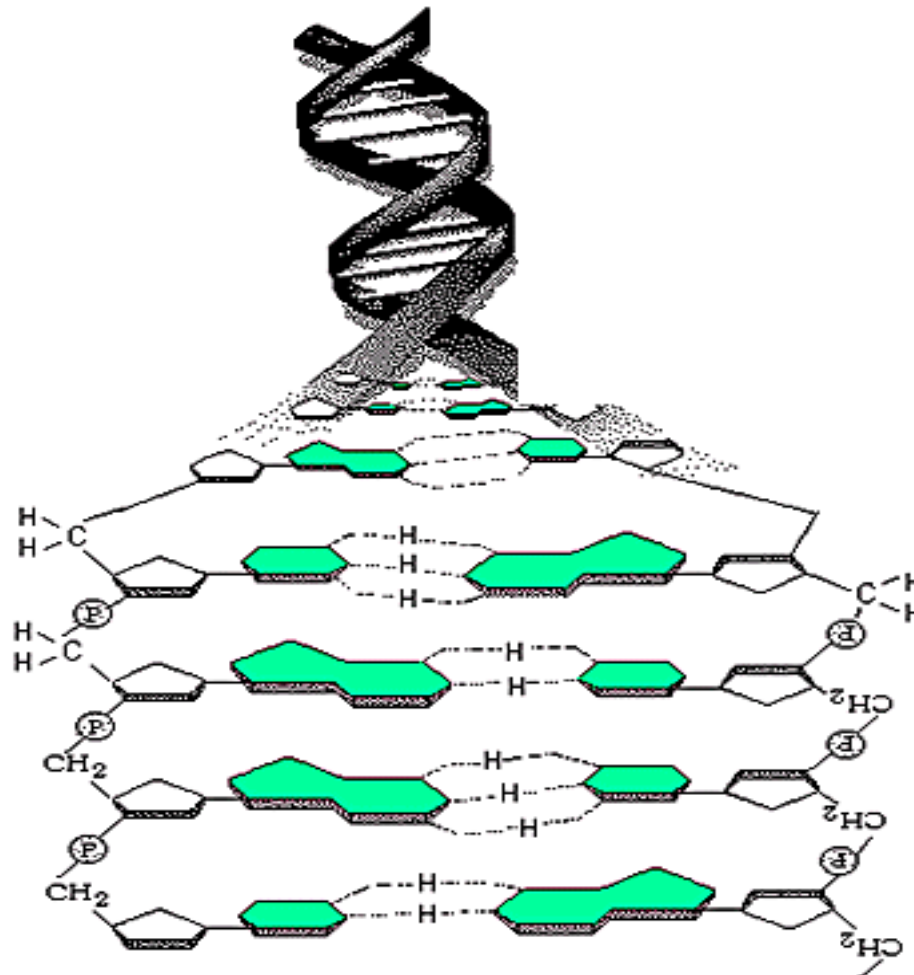




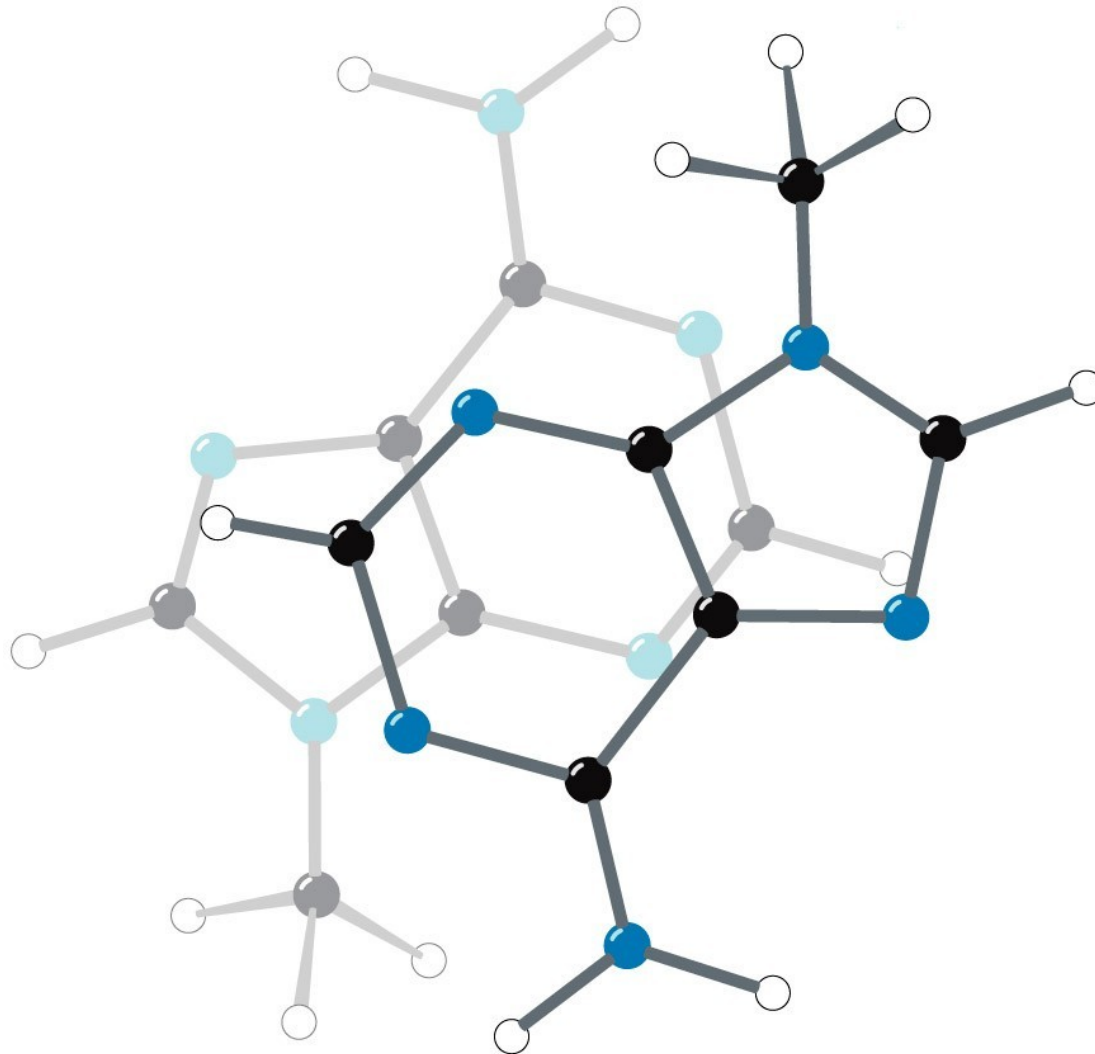
Párování basi – H můstky



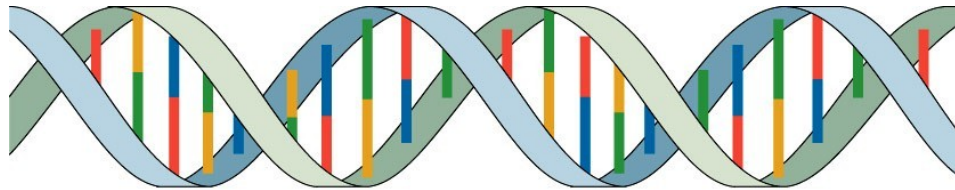
Stabilizující vazby v DNA



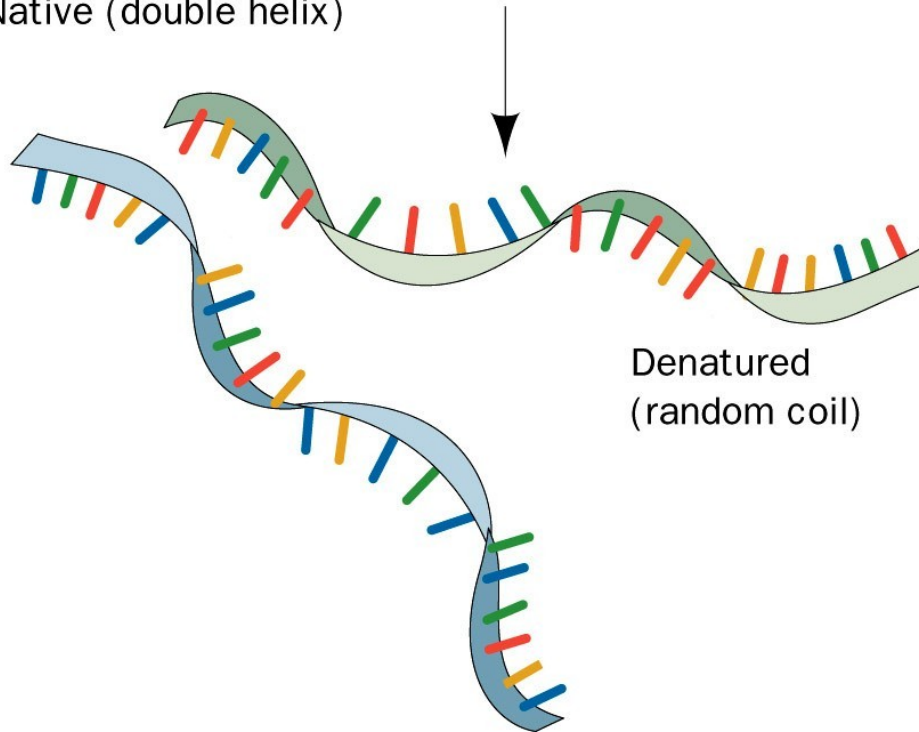
Stohování bází



Denaturace - renaturace

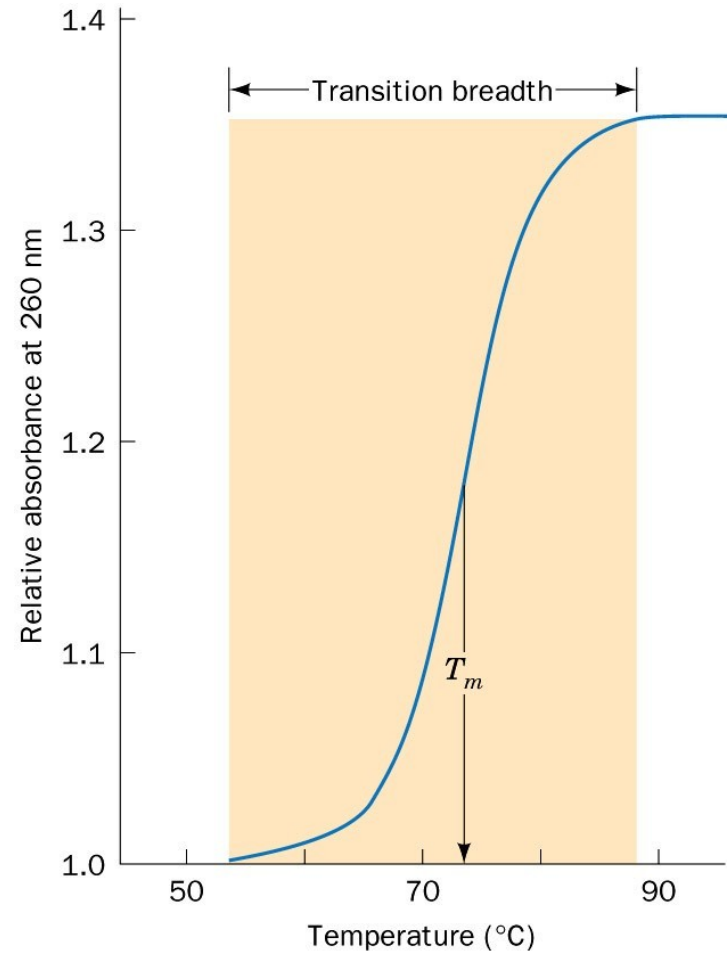
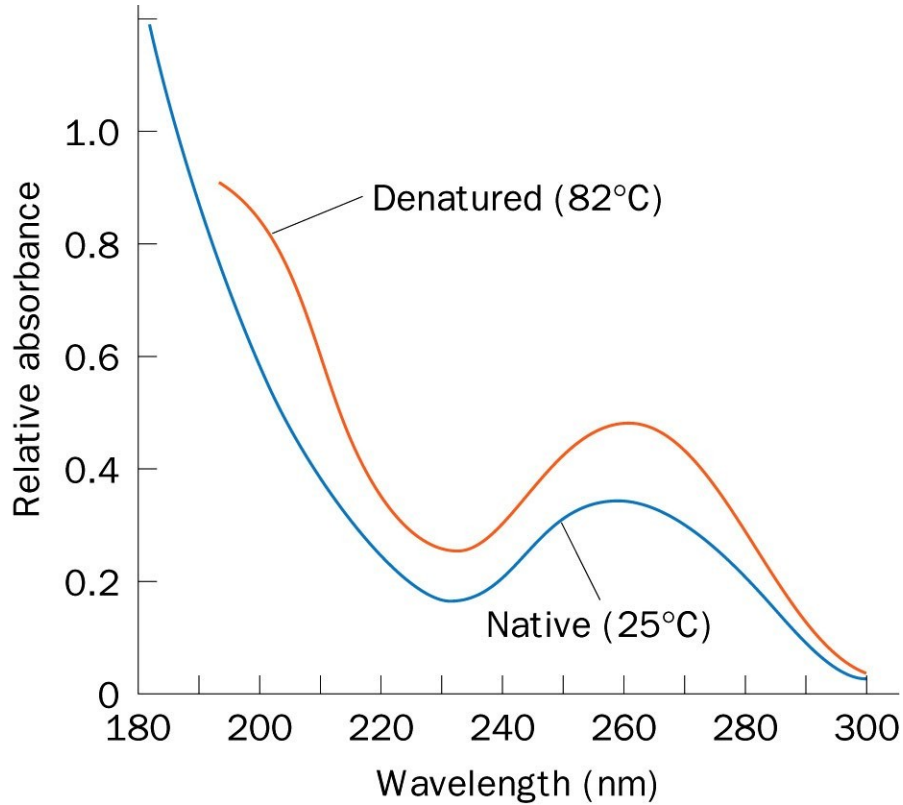


Native (double helix)



Denatured
(random coil)

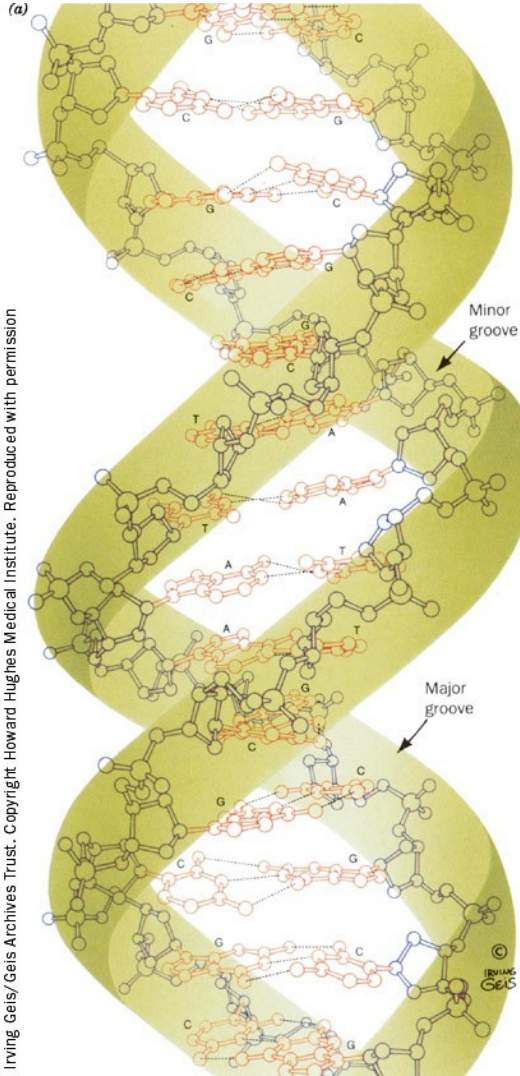
Denaturace - renaturace



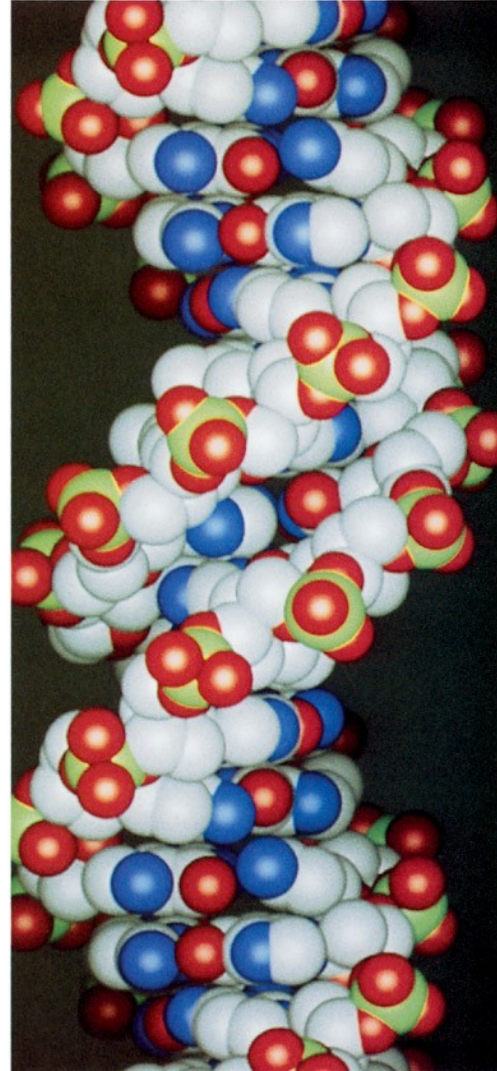
Formy DNA

- B - DNA - nativní 92 % H₂O, Na⁺
pravotočivá - 10 párů bází na závit
- A - DNA - 75 % H₂O, rovina bází 20°
pravotočivá - 11 párů bází na závit
- Z - DNA - d(CGCGCG)
levotočivá - 12 párů bází na závit

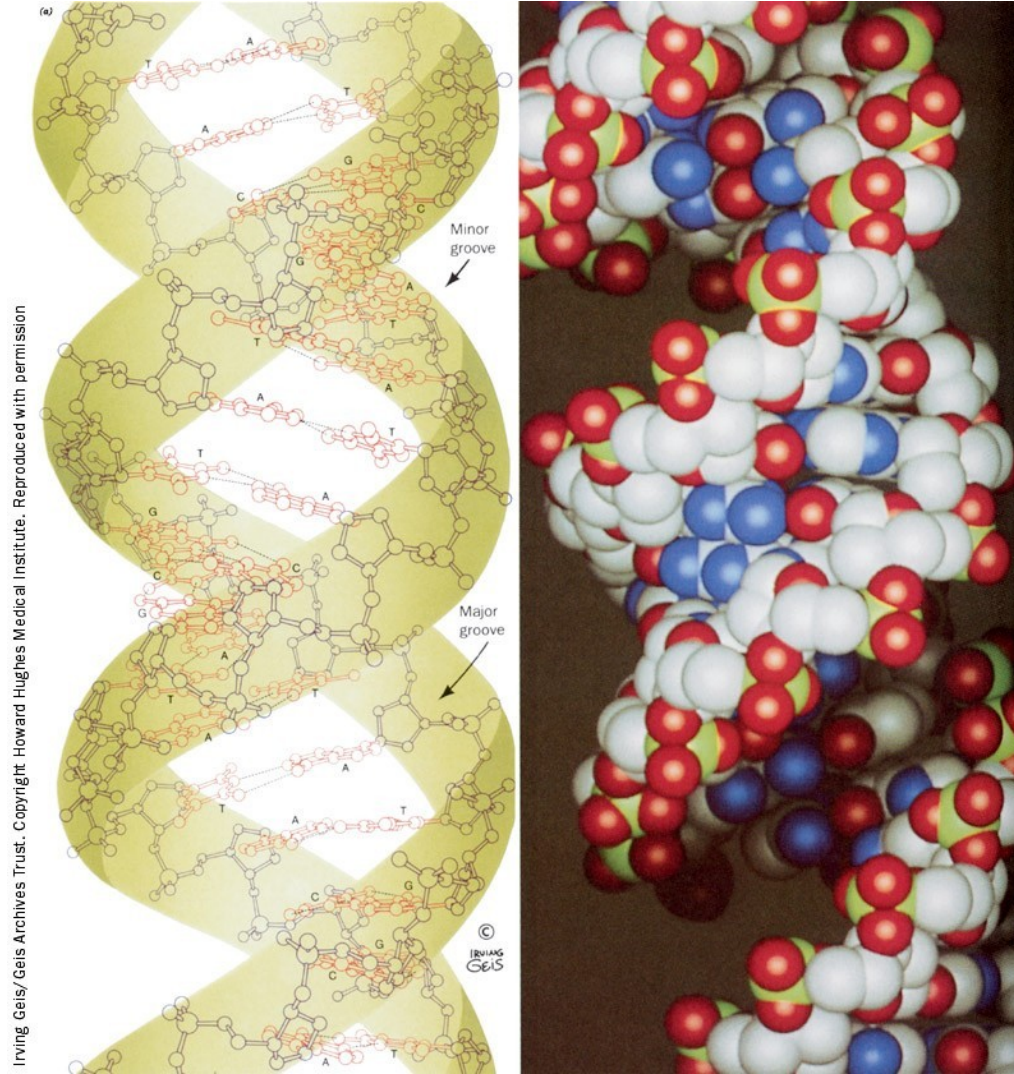
B DNA



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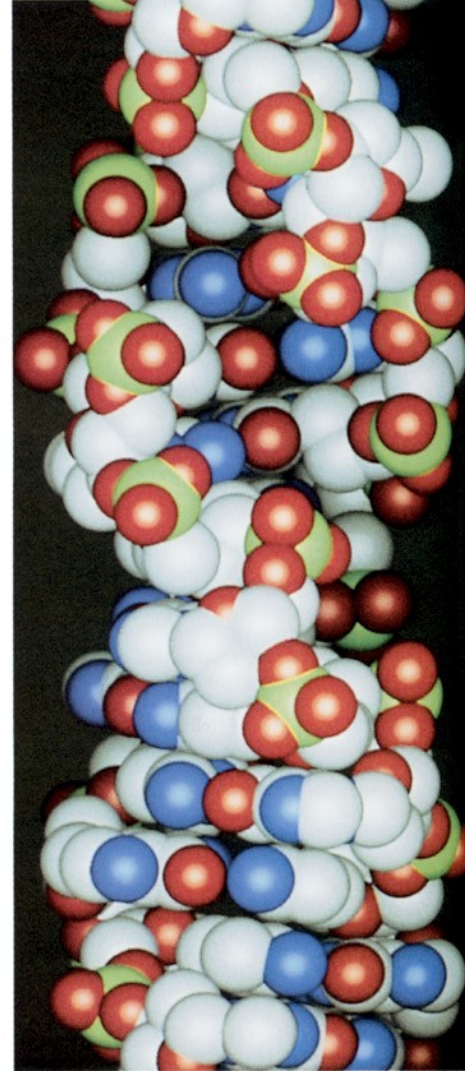
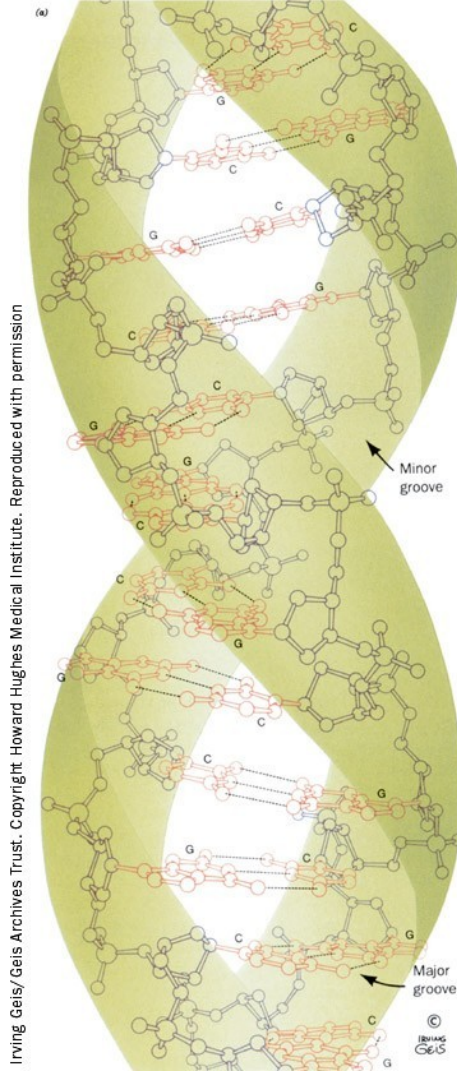


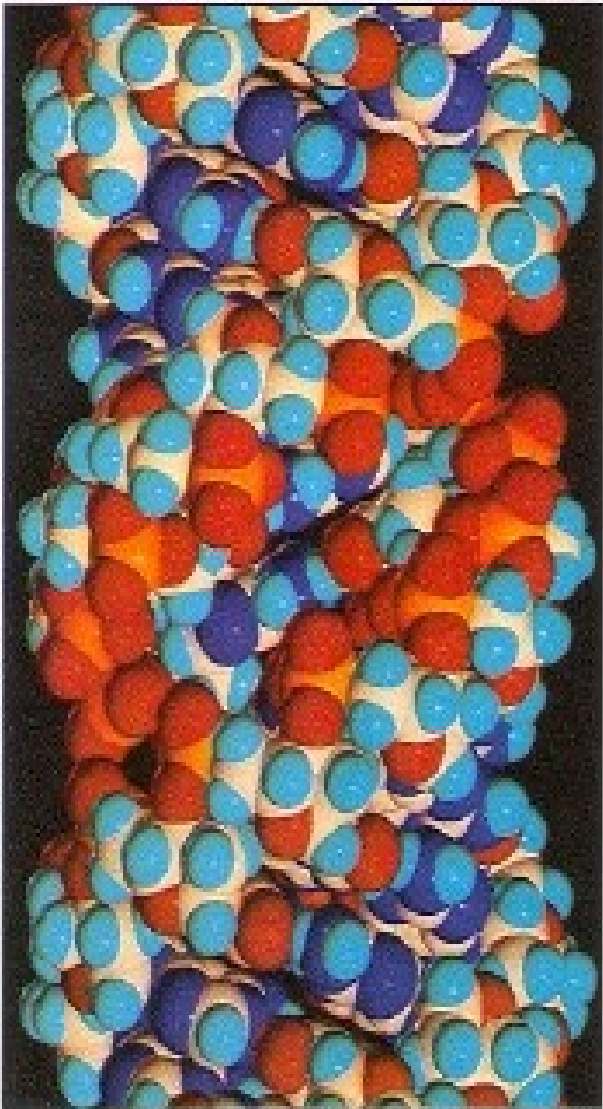
A DNA



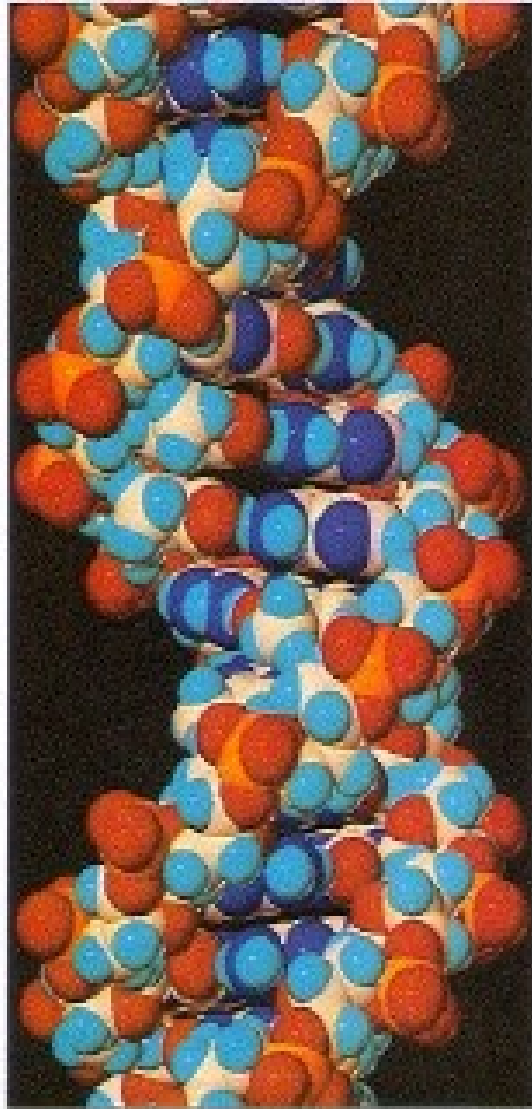
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Z DNA

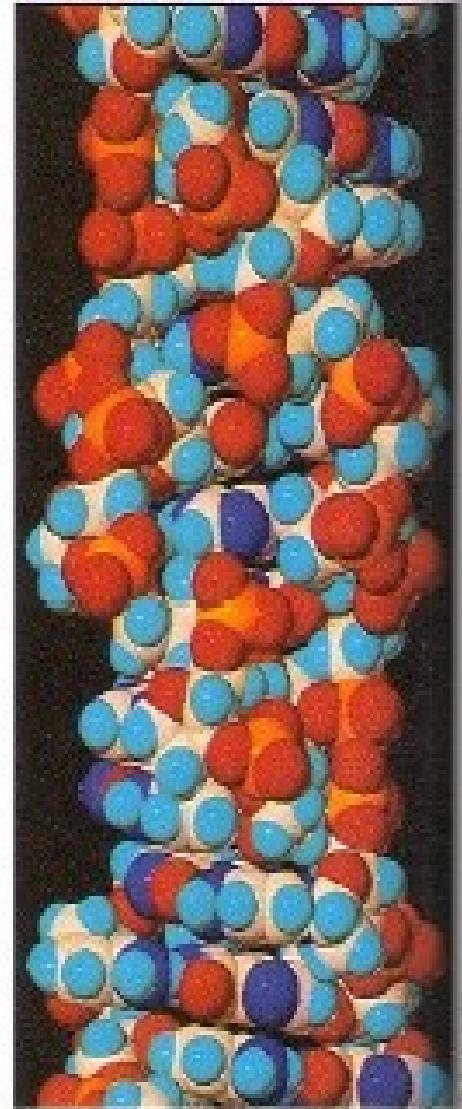




A-DNA

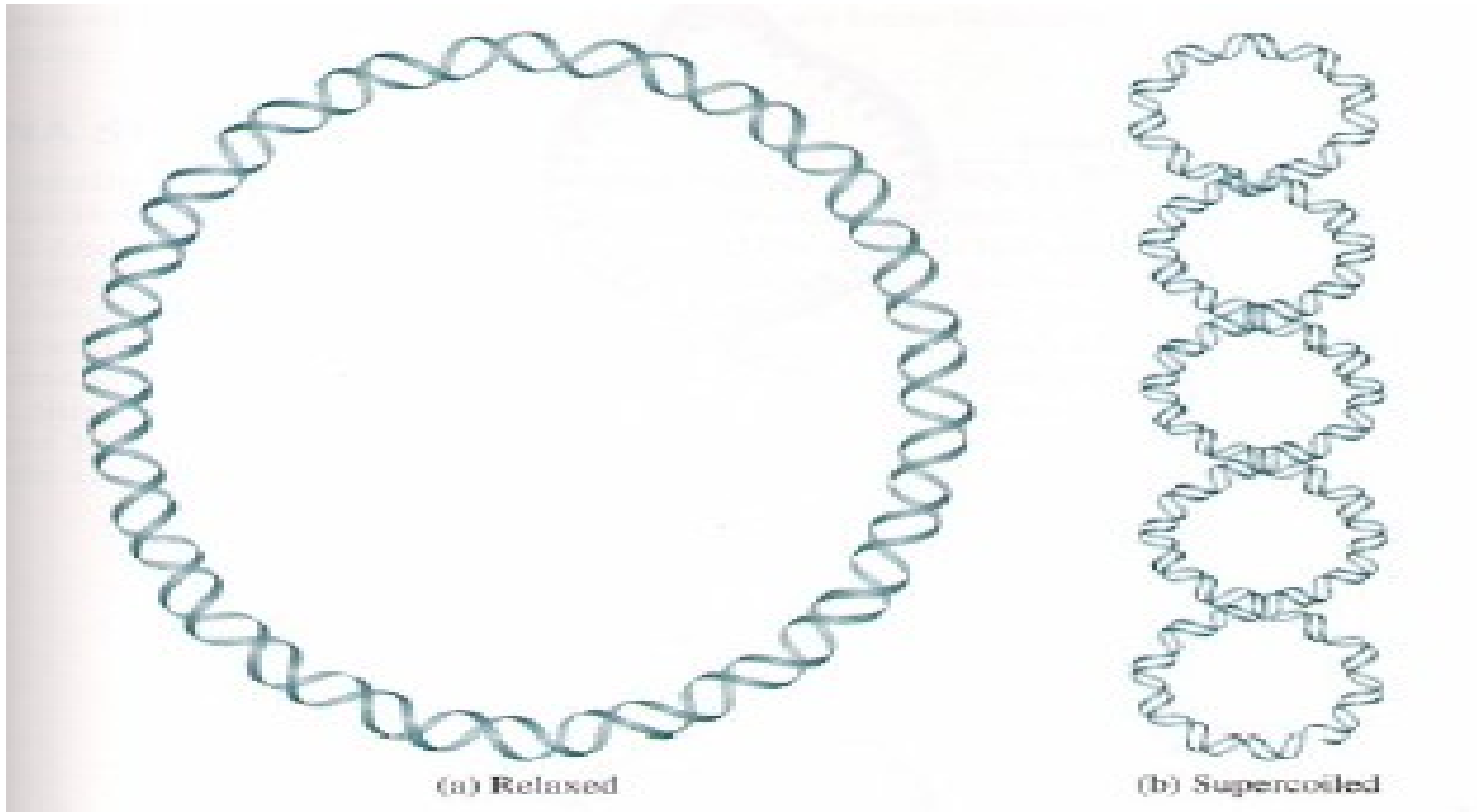


B-DNA

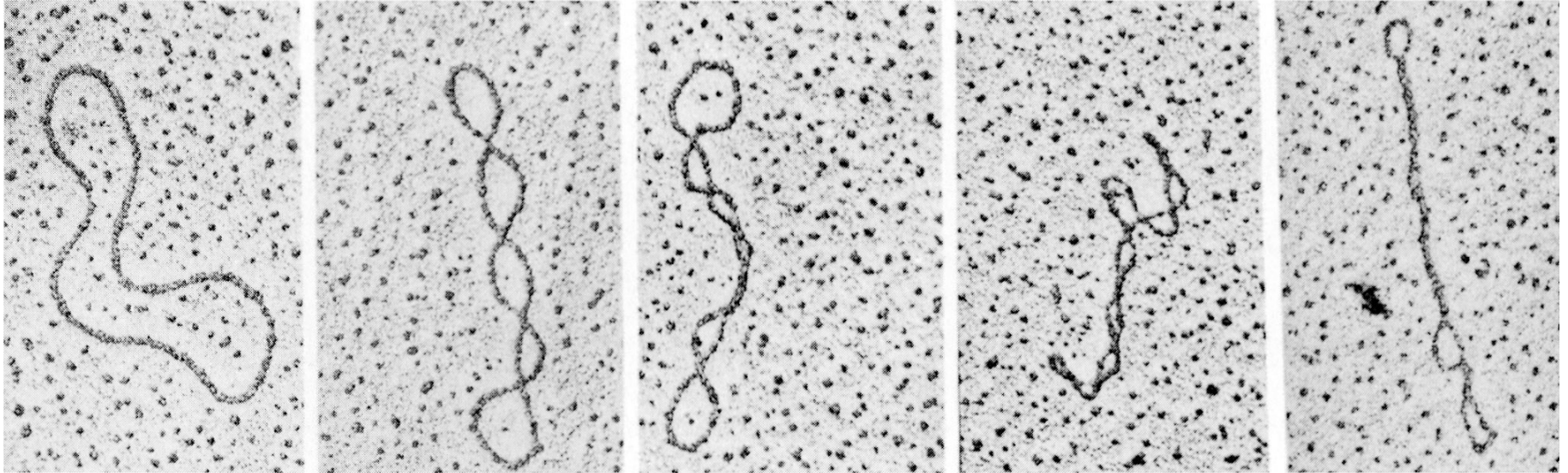


Z-DNA

Bakteriální DNA

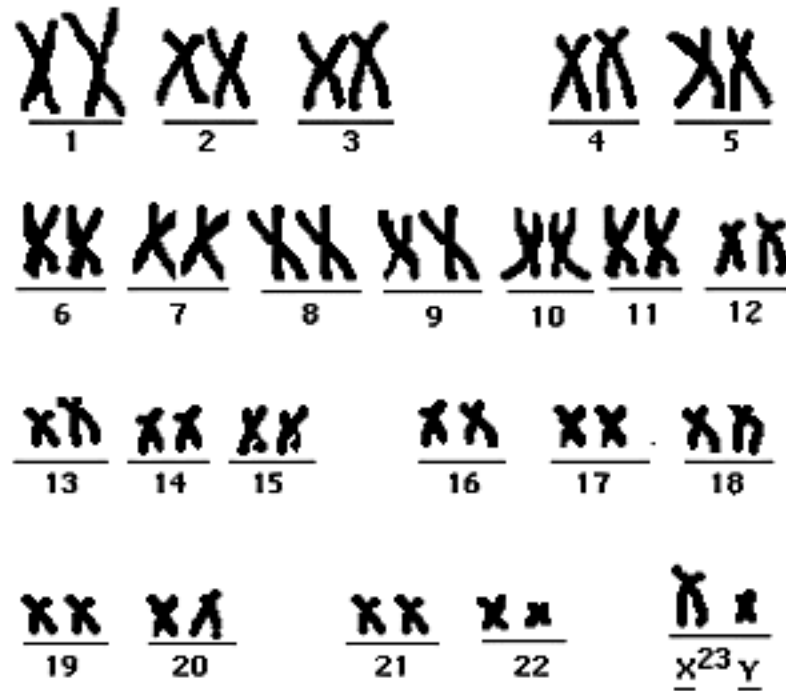


Bakteriální DNA

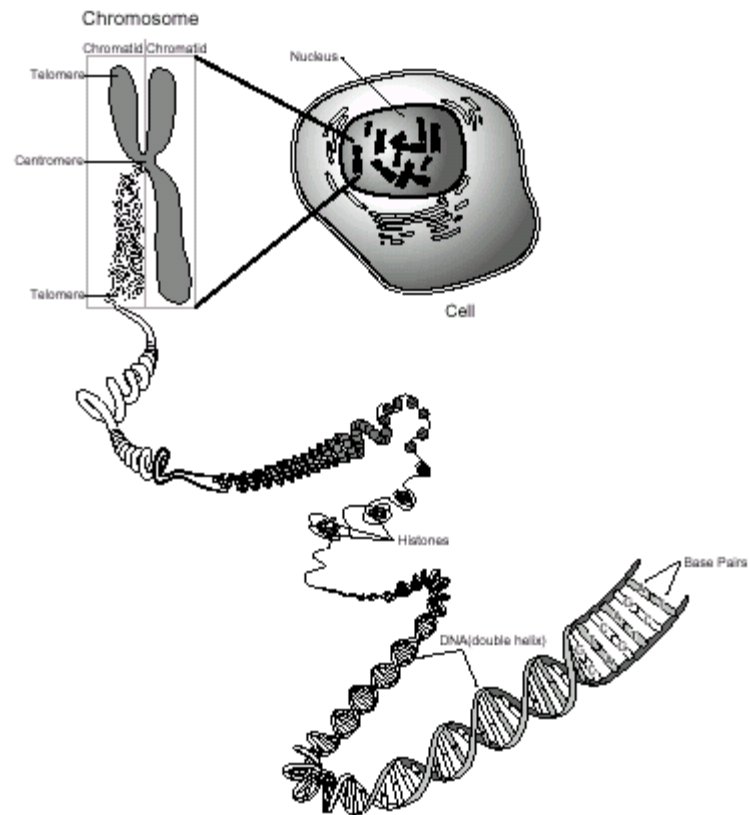


Electron micrographs by Laurien Polder. From Kornberg, A. and Baker, T.A., DNA Replication (2nd ed.), p. 36, W.H. Freeman (1992). Used with permission

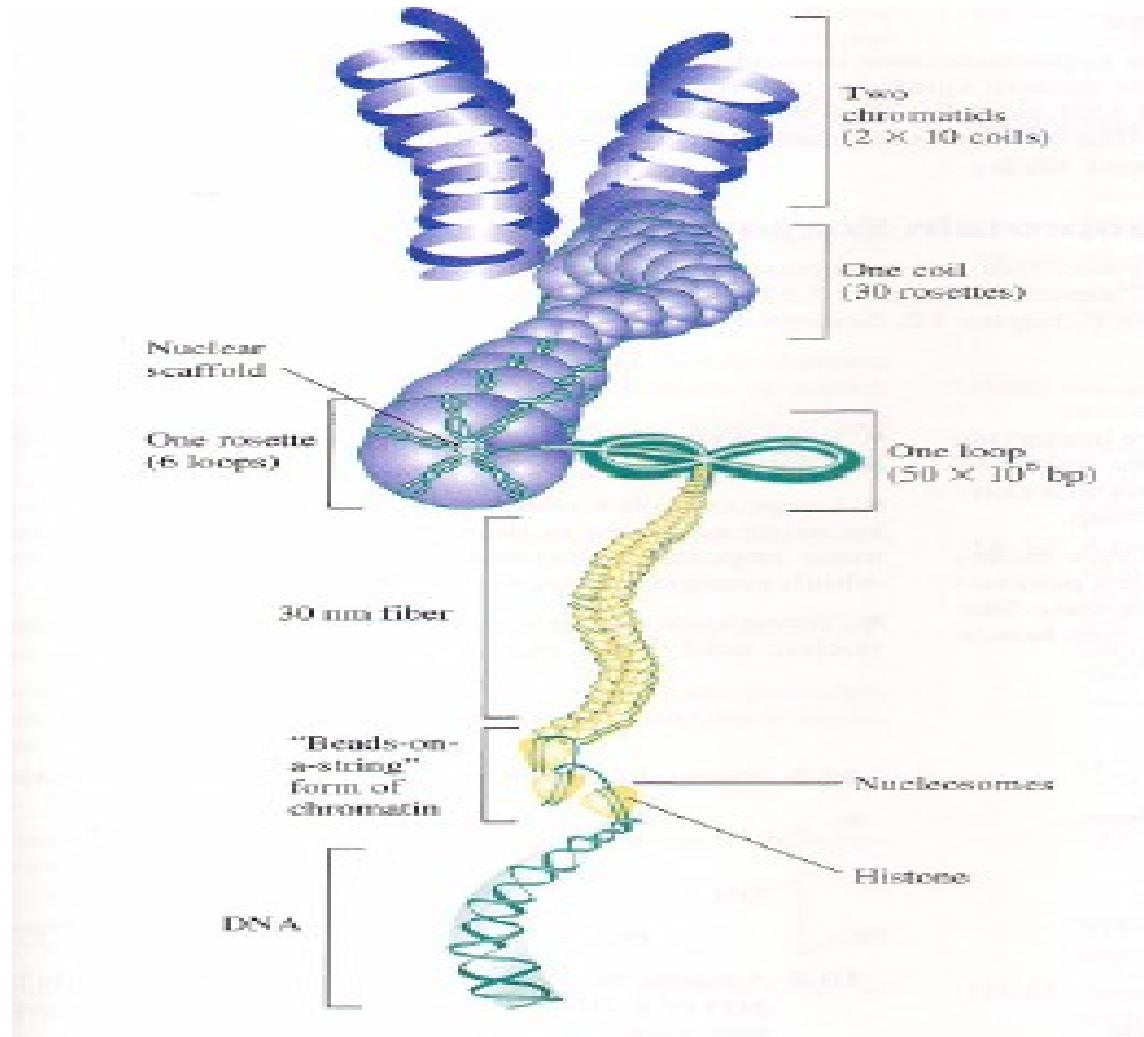
Lidské chromozomy



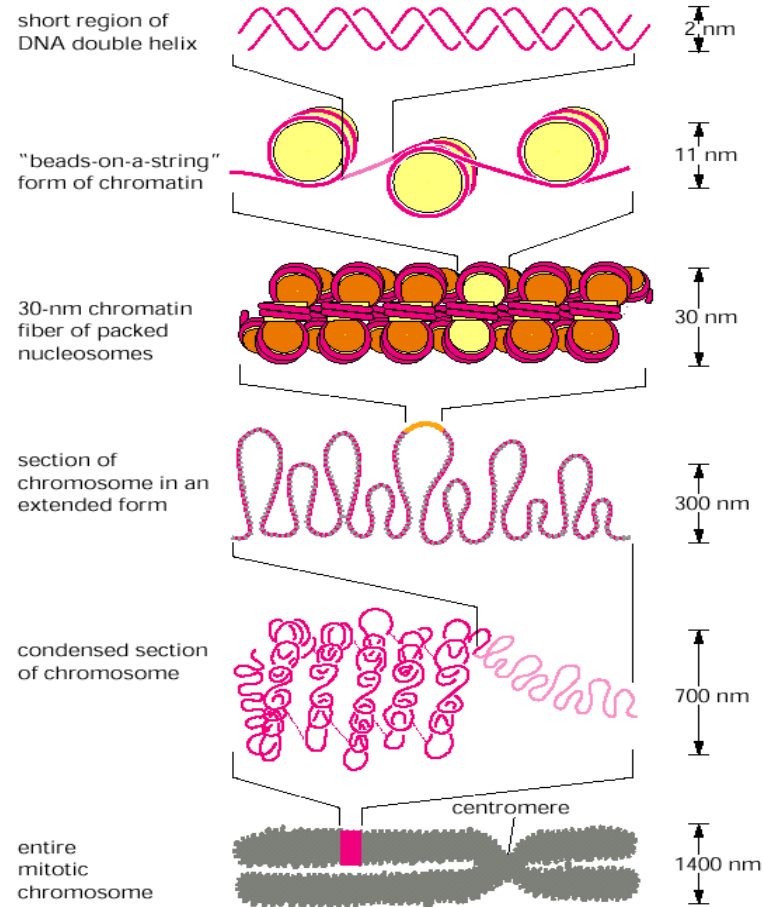
Chromozom



Chromozom

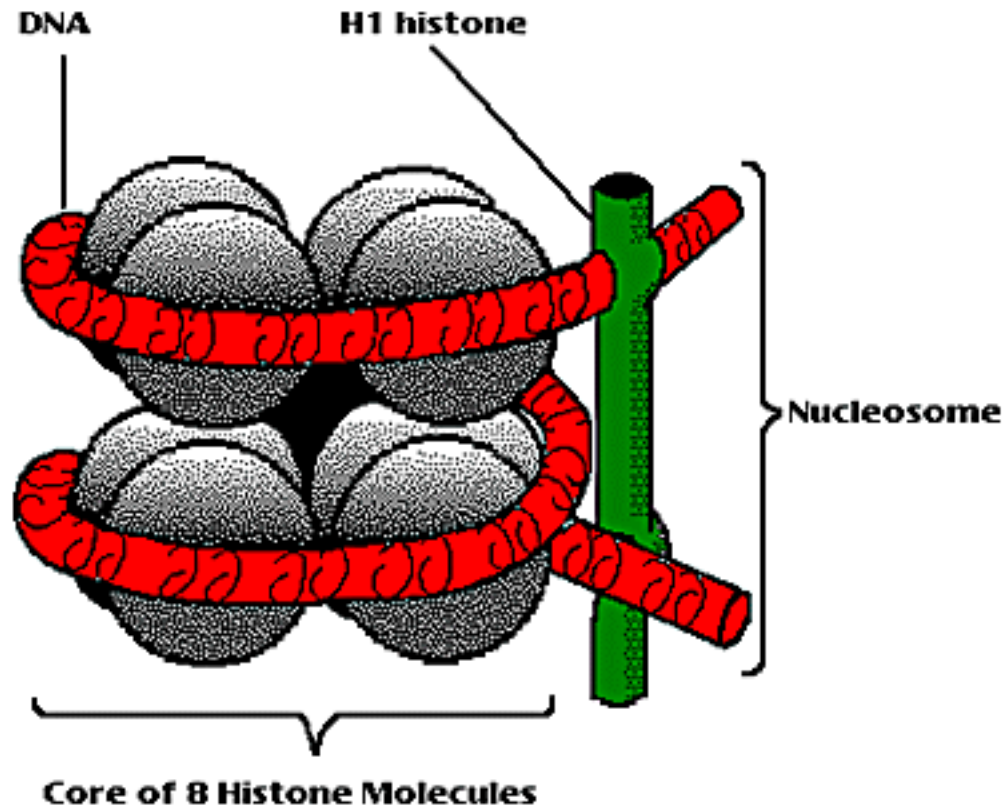


Chromatin

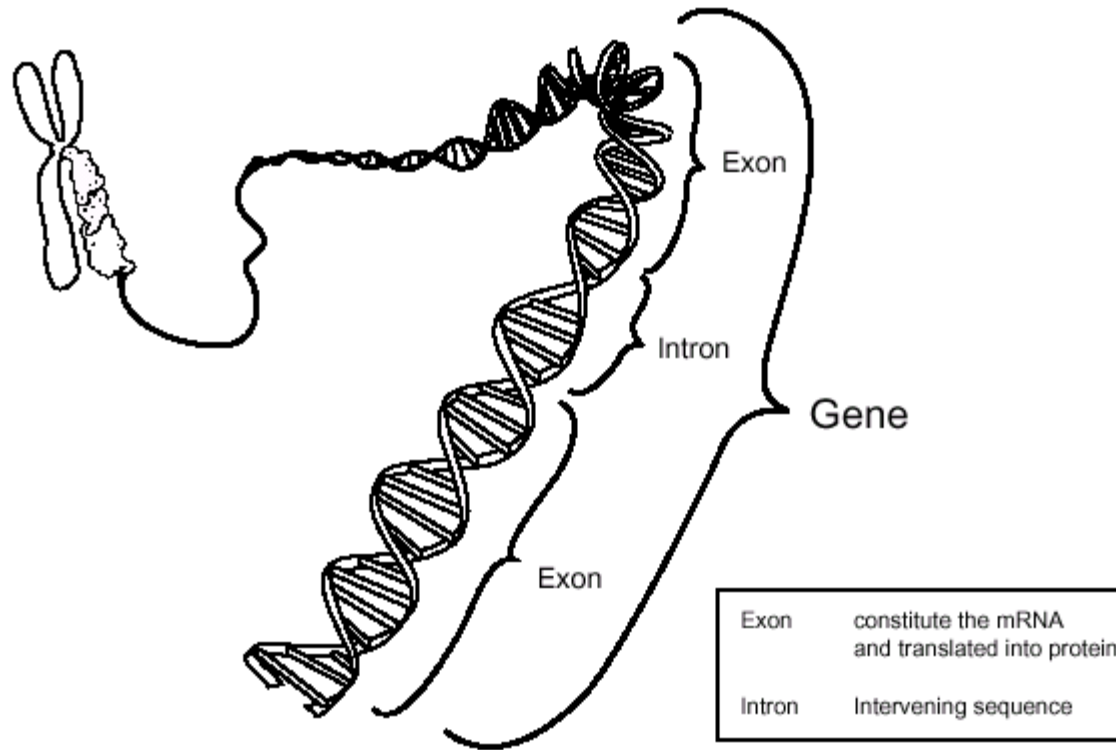


NET RESULT: EACH DNA MOLECULE HAS BEEN PACKAGED INTO A MITOTIC CHROMOSOME THAT IS 50,000x SHORTER THAN ITS EXTENDED LENGTH

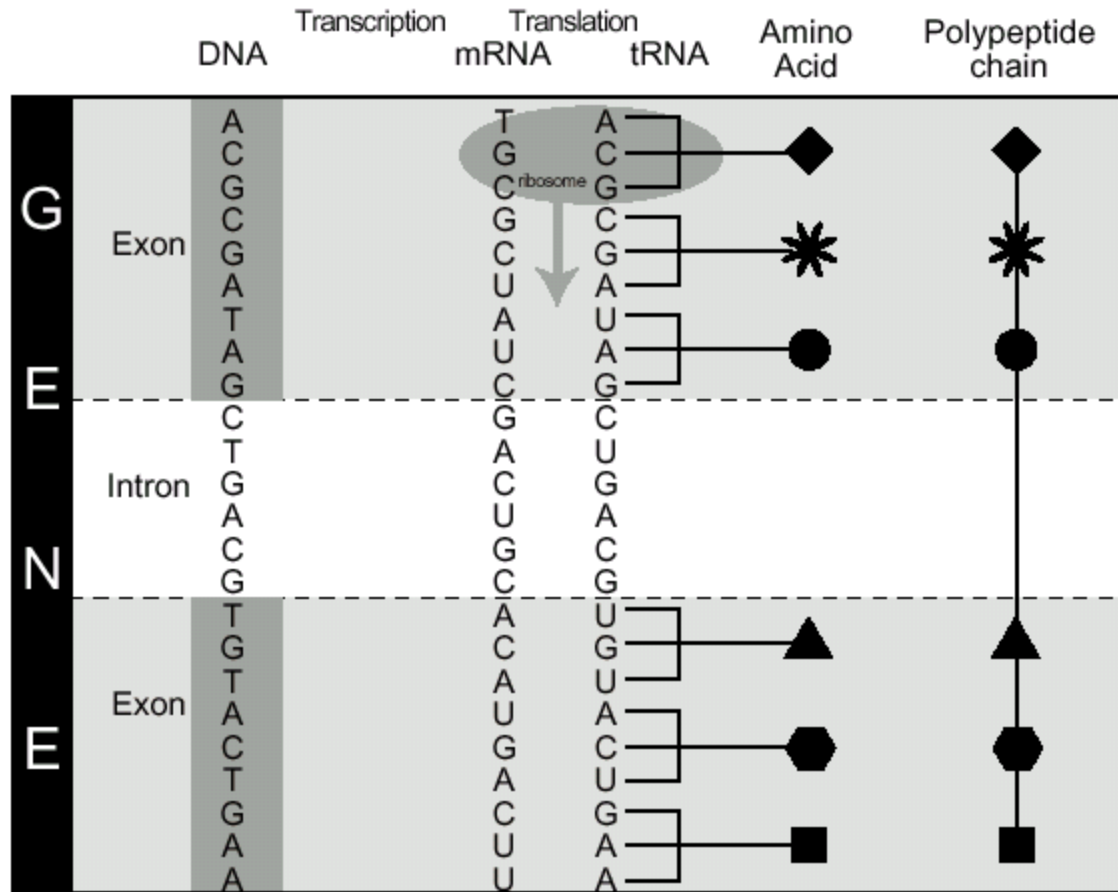
Nukleosom



Exon a intron



Exon a intron



Stanovení sekvence DNA

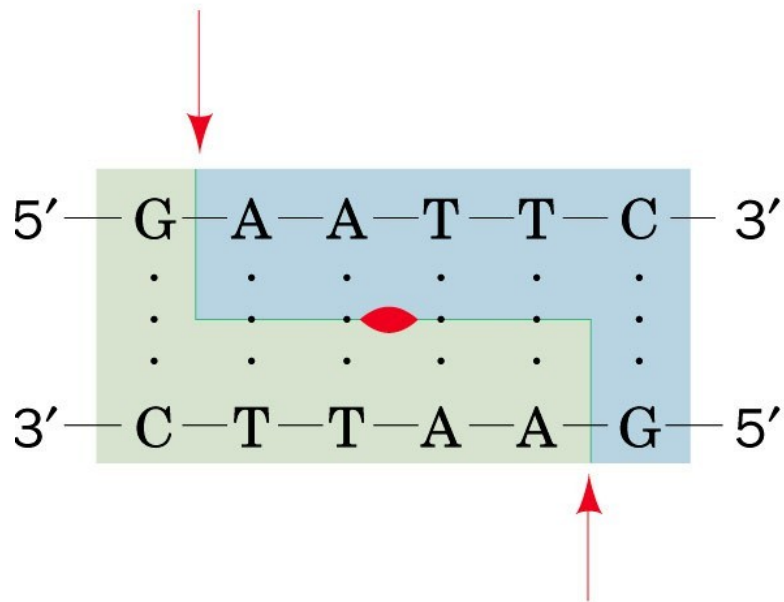
- Restrikční enzymy
- Chemické štěpení – Maxam Gilbertovo metoda
- Enzymová metoda

Restrikční enzymy

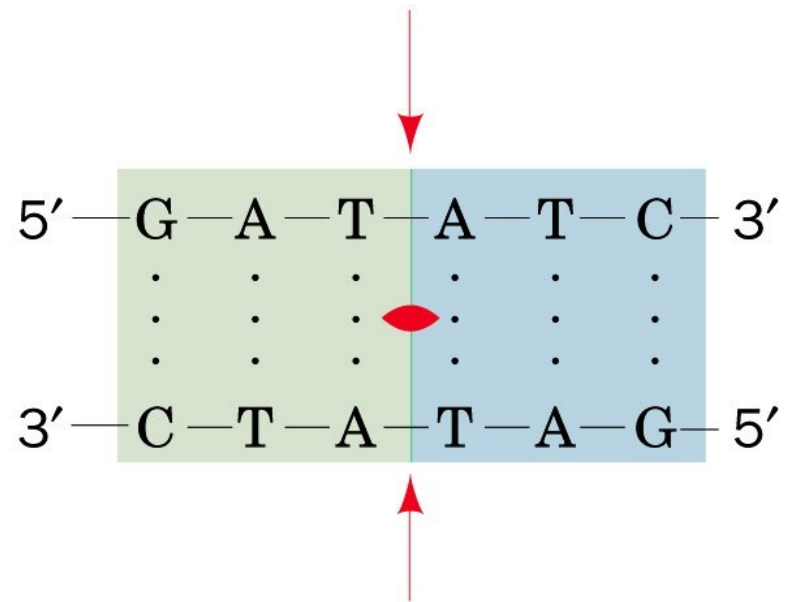
Enzyme	Recognition Sequence ^a	Microorganism
<i>AhaI</i>	AG↓C* ^a T	<i>Anthrobacter luteus</i>
<i>BamHI</i>	G↓GATC* ^a C	<i>Bacillus amyloliquefaciens</i> H
<i>BglI</i>	GCCNNNN↓NGCC	<i>Bacillus globigii</i>
<i>BglII</i>	A↓GATCT	<i>Bacillus globigii</i>
<i>EcoRI</i>	G↓AA* ^a TTC	<i>Escherichia coli</i> RY13
<i>EcoRII</i>	↓CC*(^a)GG	<i>Escherichia coli</i> R245
<i>EcoRV</i>	GA* ^a T↓ATC	<i>Escherichia coli</i> J62 pLG74
<i>HaeII</i>	RGCGC↓Y	<i>Haemophilus aegyptius</i>
<i>HaeIII</i>	GG↓C* ^a C	<i>Haemophilus aegyptius</i>
<i>HindIII</i>	A* ^a ↓AGCTT	<i>Haemophilus influenzae</i> R ₄
<i>HpaII</i>	C↓C* ^a GG	<i>Haemophilus parainfluenzae</i>
<i>MspI</i>	C* ^a ↓CGG	<i>Moraxella</i> species
<i>PstI</i>	CTGCA* ^a ↓G	<i>Providencia stuartii</i> 164
<i>PvuII</i>	CAG↓C* ^a TG	<i>Proteus vulgaris</i>
<i>SalI</i>	G↓TCGAC	<i>Streptomyces albus</i> G
<i>TaqI</i>	T↓CGA* ^a	<i>Thermus aquaticus</i>
<i>XhoI</i>	C↓TCGAG	<i>Xanthomonas holcicola</i>

Restrikční enzymy

(a) *EcoRI*



(b) *EcoRV*



 Cleavage site

 Twofold symmetry axis

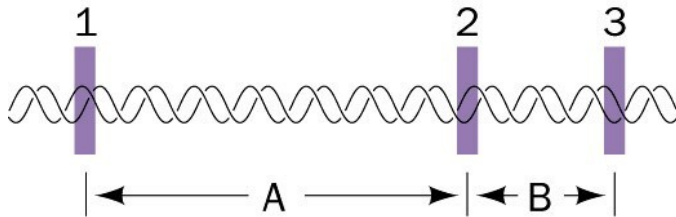
Restrikční enzymy



Restrikční enzymy

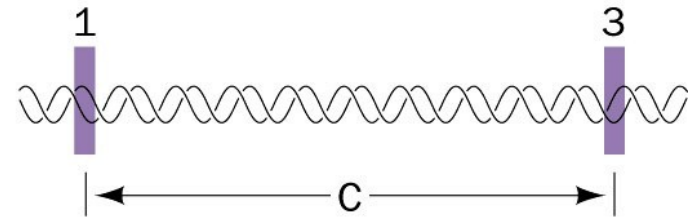
Chromosome I

DNA has
3 target sites



Chromosome II

DNA has only
2 of the target sites



Cleave with
restriction enzyme
and electrophoresis

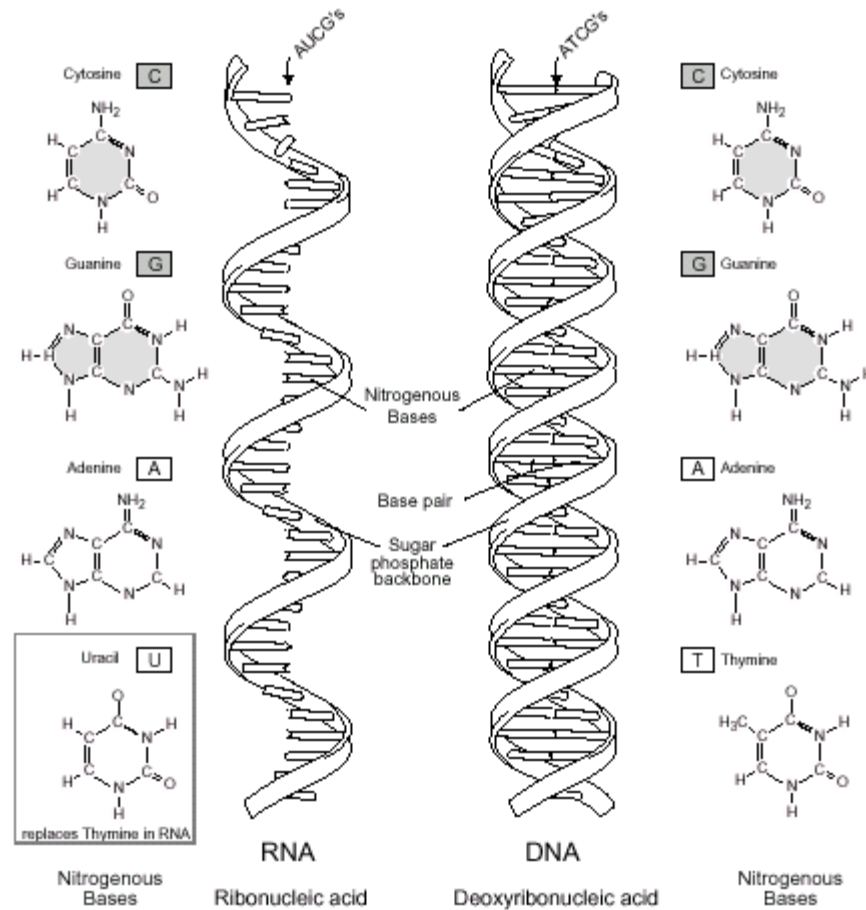
Fragment C is
the size of
A + B combined

Maxam Gilbertova metoda

Maxam-Gilbertova metoda

- značení 5' konce ^{32}P
- specifické chemické štěpení
- elektroforéza

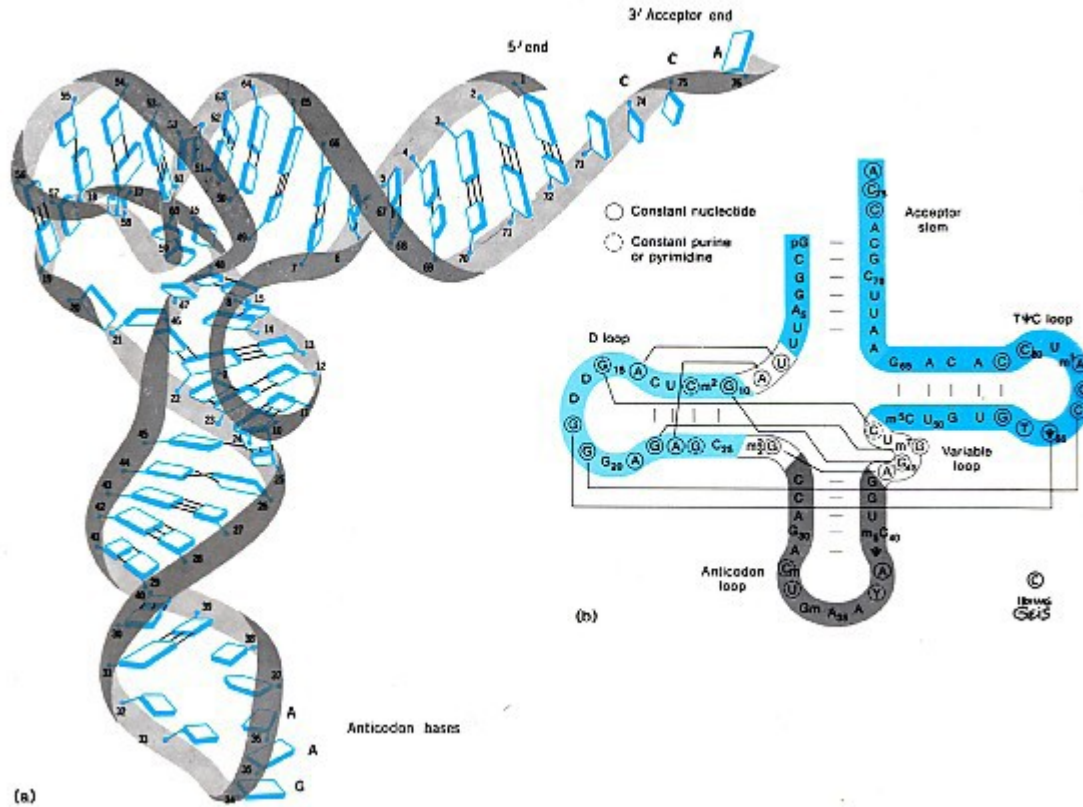
RNA versus DNA



Formy RNA

- mRNA – mediátorová, messenger,
informační – 5-10 %
- rRNA – ribosomální – 80 %
- tRNA – transferová, přenosová – 10-15 %
60 tRNA

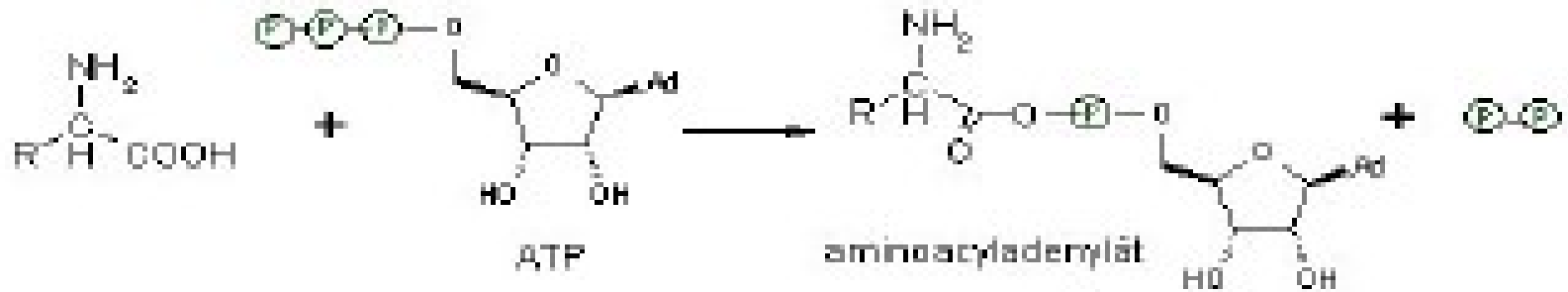
tRNA



(a)

(b)

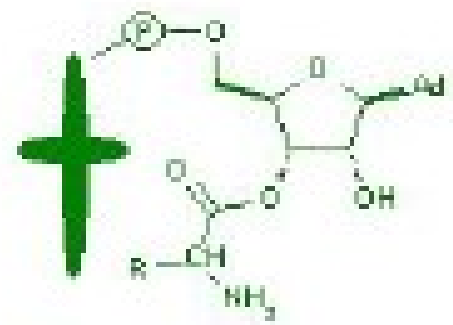
Anticodon bases



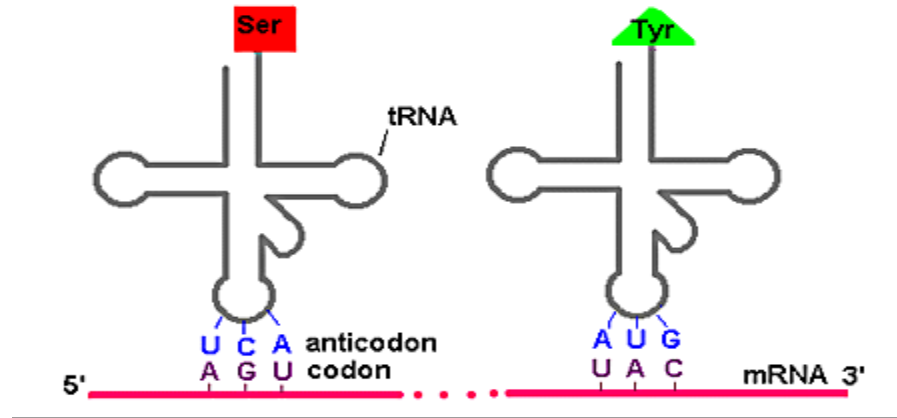
tRNA



aminoacyl-tRNA + AMP



Genetický kod



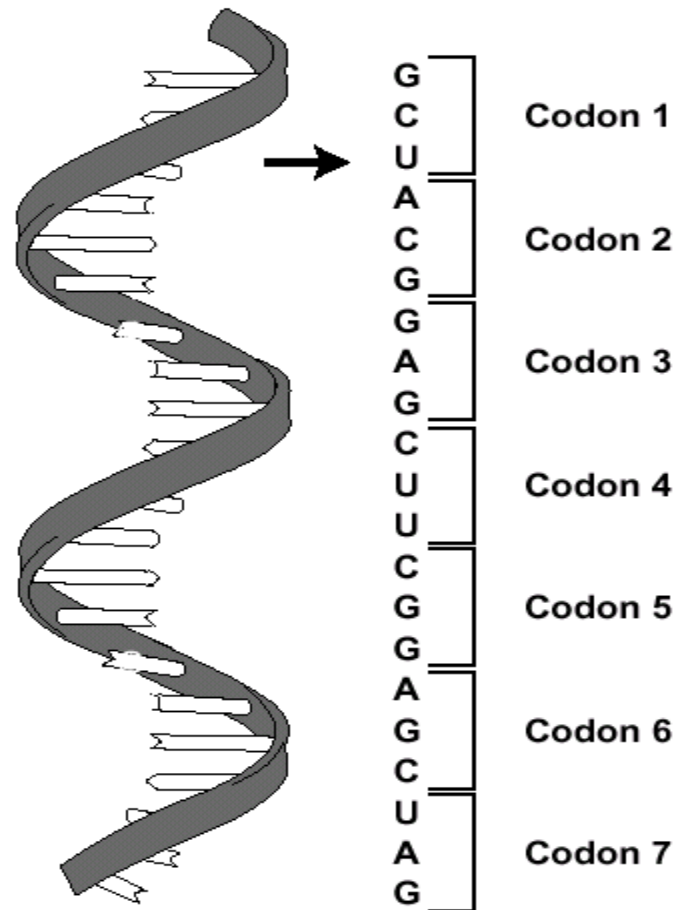
2nd base in codon

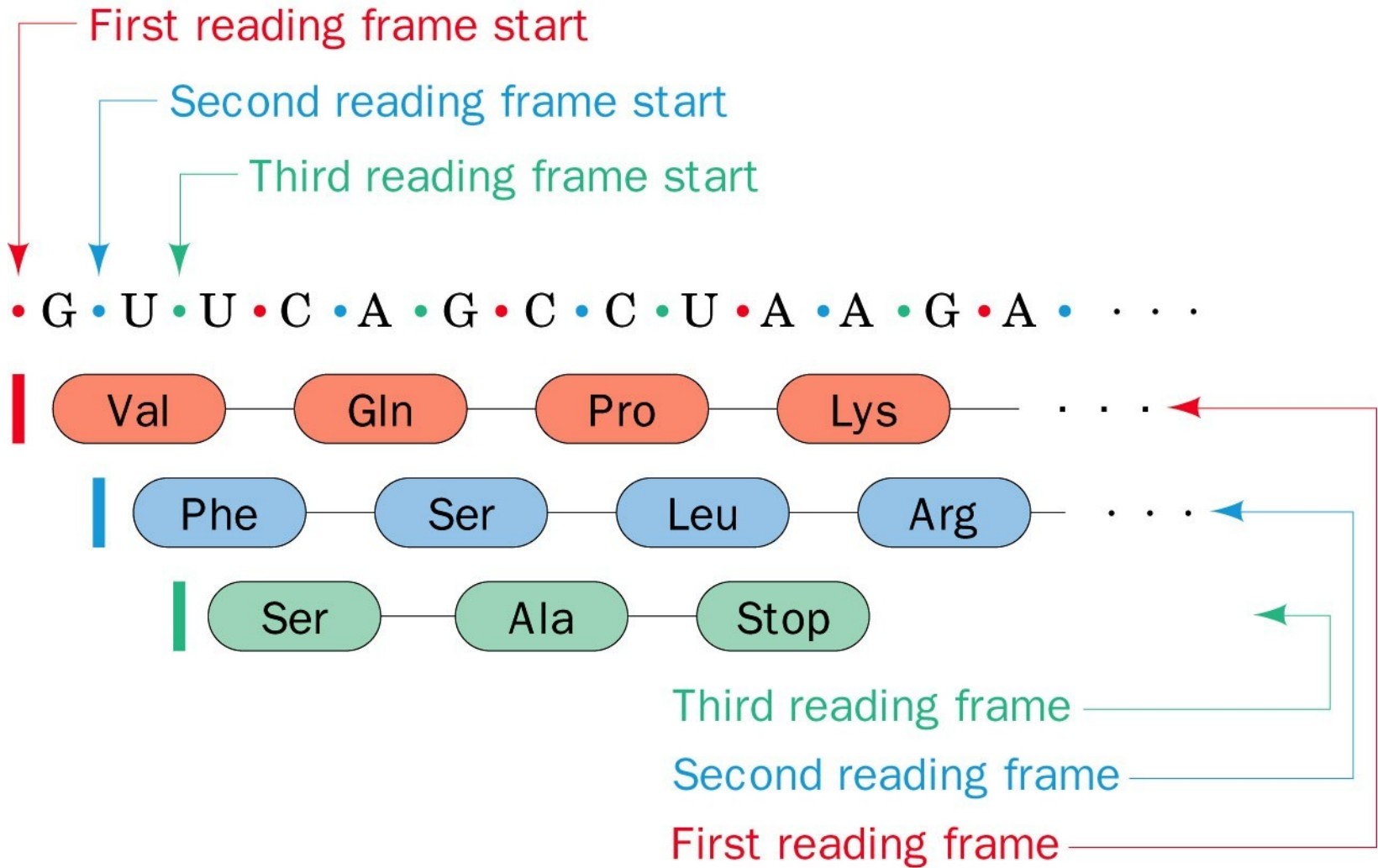
	U	C	A	G	
U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr STOP STOP	Cys Cys STOP Trp	U C A G
C	Leu Leu Leu Leu	Pro Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G
A	Ile Ile Ile Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G
G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G

1st base in codon

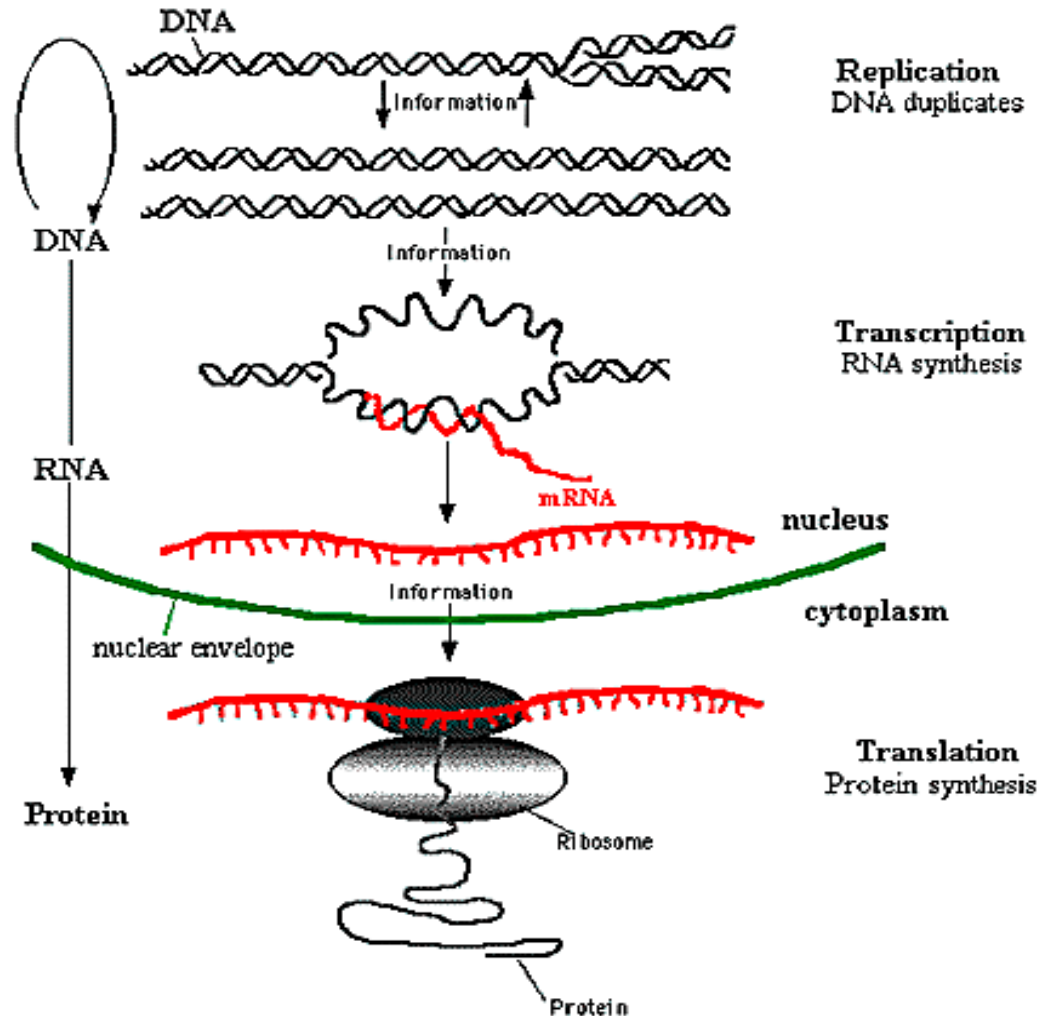
3rd base in codon

Kodón

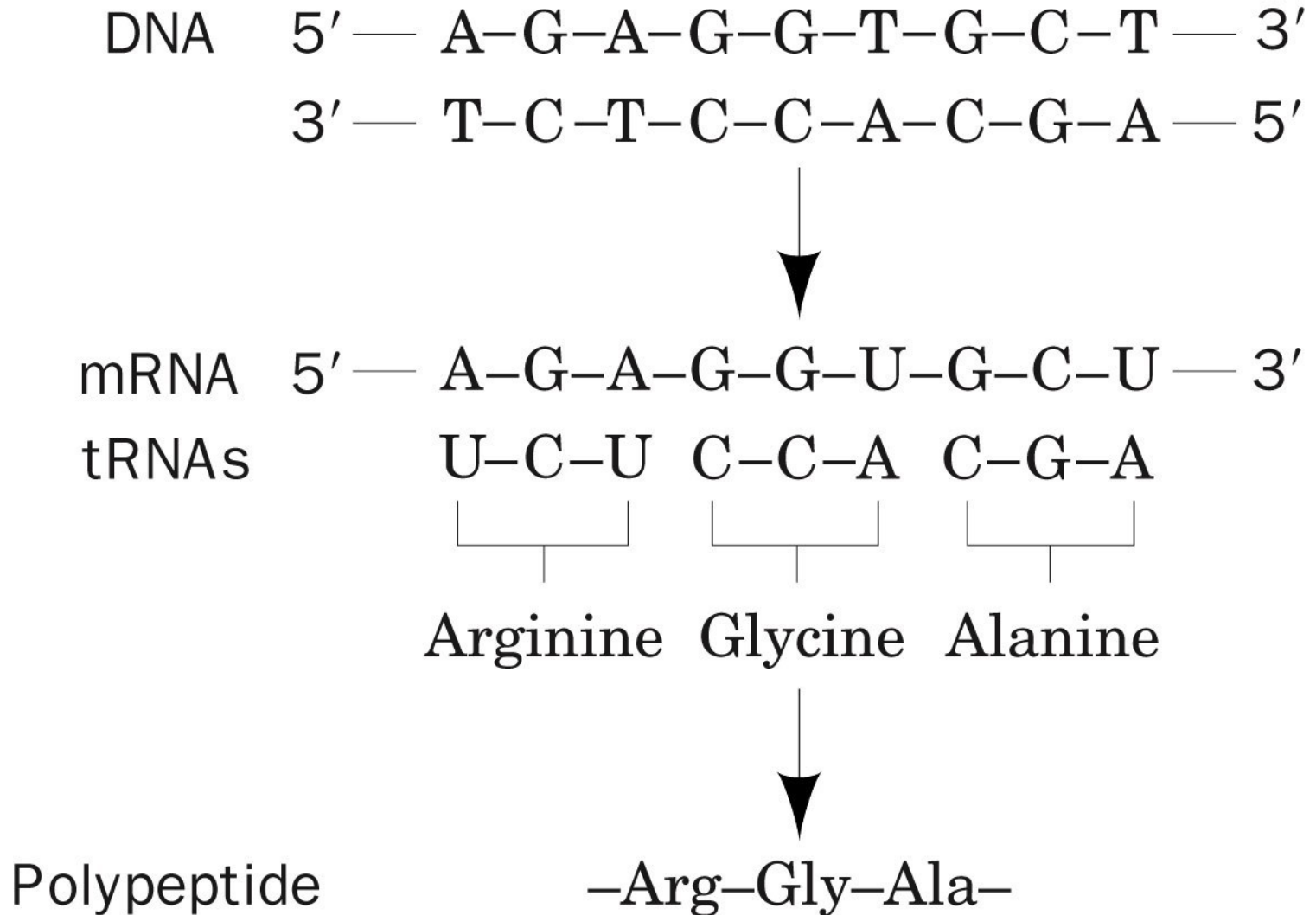




Centrální dogma mol.biologie

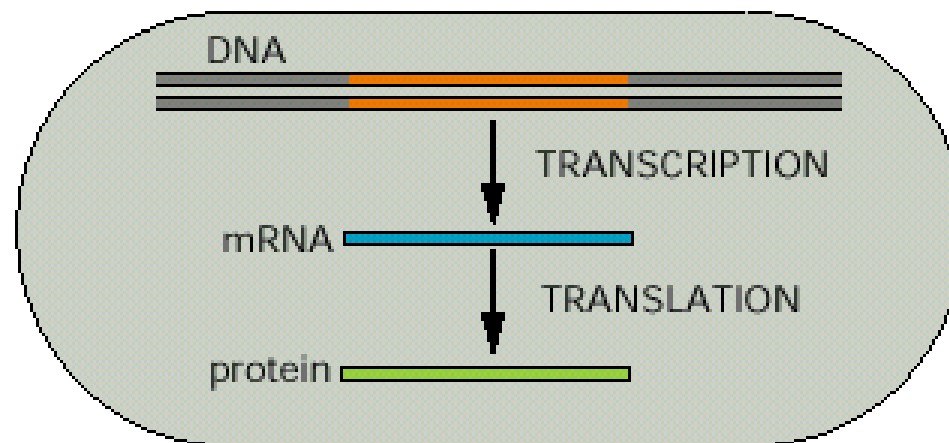


Centrální dogma mol.biologie



Prokaryota

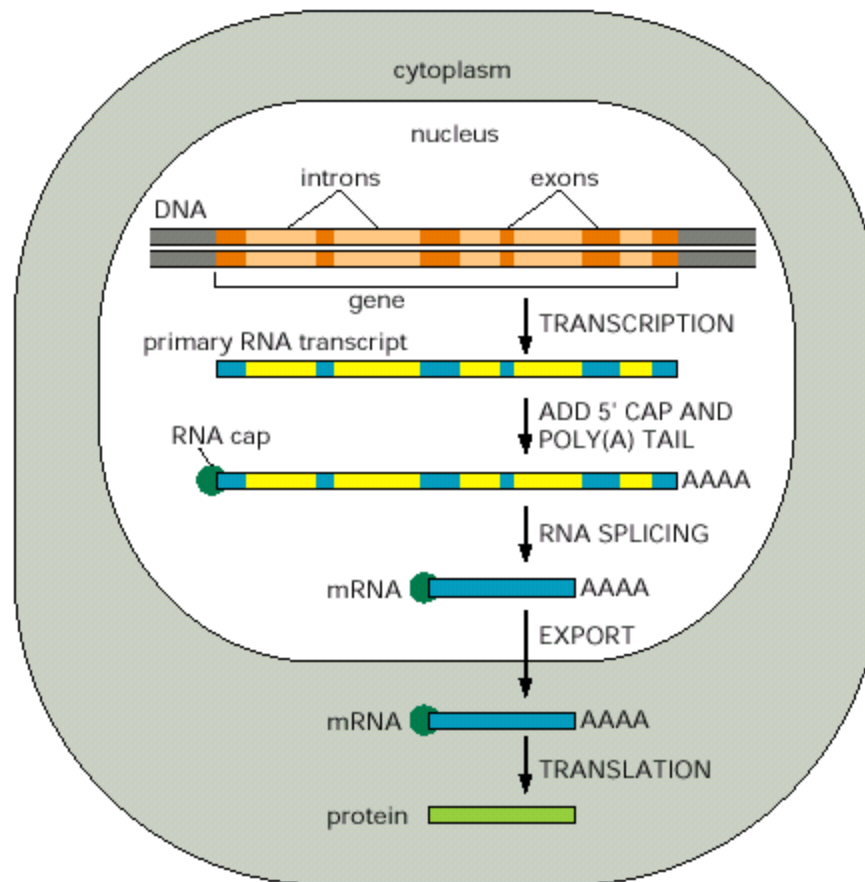
(B) PROCARYOTES



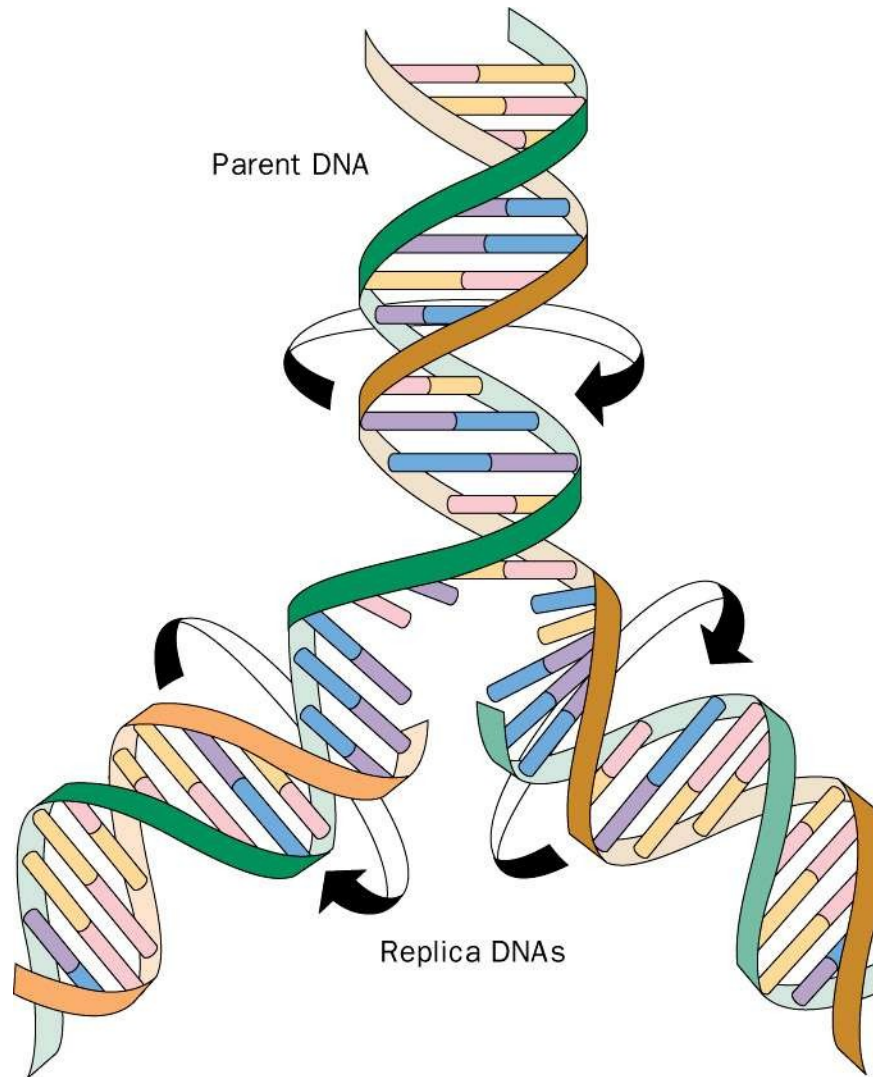
Eukaryota

(A) EUCARYOTES

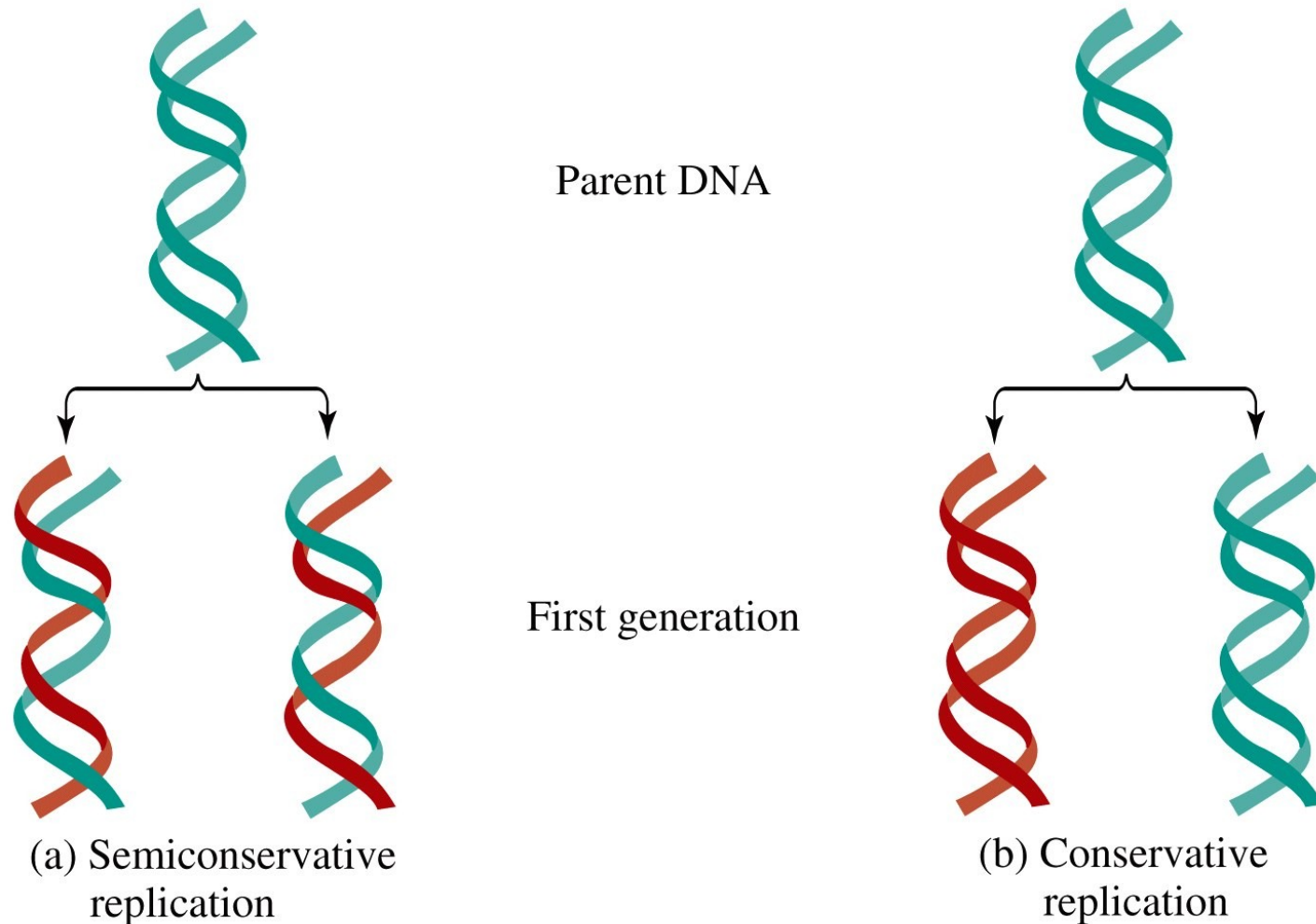
(B)



Replikace DNA



Replikace DNA



Meselsonův a Stahlův experiment

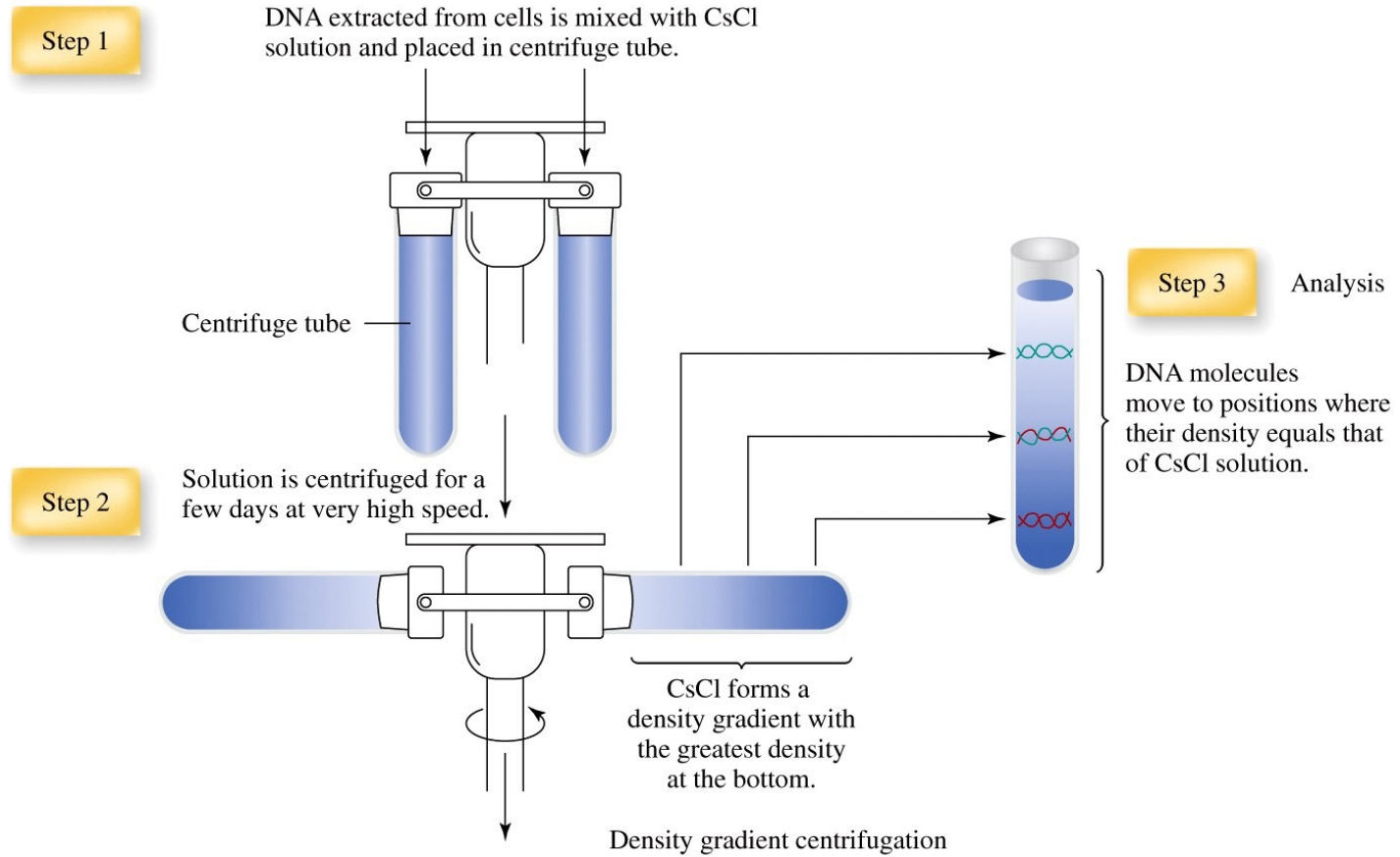


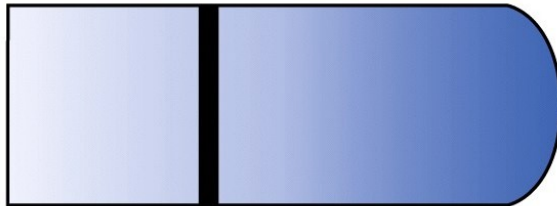
Figure 11-2a Concepts in Biochemistry, 3/e
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Meselsonův a Stahlův experiment

Direction of sedimentation



Heavy DNA



Light DNA



Parent ^{15}N -DNA
(both strands
heavy)

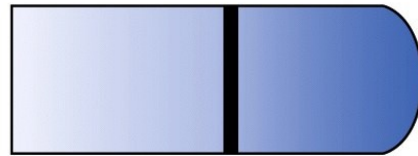


Normal ^{14}N -DNA
(with two light
strands)

Preliminary experiment

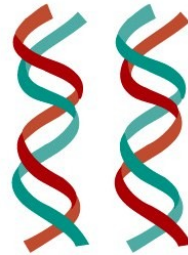
Meselsonův a Stahlův experiment

Experimental results



Hybrid DNA

After one generation on $^{14}\text{N-NH}_4\text{Cl}$



Conclusions

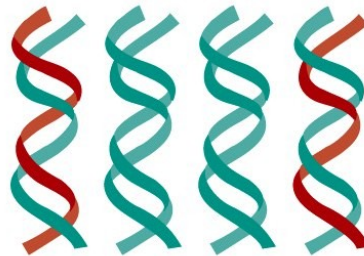
First generation:
Both DNAs contain one light and one heavy strand.



Light DNA

Hybrid DNA

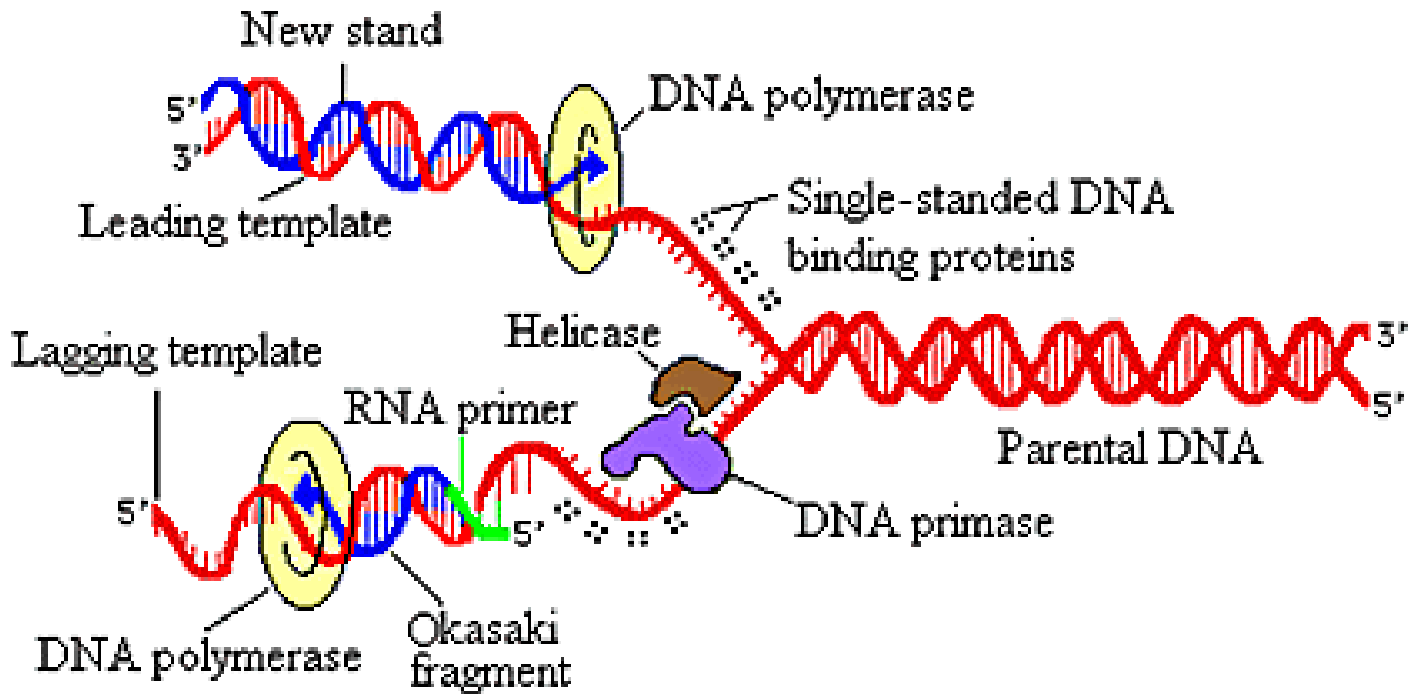
After two generations on $^{14}\text{N-NH}_4\text{Cl}$



Second generation:
Two hybrid DNAs and two light DNAs are formed.

Actual experiment

Účast enzymů na replikaci



Účast enzymů na replikaci

Table 11.2

Proteins necessary for DNA replication in *E. coli*

Protein	Function
Helicase	Begins unwinding of DNA double helix
DNA gyrase	Assists unwinding
SSB proteins	Stabilize single strands of DNA
Primase	Synthesis of RNA primer
DNA polymerase III	Elongation of chain by DNA synthesis
DNA polymerase I	Removal of RNA primer and filling in gap with DNA
DNA ligase	Closes last phosphoester gap to form phosphodiester bond

Prokaryontní replikaci

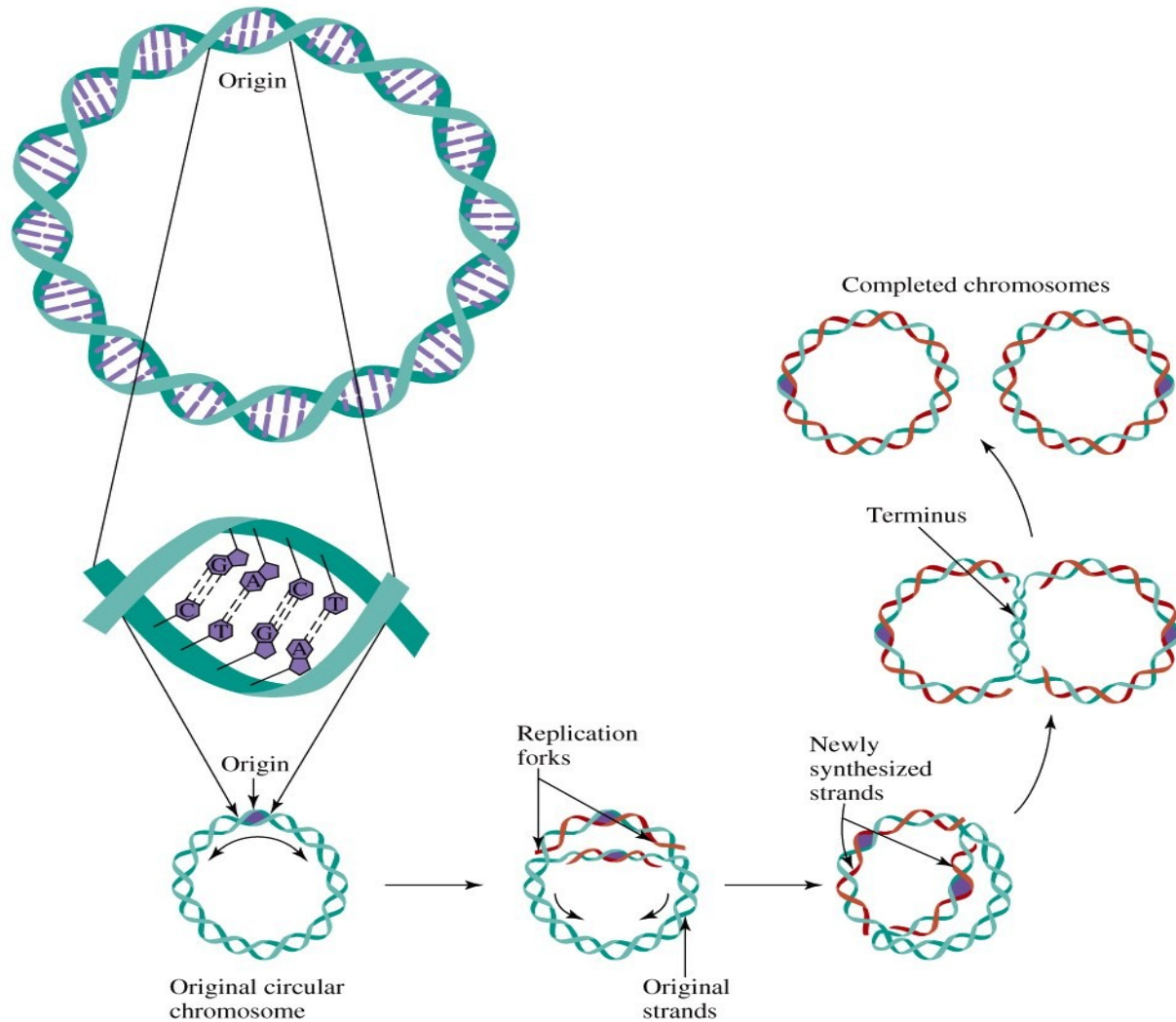
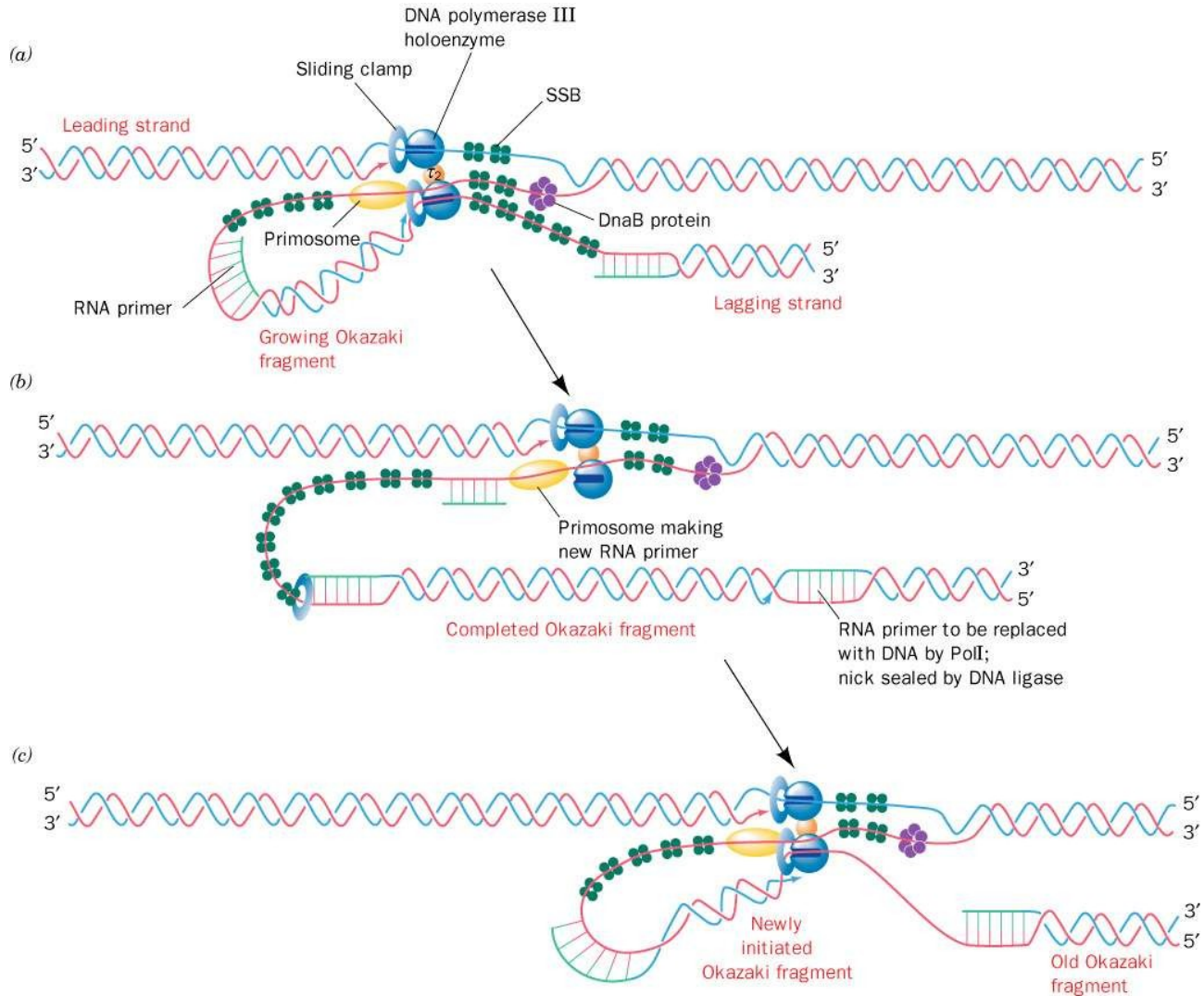


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Prokaryontní replikaci



Eukaryontní replikaci

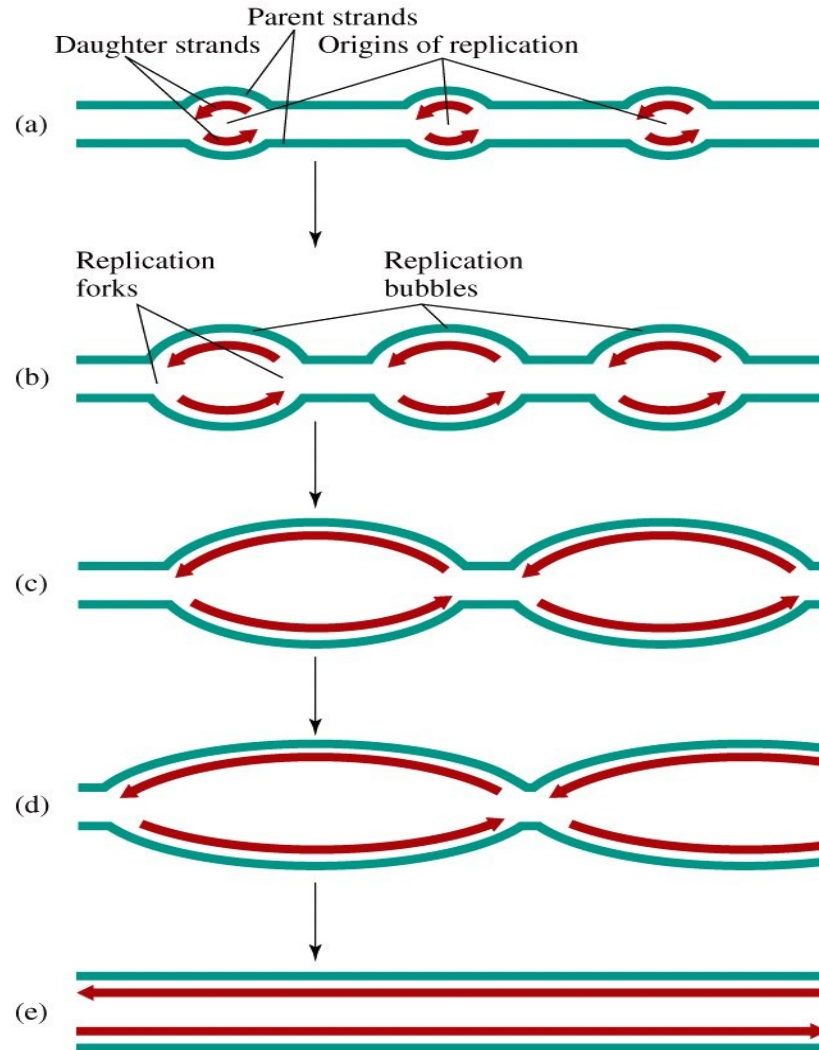
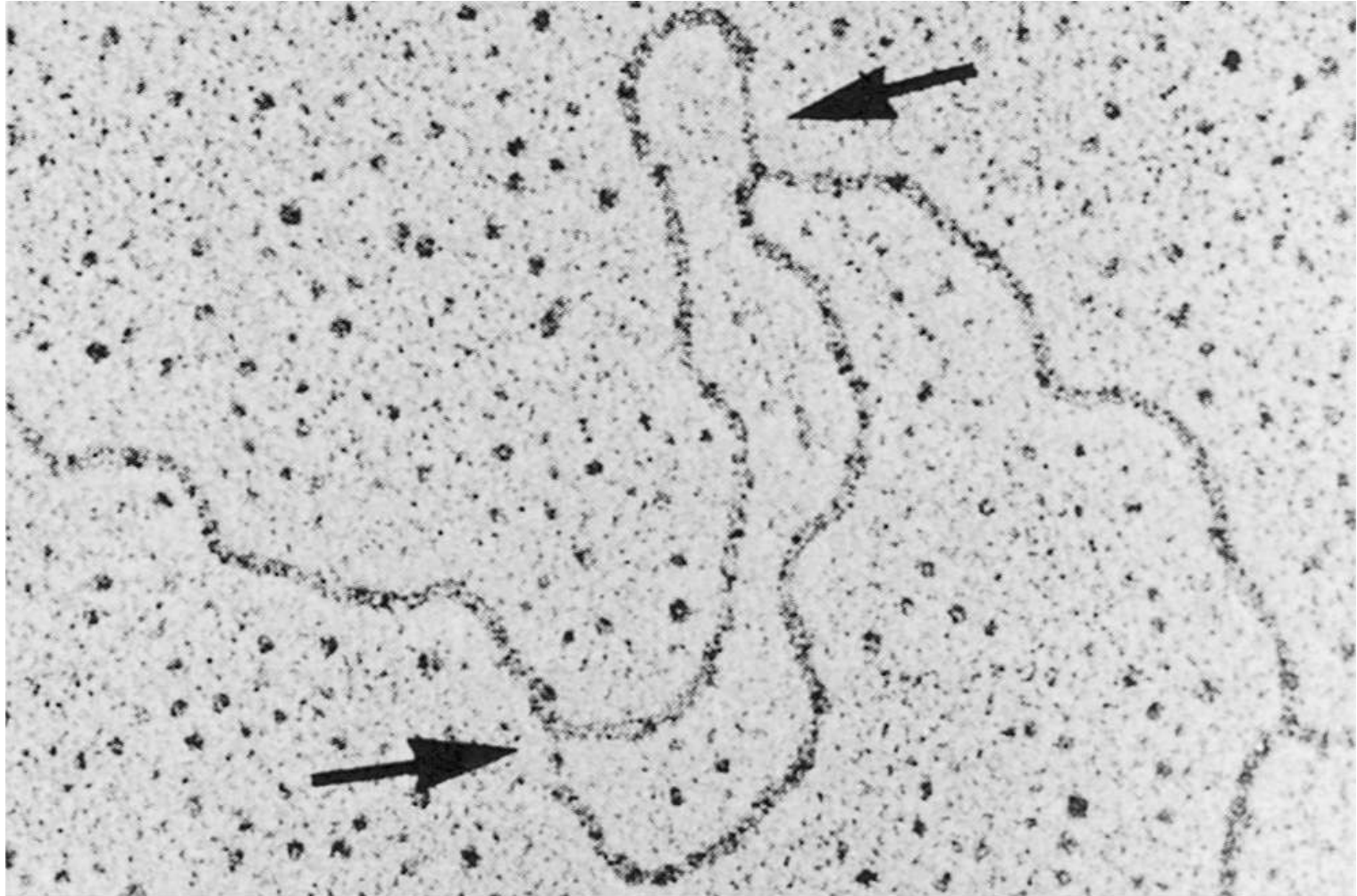
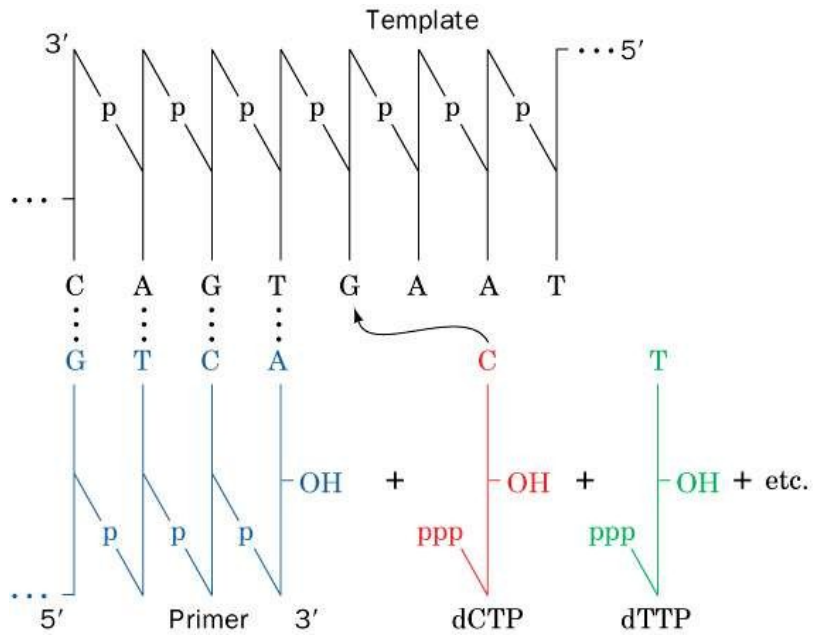


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Replikace u drosofil

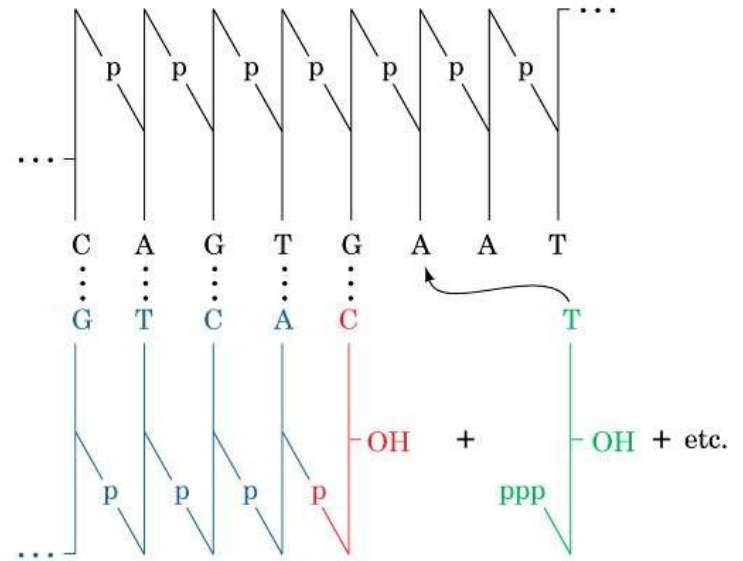


From Kreigstein, H.J. and Hogness, D.S., *Proc. Natl. Acad. Sci.* **71**, 173 (1974)



DNA
polymerase

PP_i



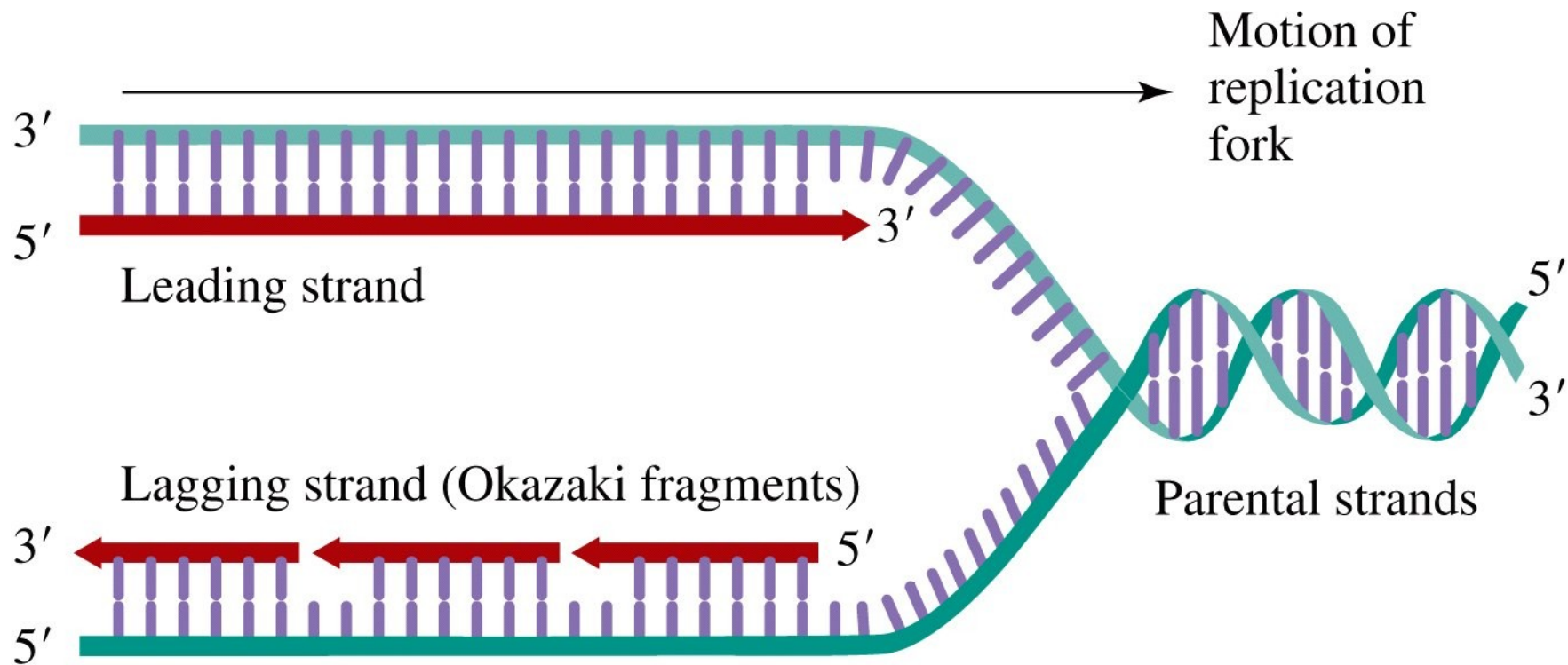


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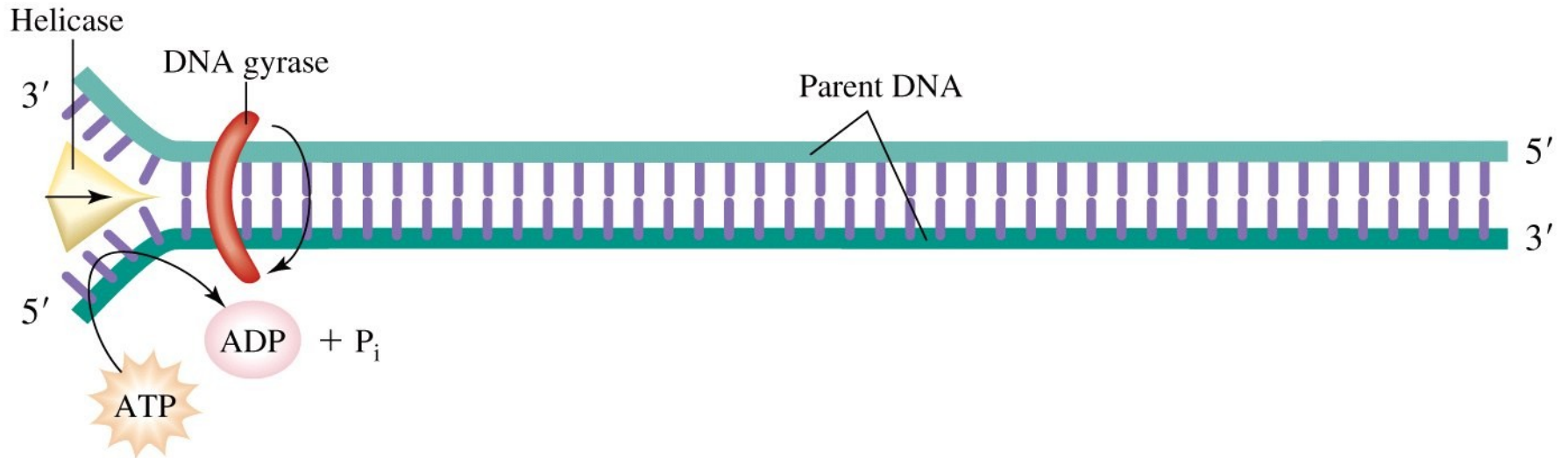


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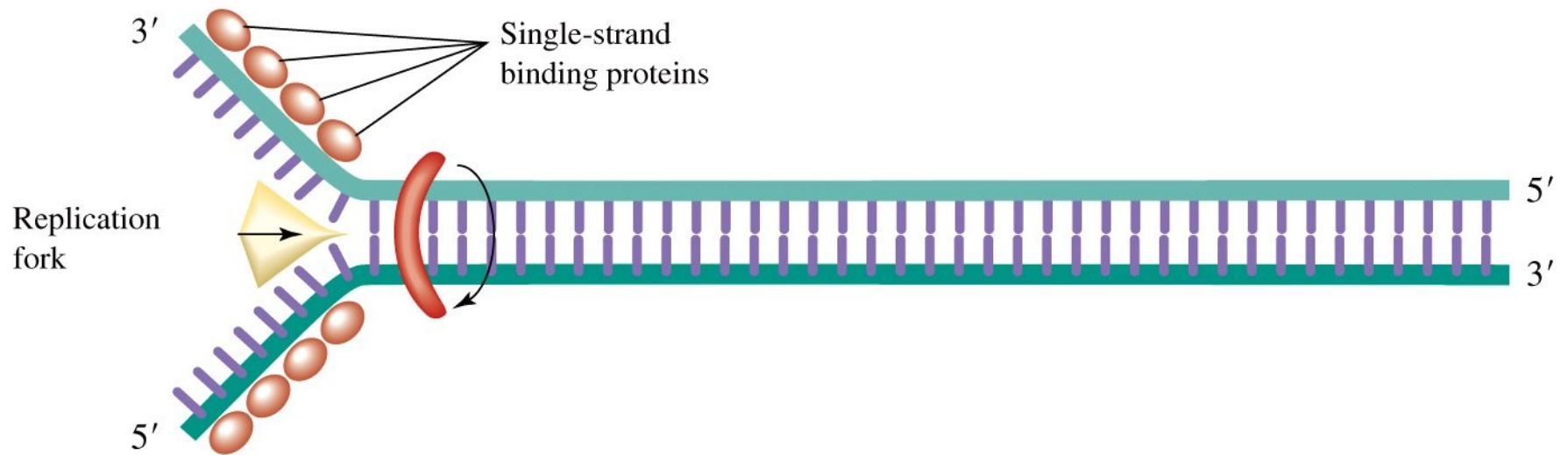


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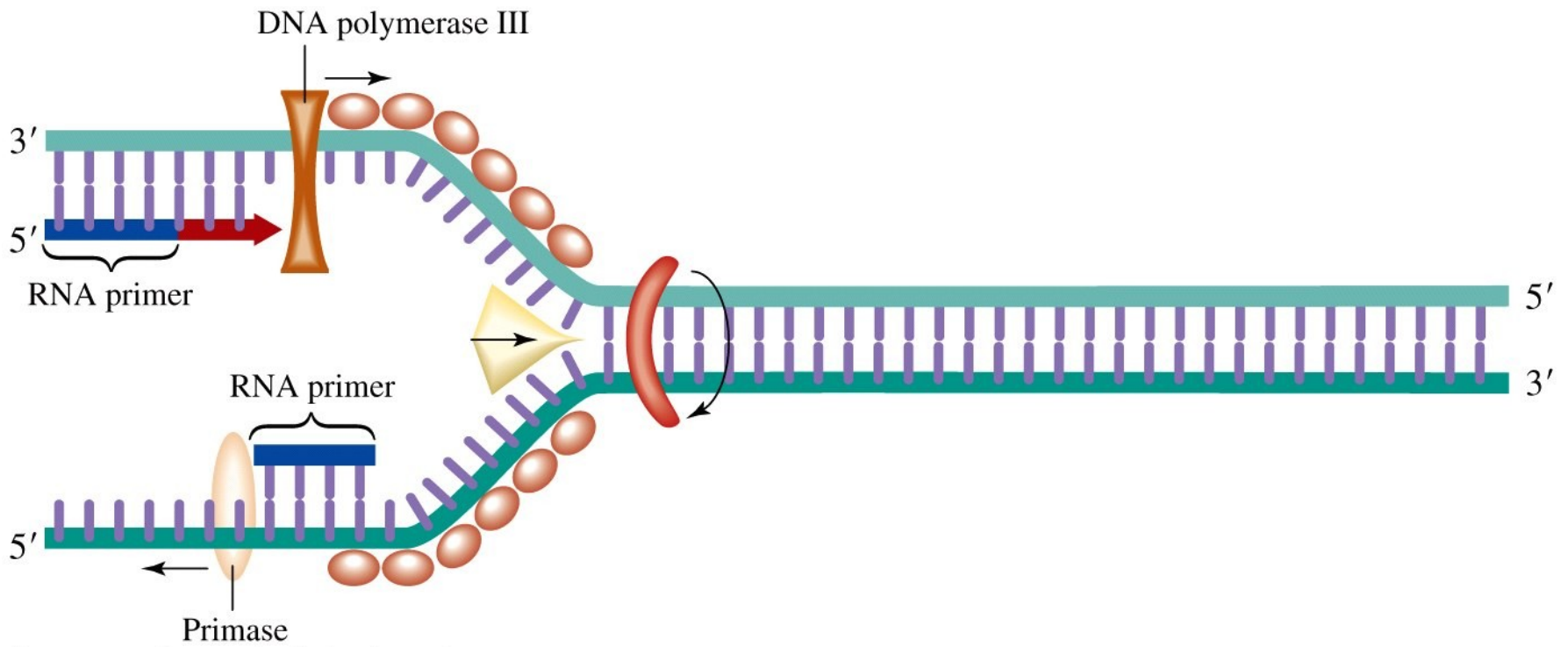


Figure 11-10c Concepts in Biochemistry, 3/e
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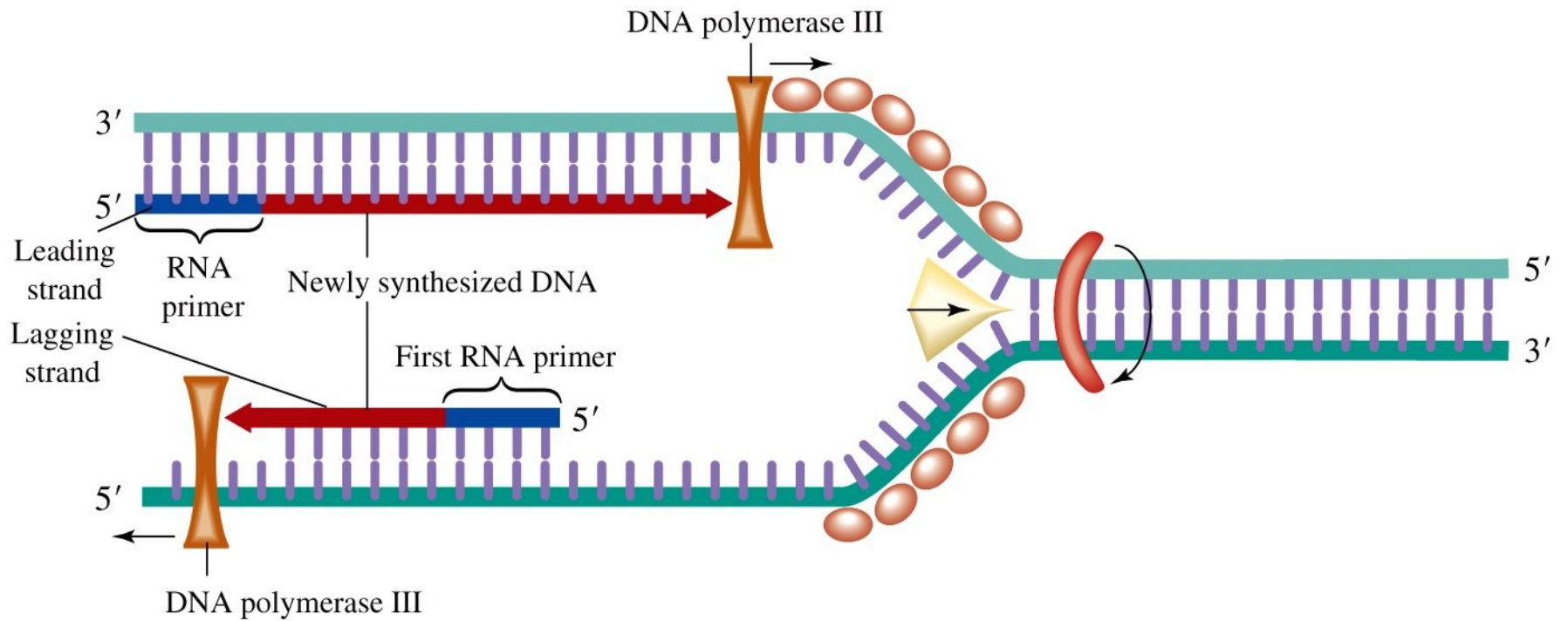


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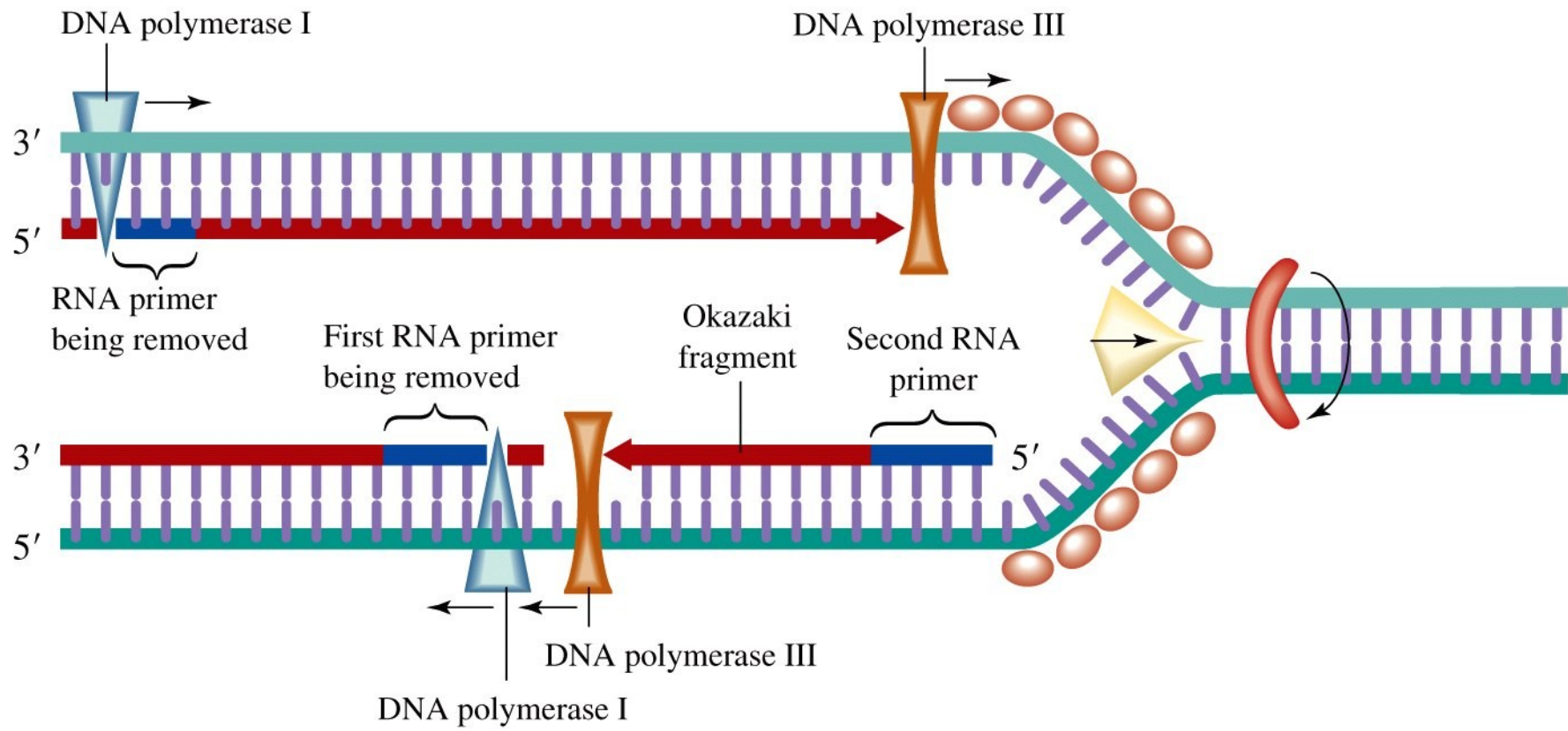


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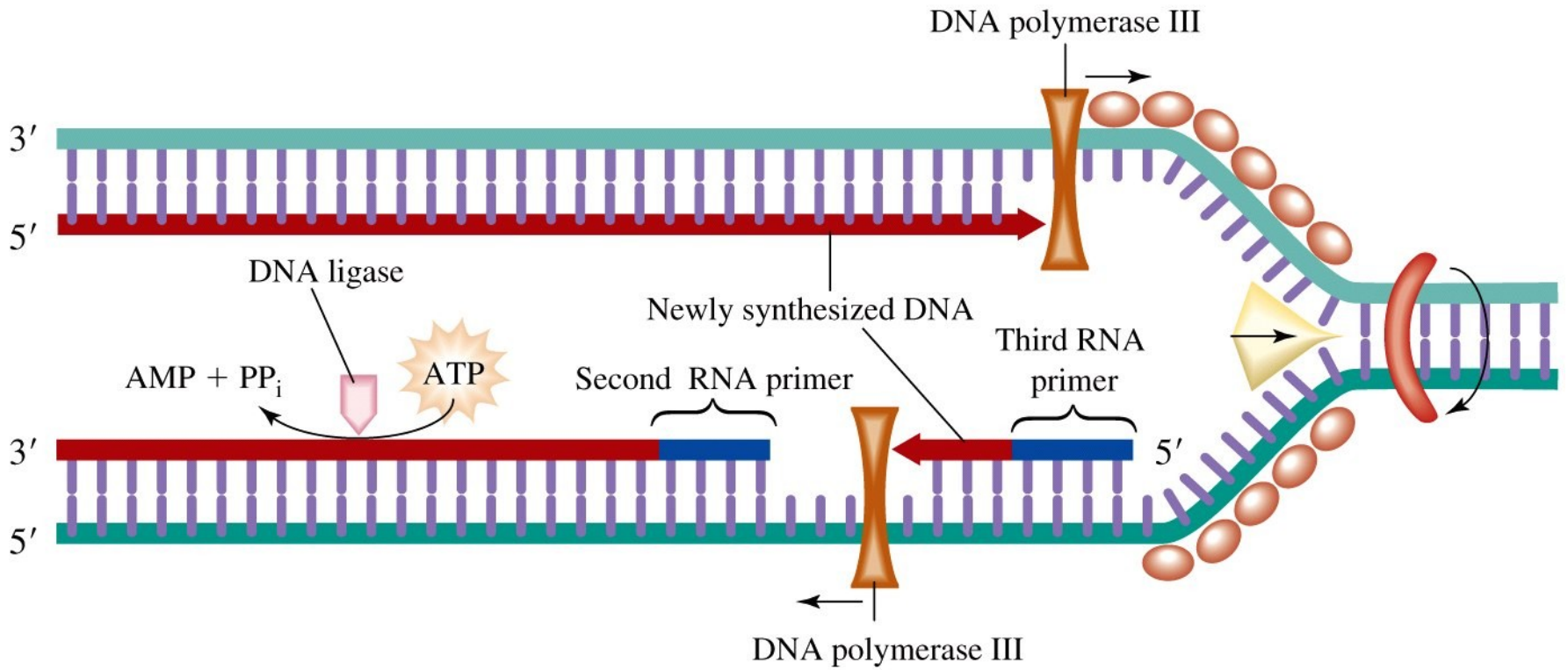
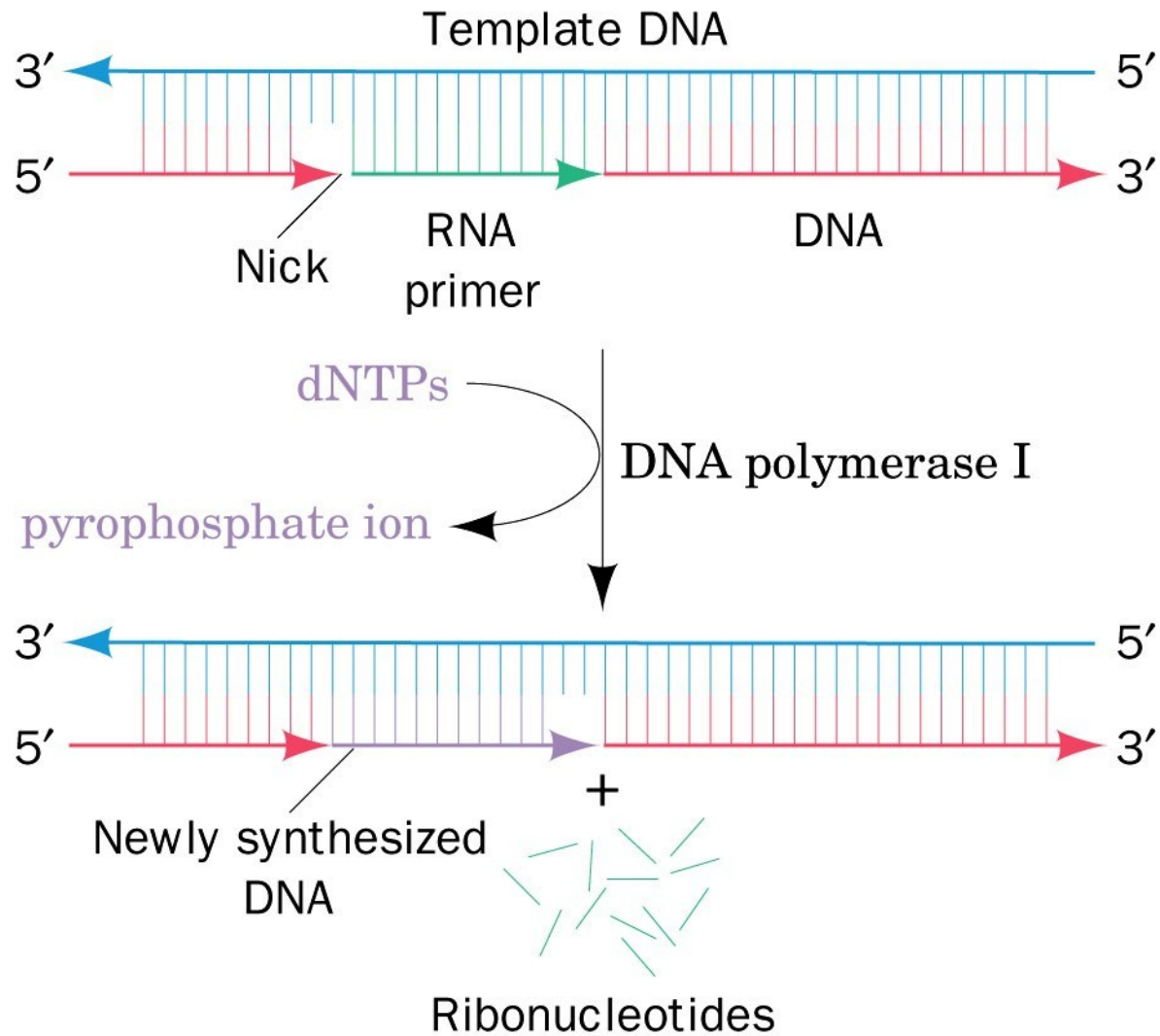
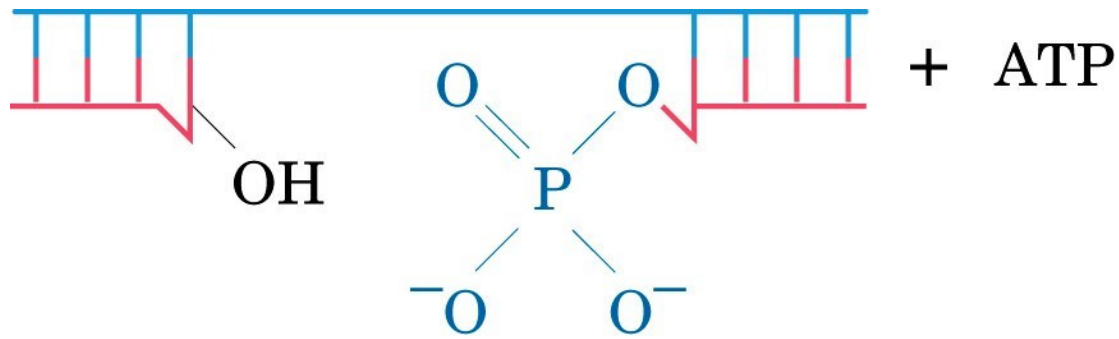
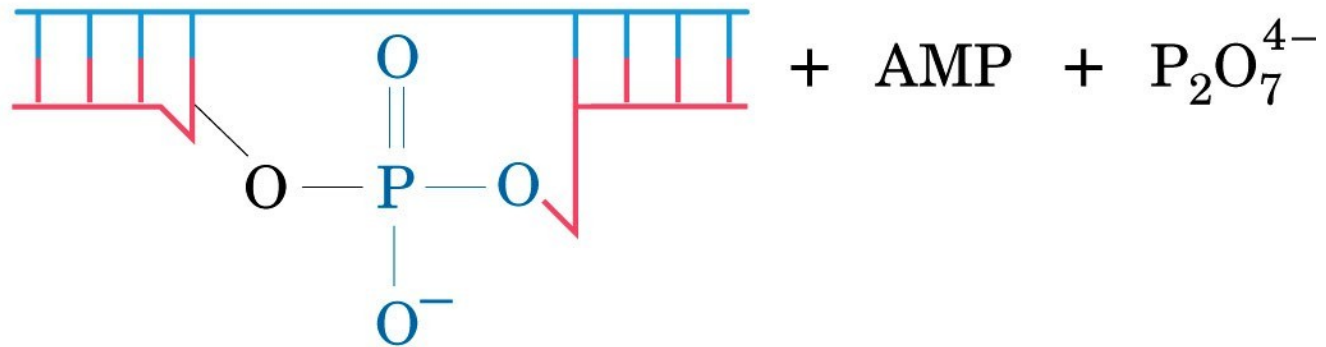


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DNA ligase



Maxam Gilbertova metoda

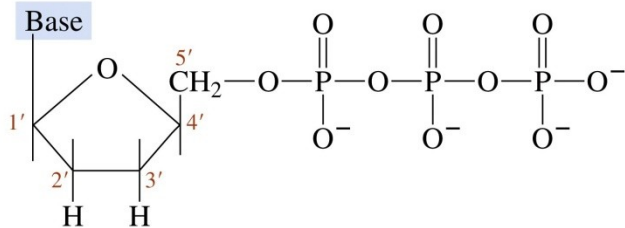


Figure 11-29 Concepts in Biochemistry, 3/e
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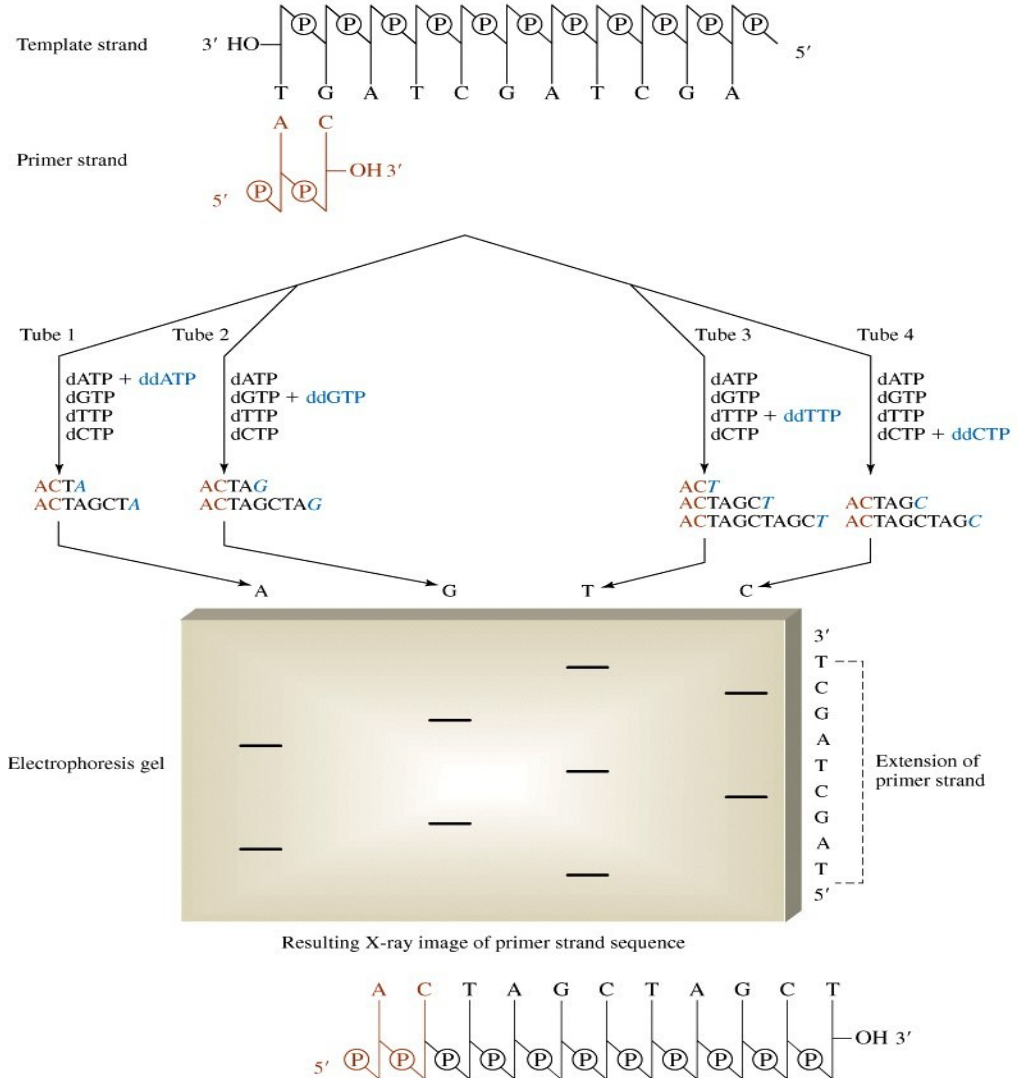
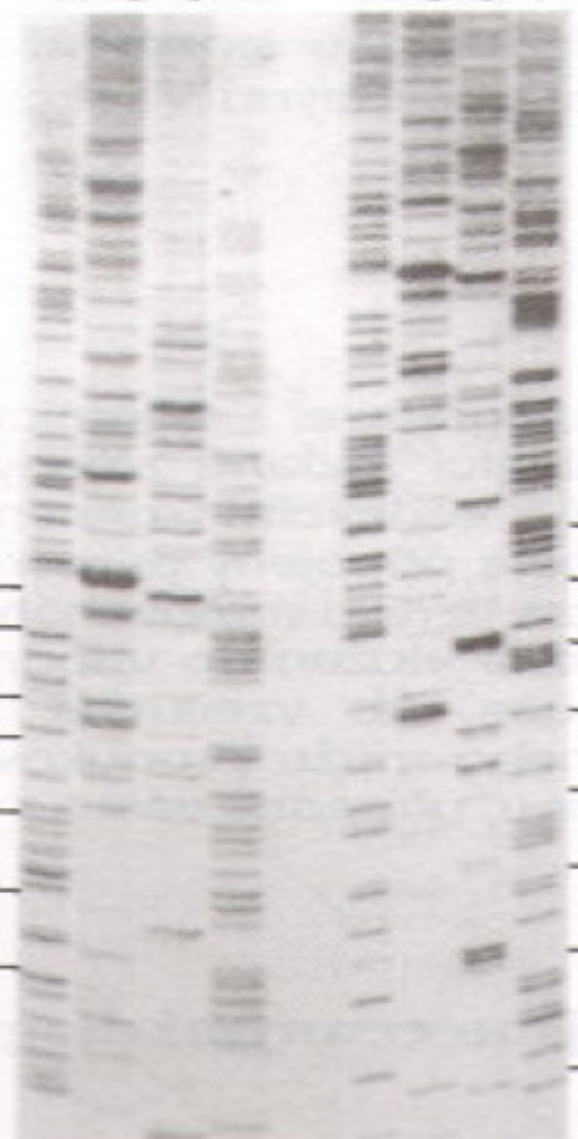


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A G C T

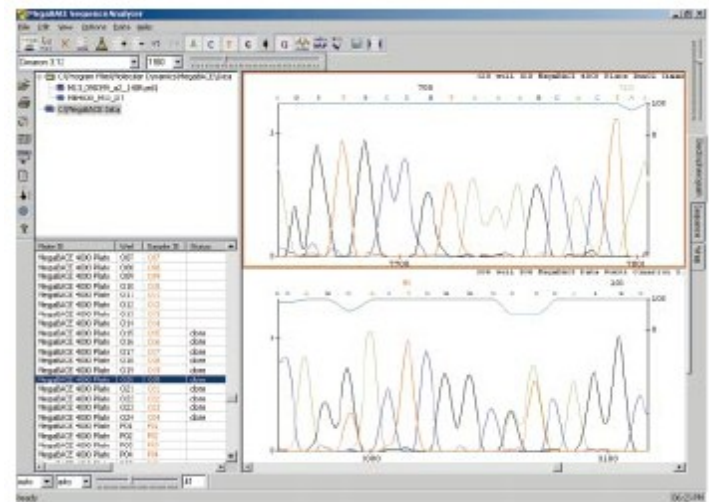
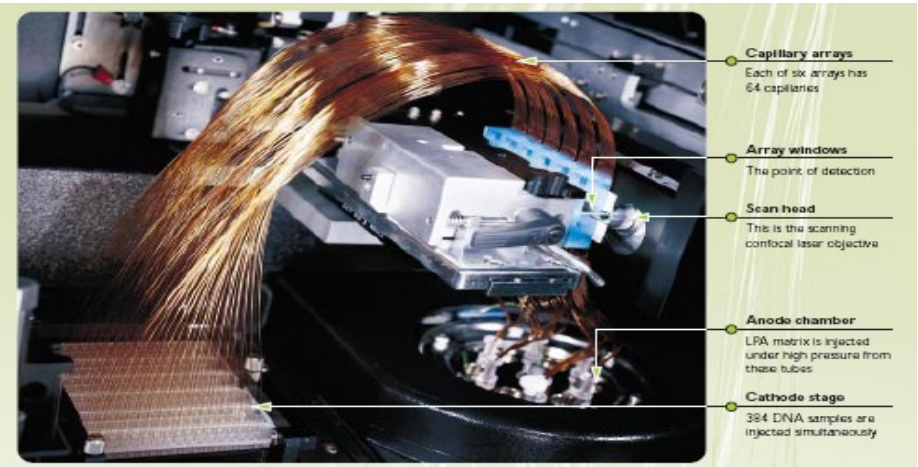
A G C T

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C T T T A T G T A T
G T A A C A G A A G
G C A T T T C G C G
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T A A T A A T A T G
T C C A A G T T T A



80
A T T T G A A C C T
C A G A T A G T A A
T T C C C T G A A T
C C T G G G A G T T
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T A C C C A T C A T
T C G T A C T T G T

2003 - Projekt lidského genomu



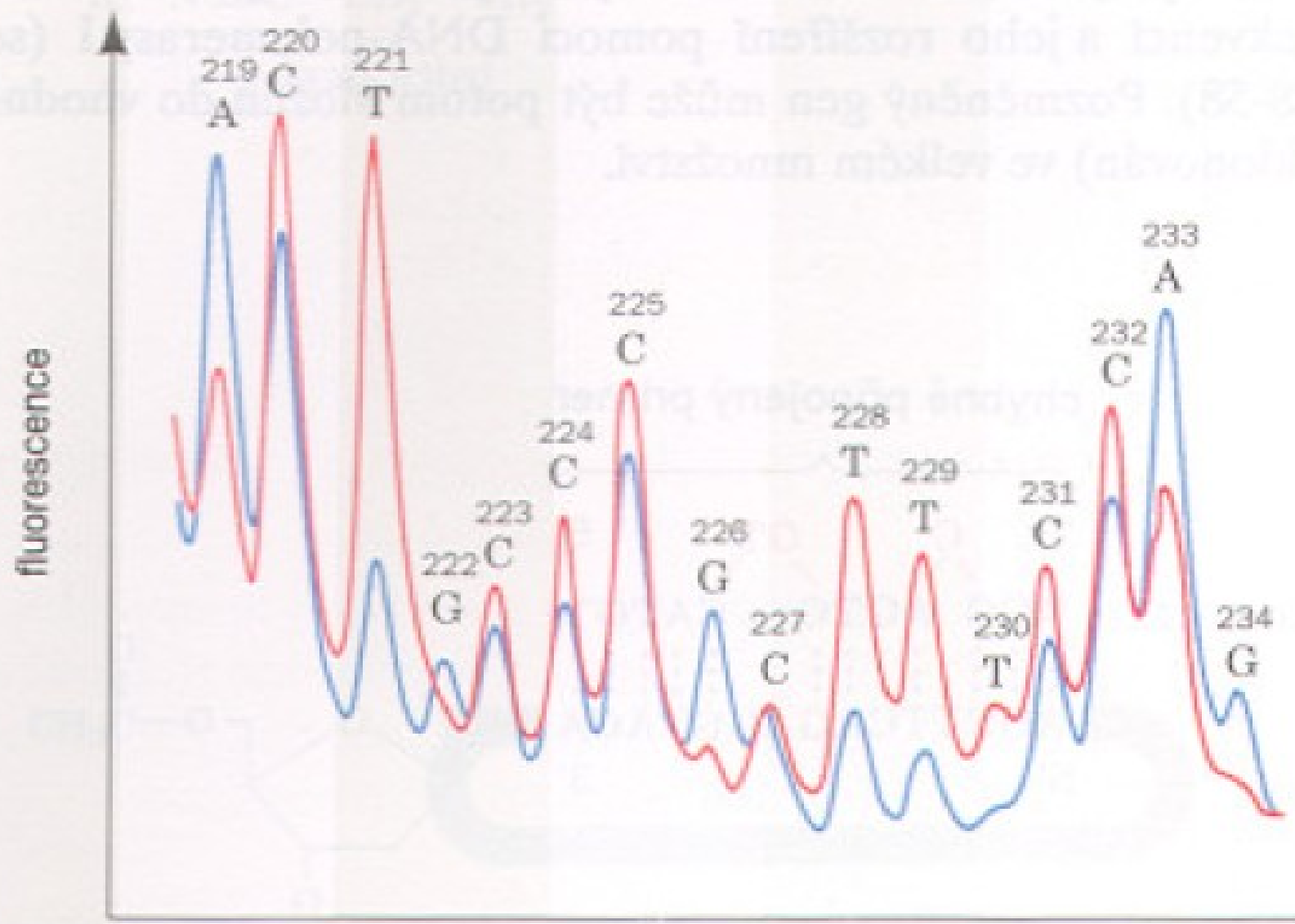
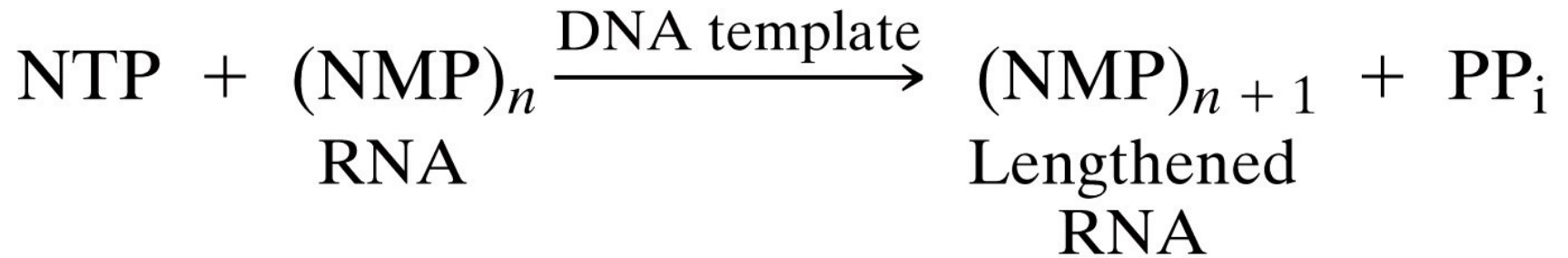


Table 13.3
Some Sequenced Genomes

Organism	Genome size (kb)	Number of Chromosomes
<i>Mycoplasma genitalium</i> (human parasite)	580	1
<i>Borrelia burgdorferi</i> (agent of Lyme disease)	1444	1
<i>Haemophilus influenzae</i> (human pathogenic bacterium)	1830	1
<i>Mycobacterium tuberculosis</i> (cause of tuberculosis)	4412	1
<i>Escherichia coli</i> (bacterium)	4639	1
<i>Saccharomyces cerevisiae</i> (yeast)	11,700	16
<i>Drosophila melanogaster</i> (fruit fly)	137,000	4
<i>Oryza sativa</i> (rice)	430,000	12
<i>Homo sapiens</i> (human)	3,200,000	23



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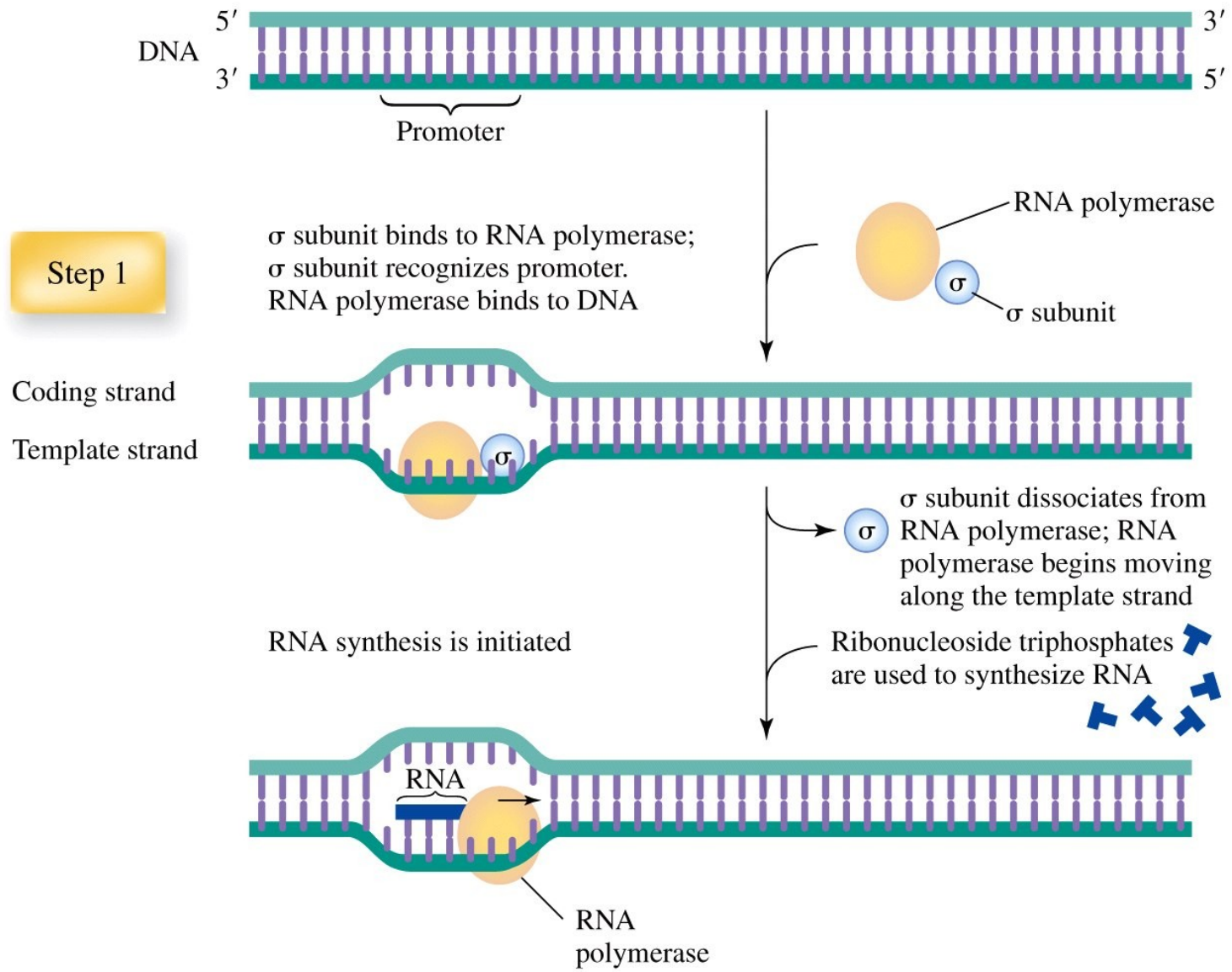


Figure 11-22 part 1 Concepts in Biochemistry, 3/e
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Step 2

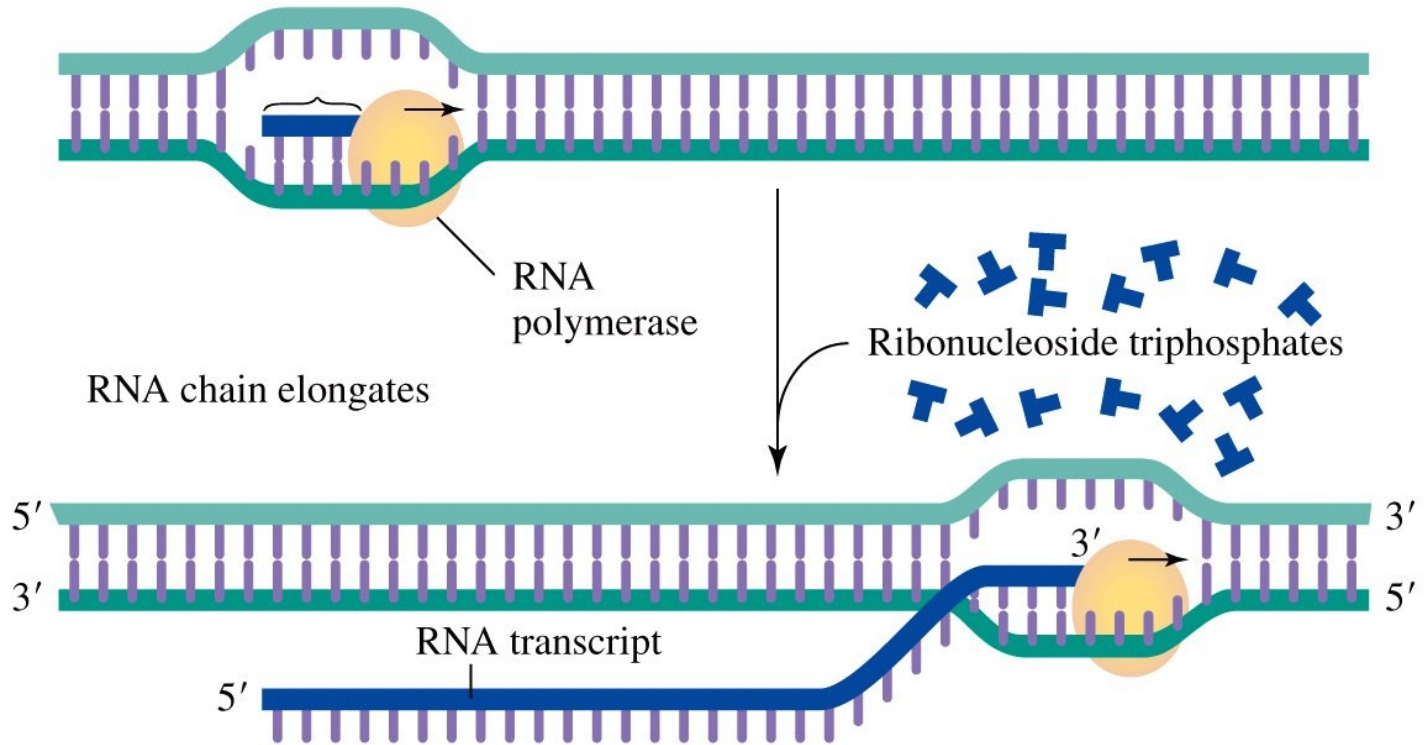


Figure 11-22 part 2 Concepts in Biochemistry, 3/e
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Step 3

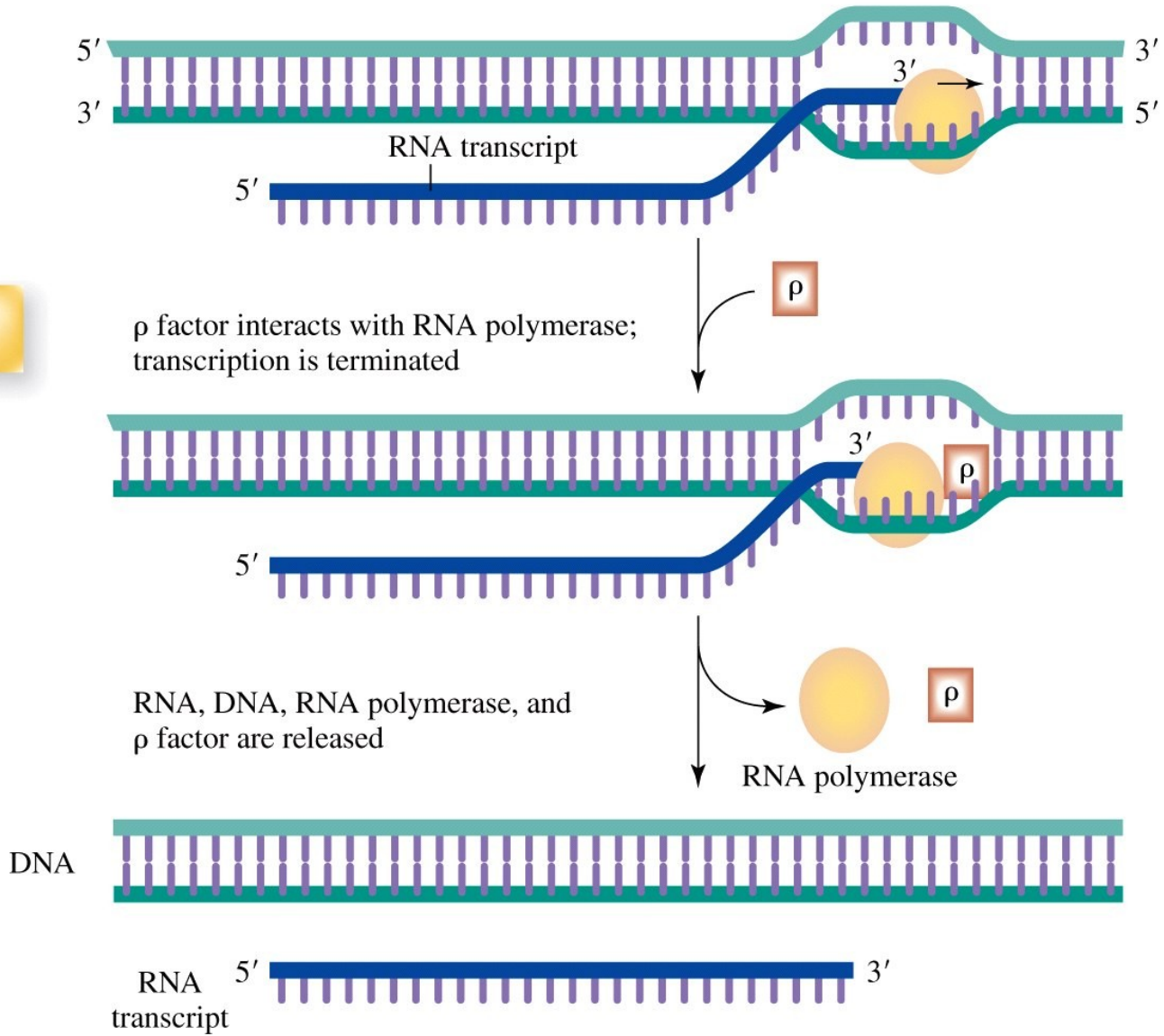
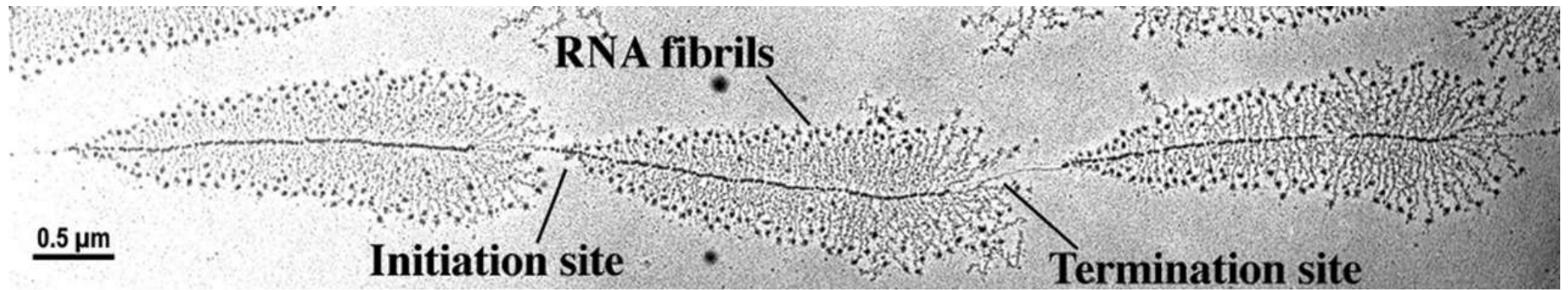
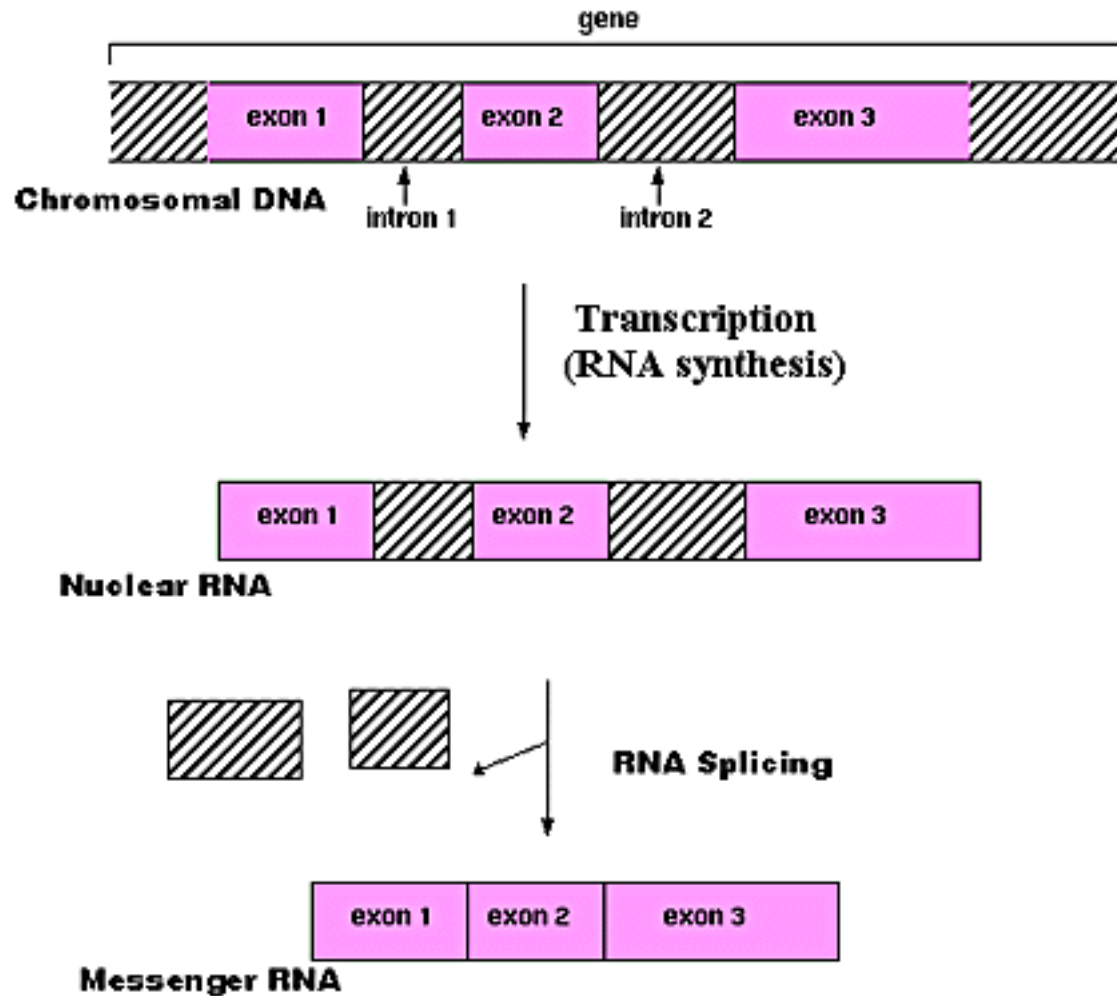


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Courtesy of Ulrich Scheer, University of Würzburg, Germany

Syntéza RNA



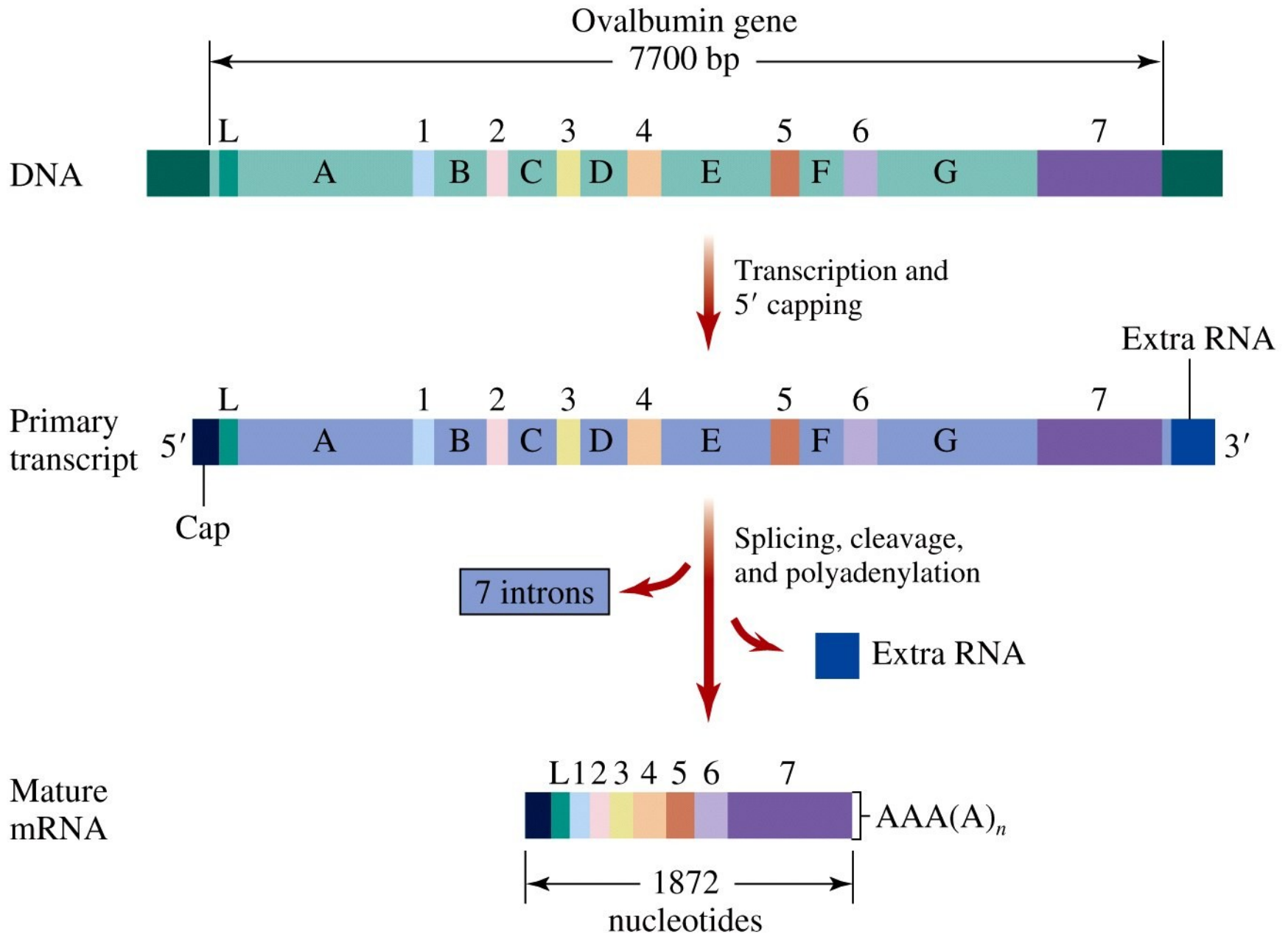
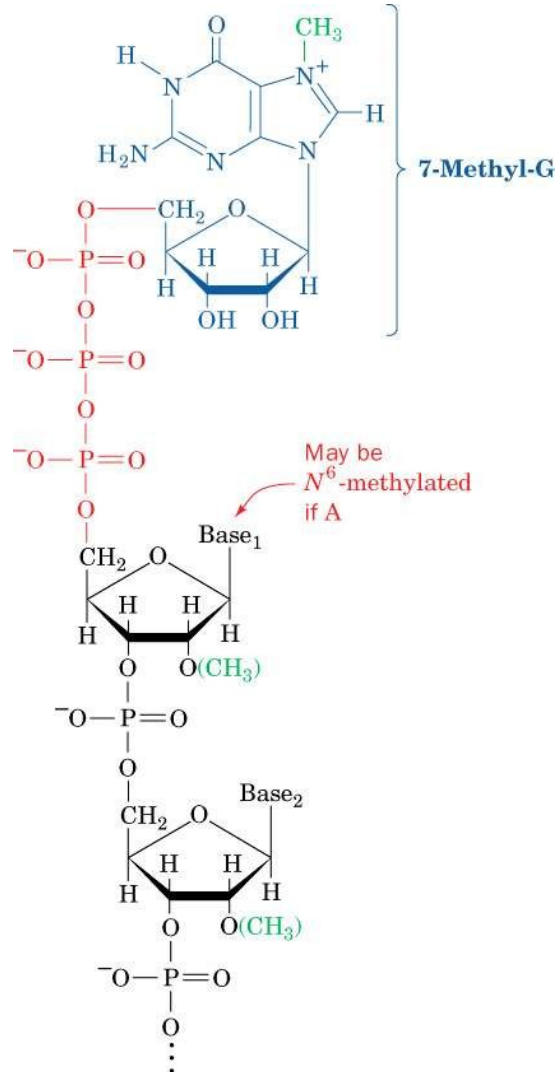


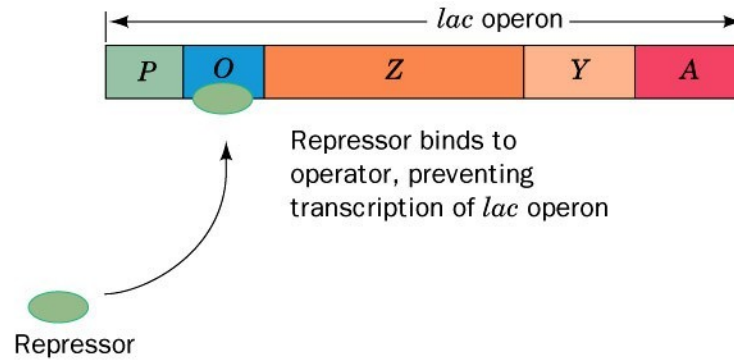
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Cap

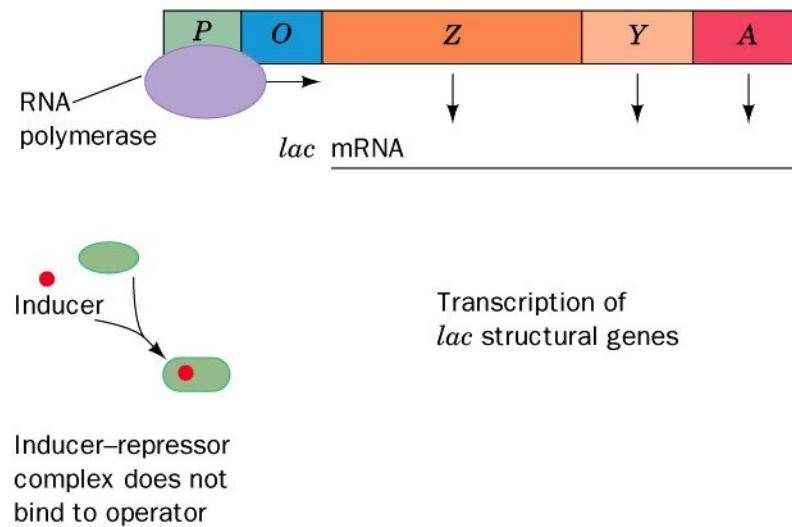


Regulate transcribe

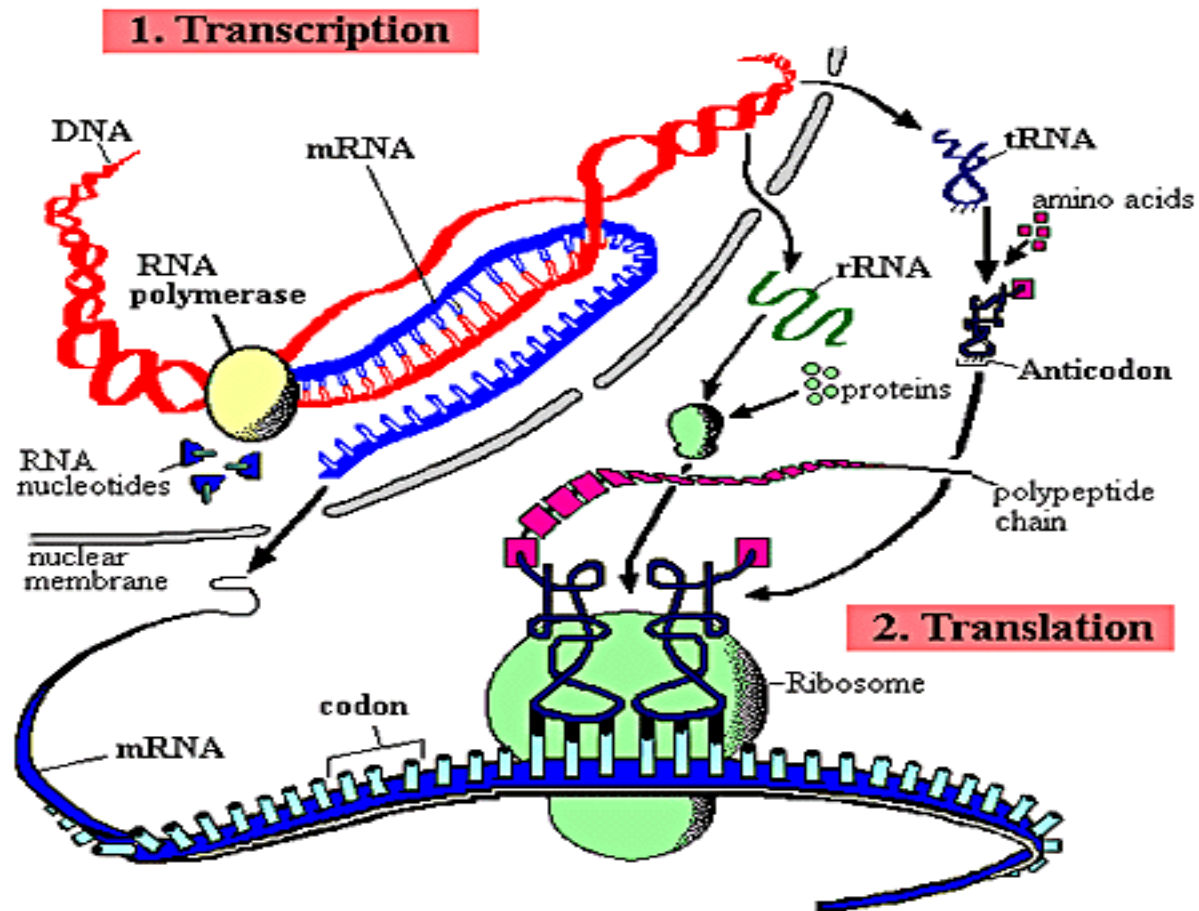
(a) Absence of inducer

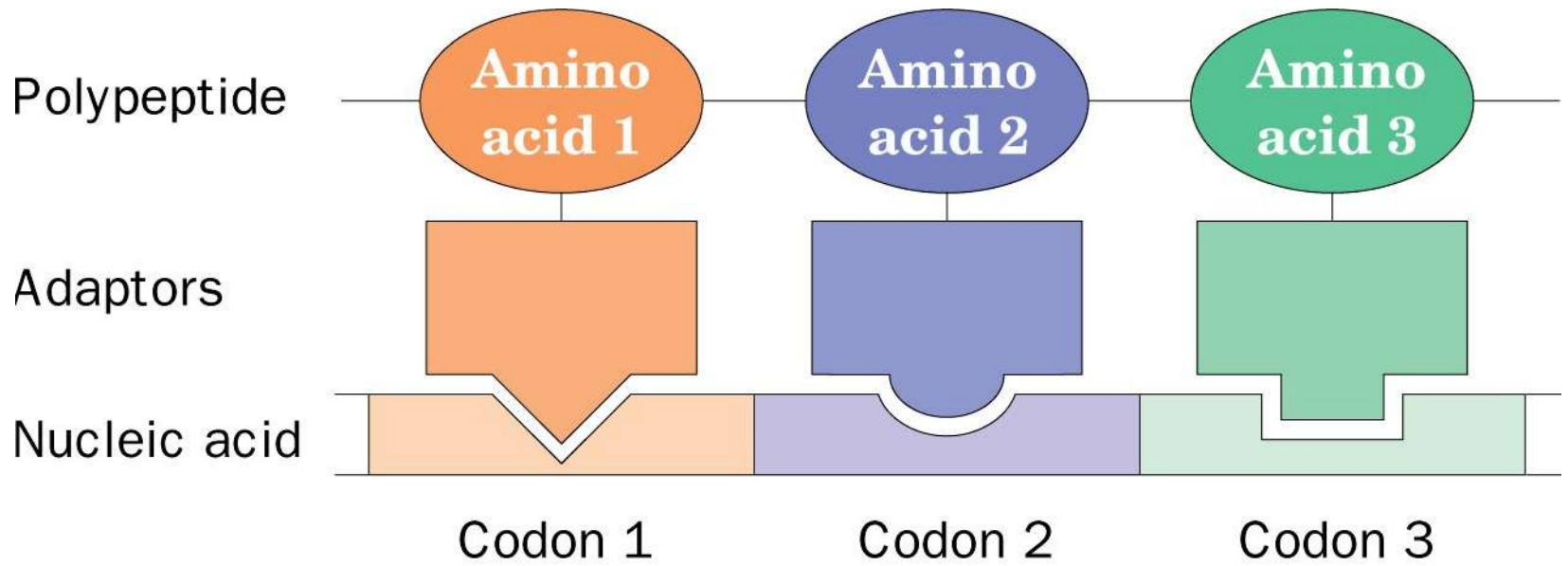


(b) Presence of inducer



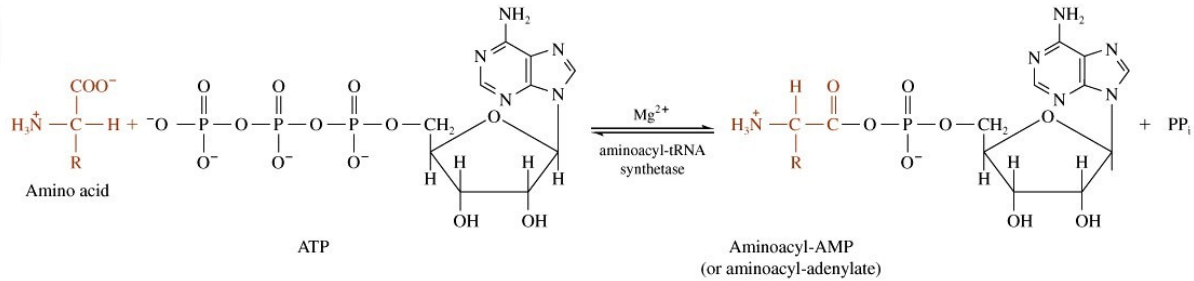
Syntéza bílkovin



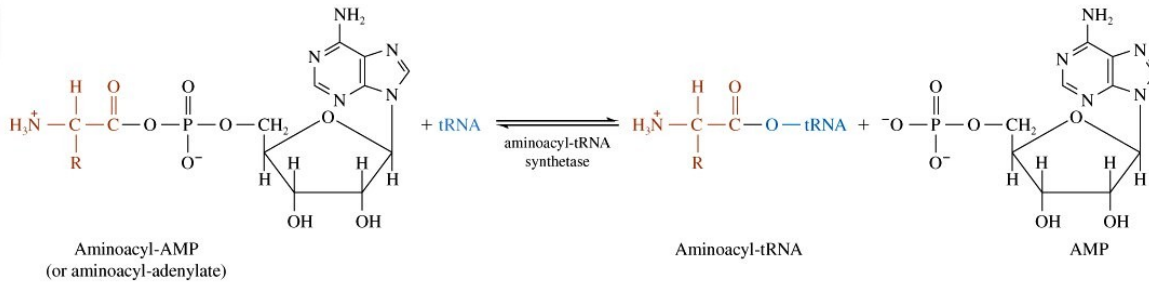


Aktivace AMK

Step 1



Step 2



Overall

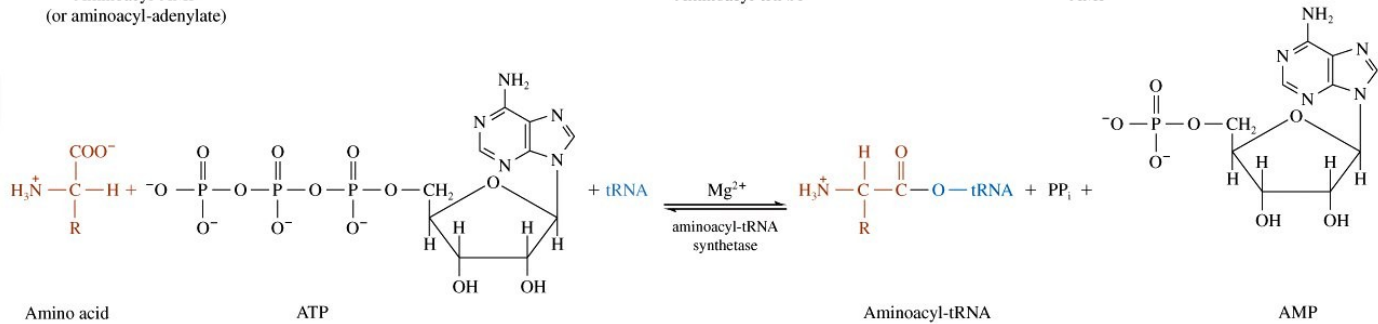


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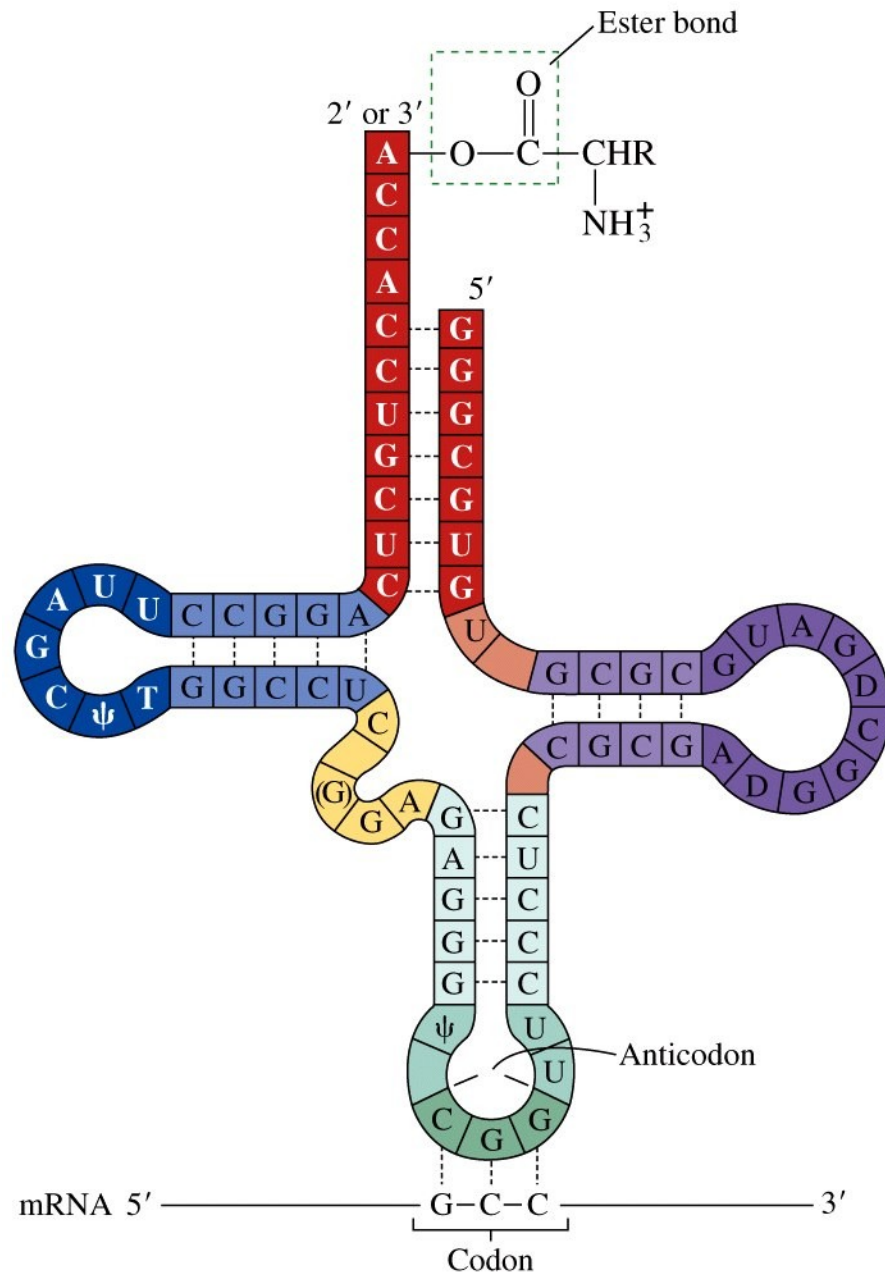
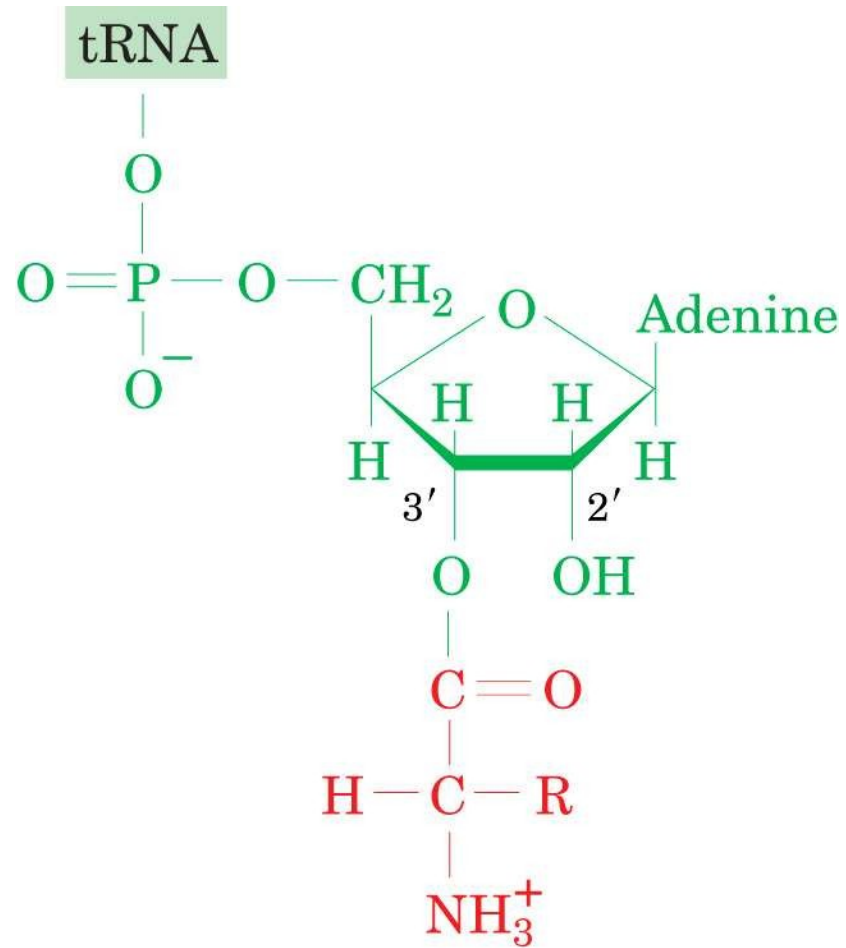
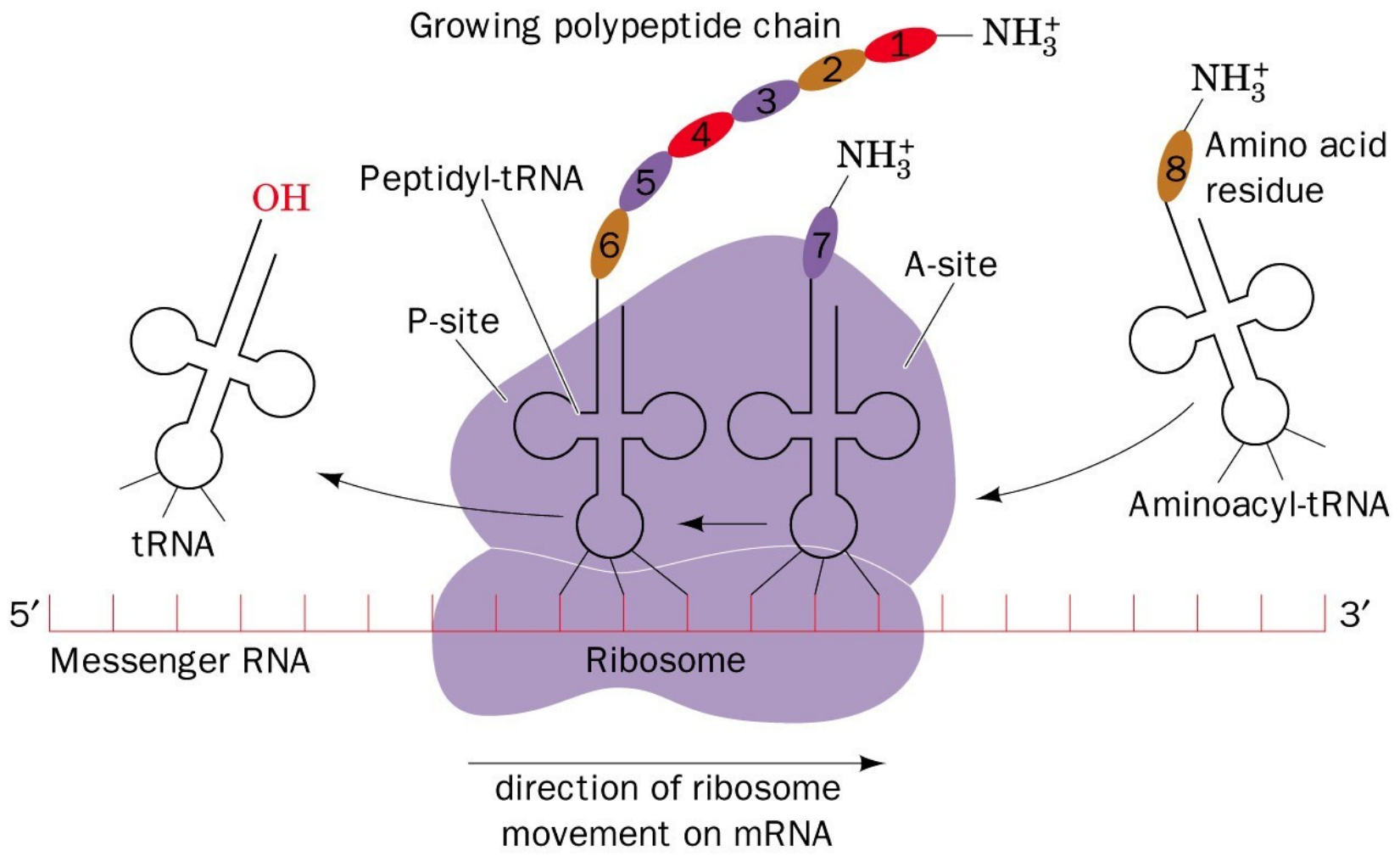


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Aminoacyl-tRNA



Ribozomy

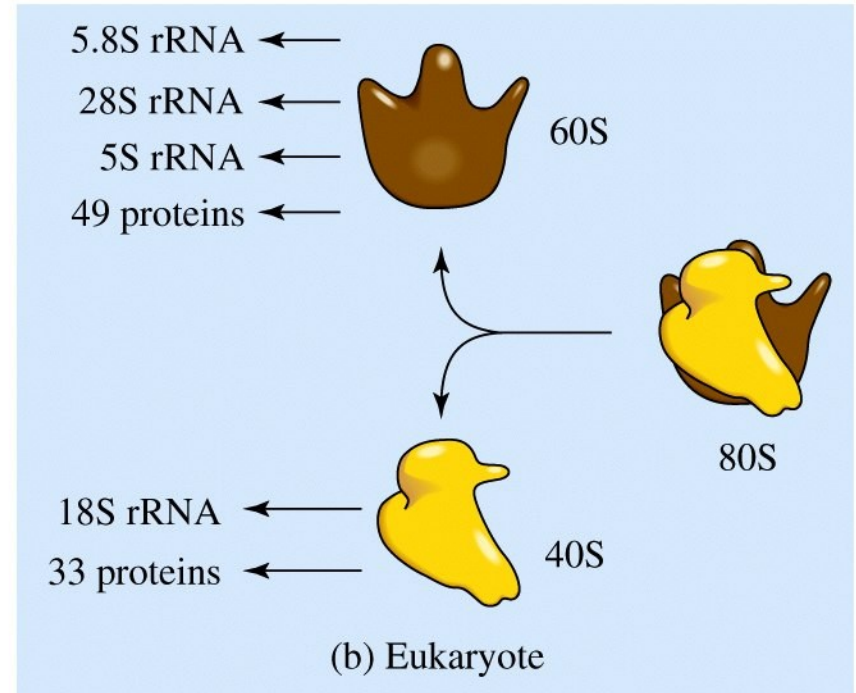
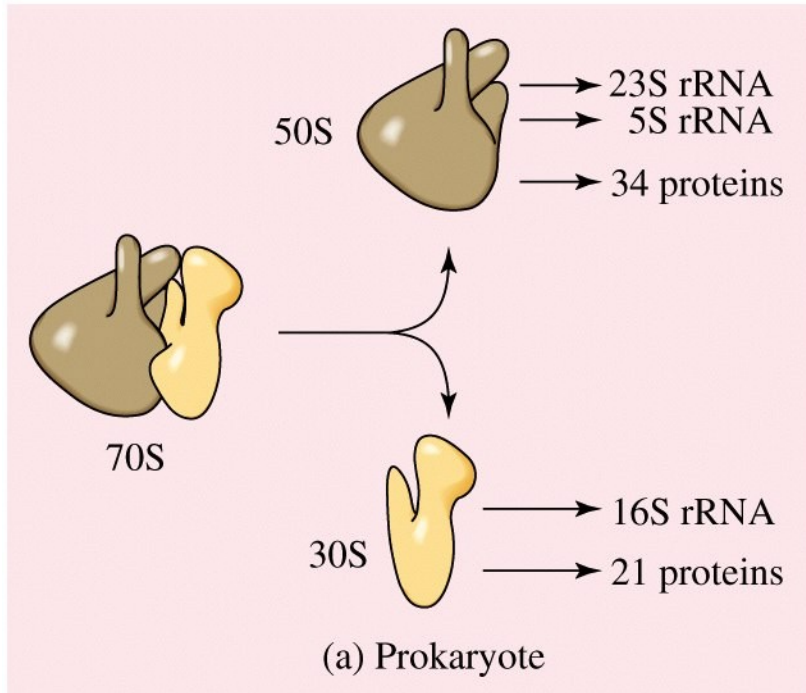
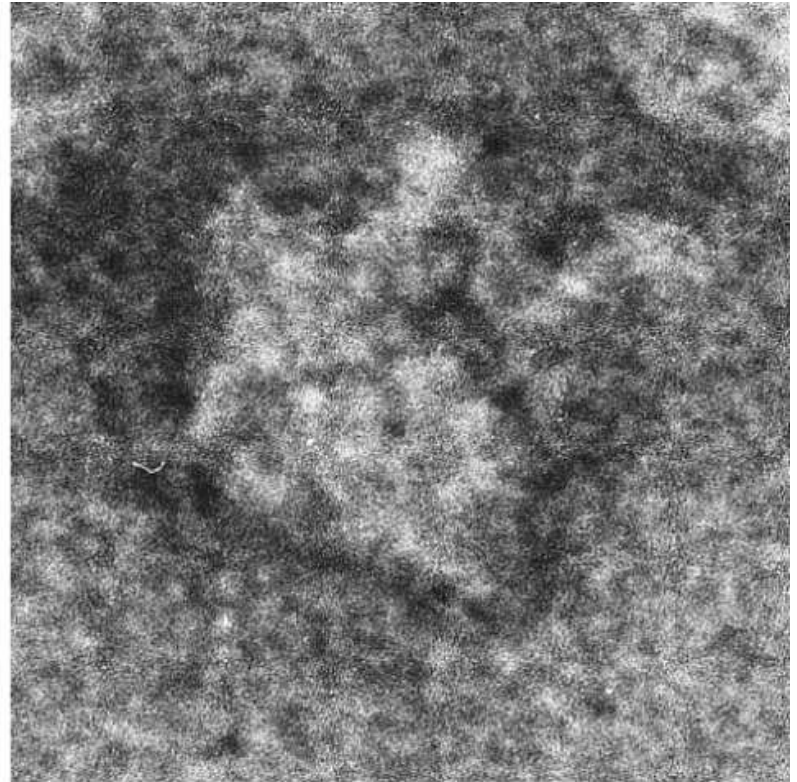
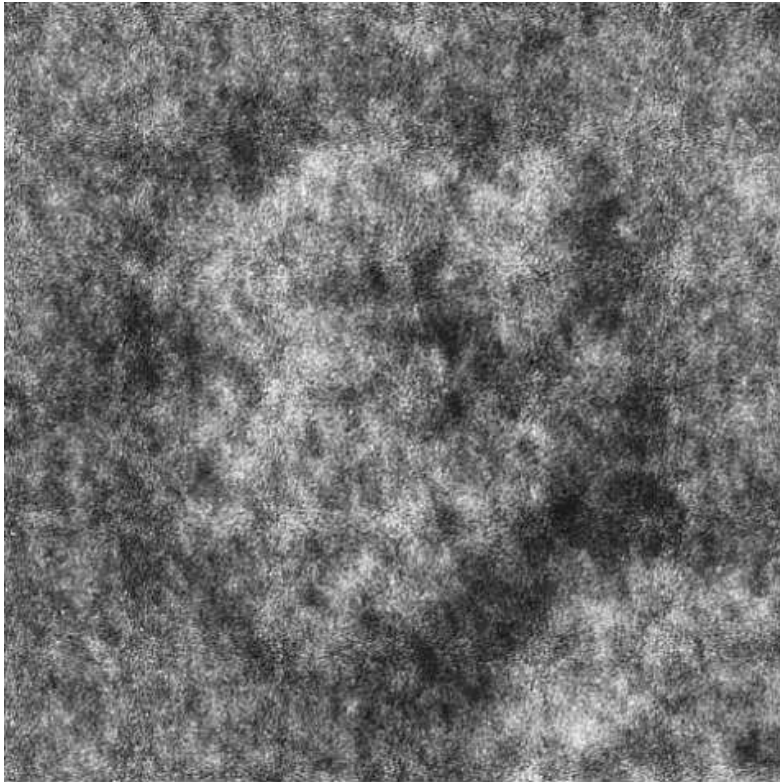


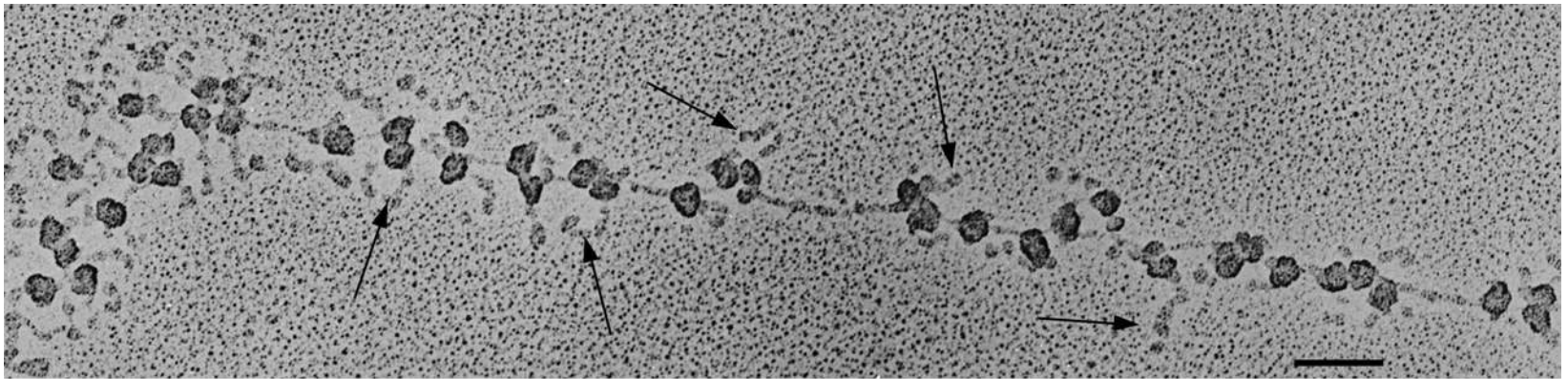
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Ribozomy E.coli



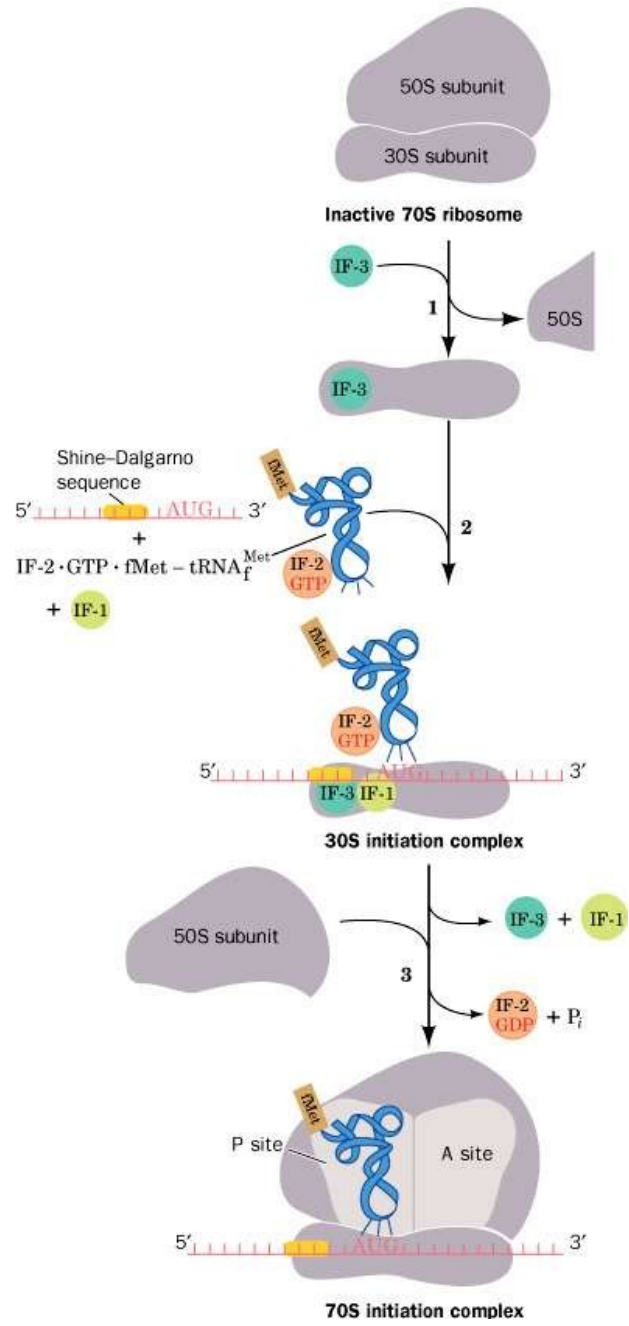
Courtesy of James Lake, UCLA

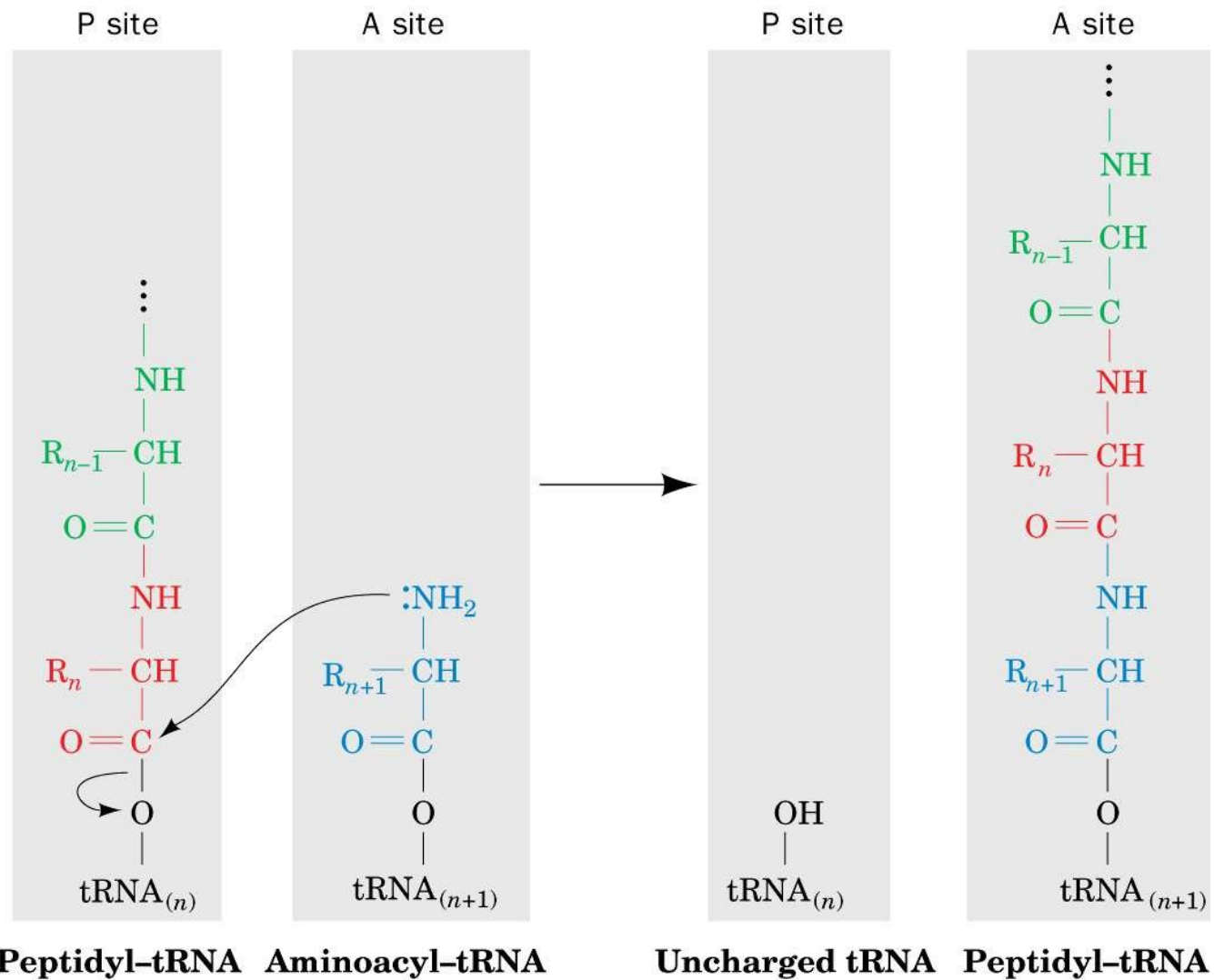
Polyribozomy *Bombyx mori*



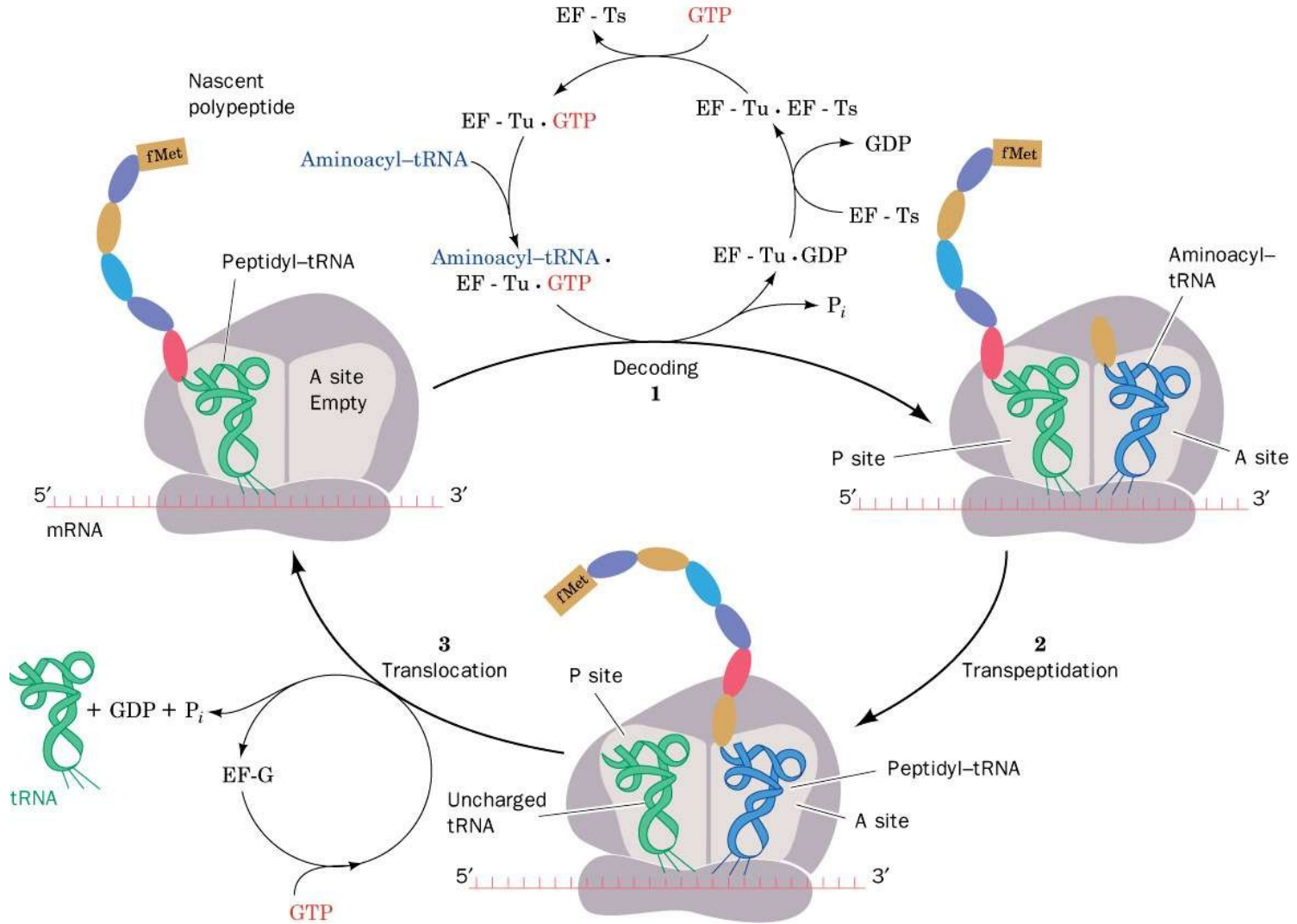
Courtesy of Oscar L. Miller, Jr. and Steven L. McKnight, University of Virginia

Inicializace

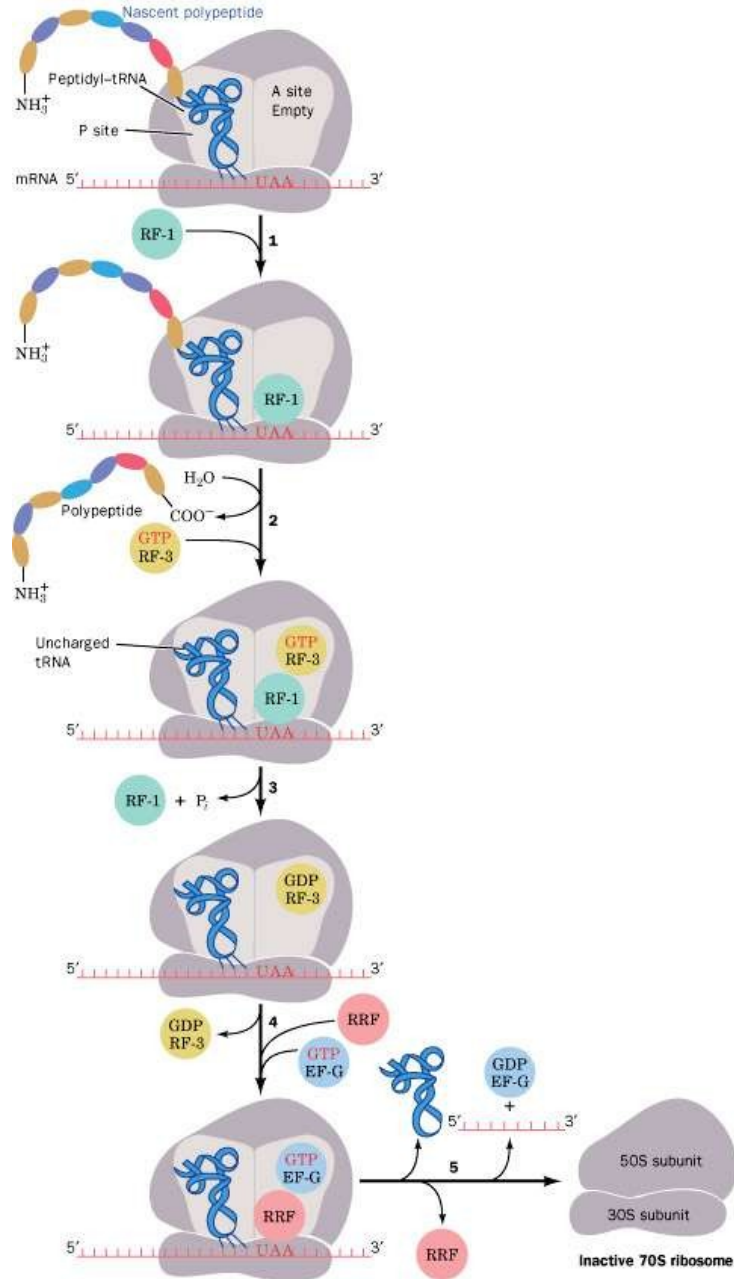




Elongate



Termination



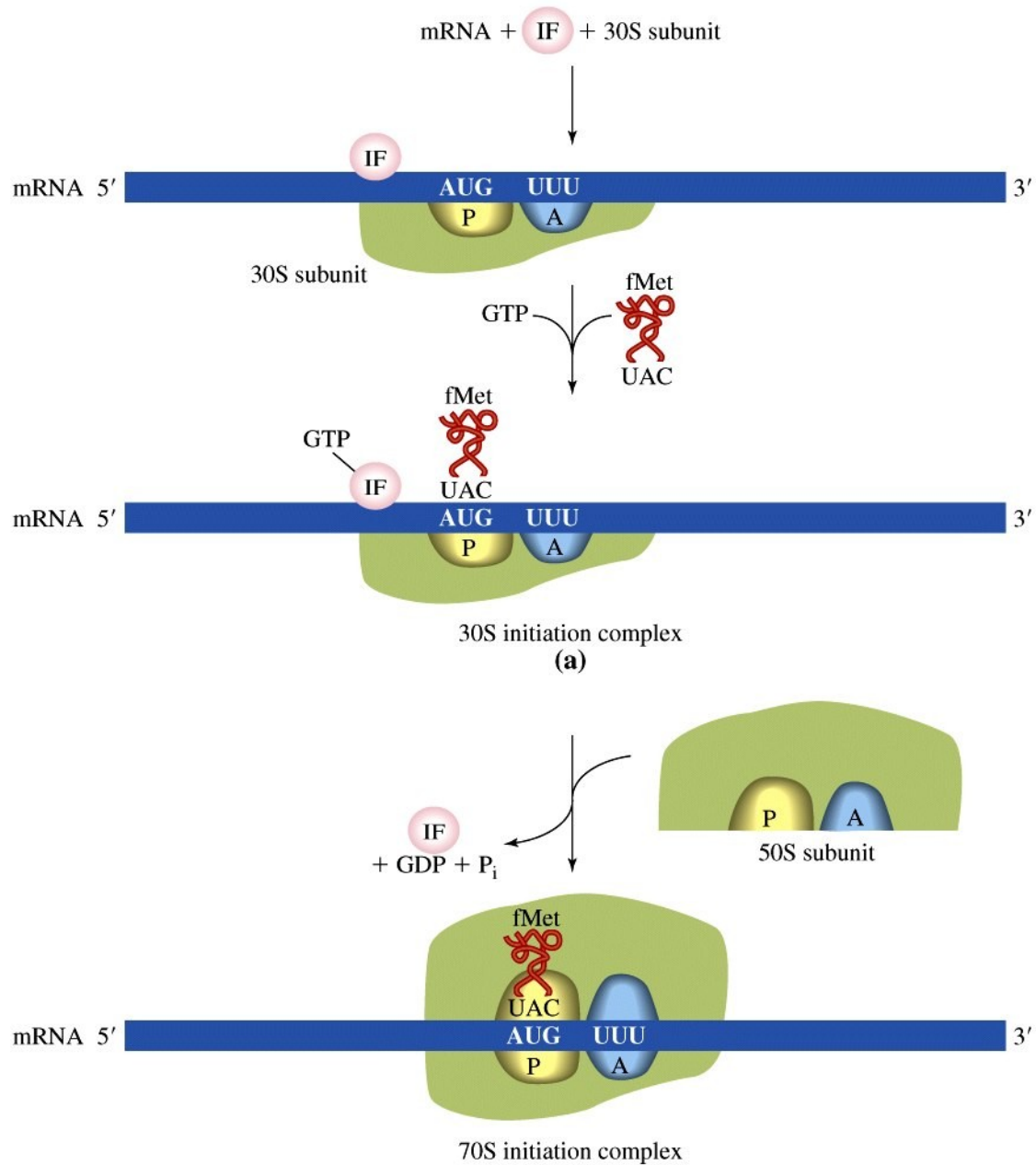


Figure 12-5a Concepts in Biochemistry, 3/e
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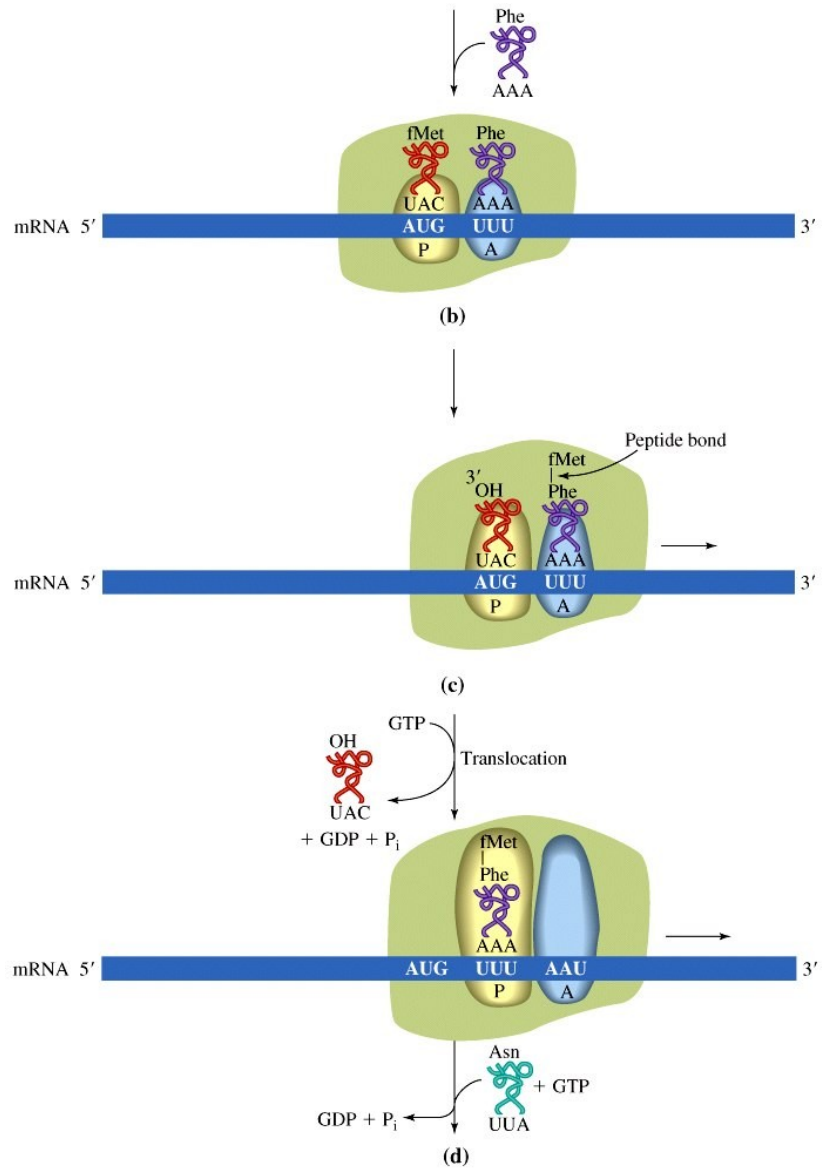


Figure 12-5b Concepts in Biochemistry, 3/e
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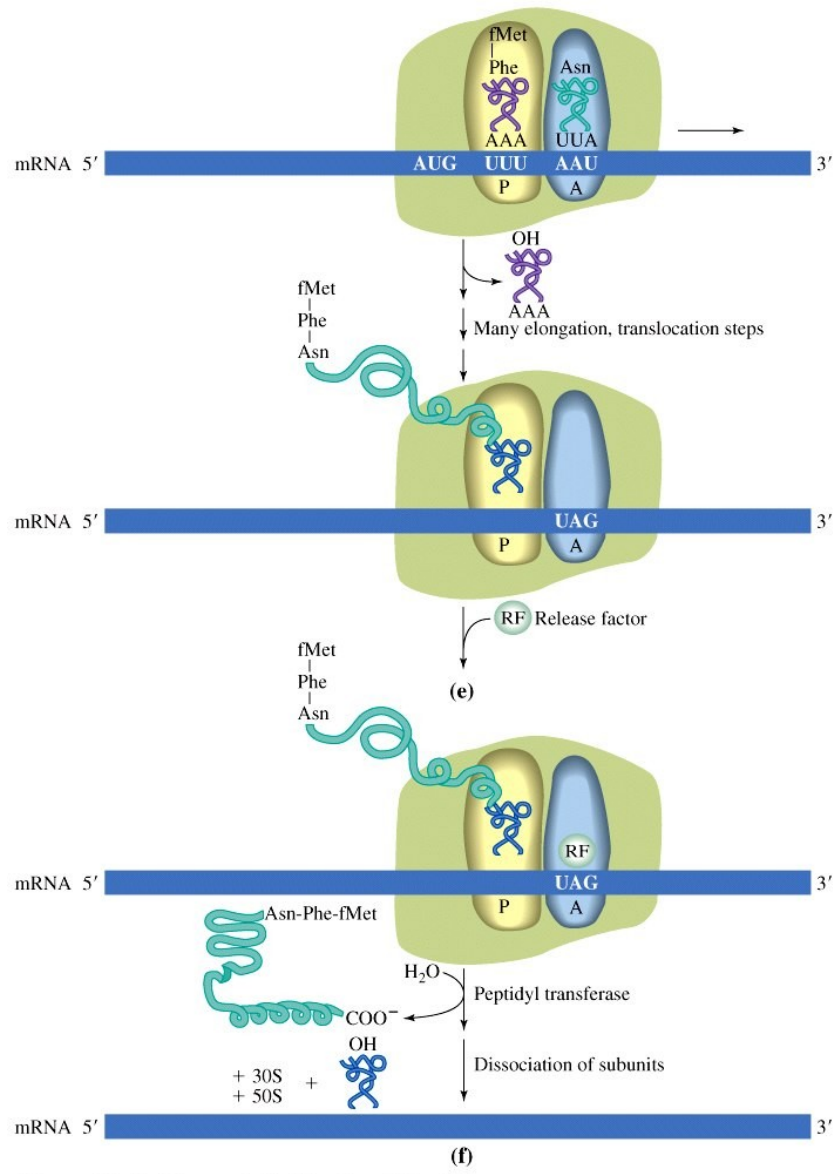


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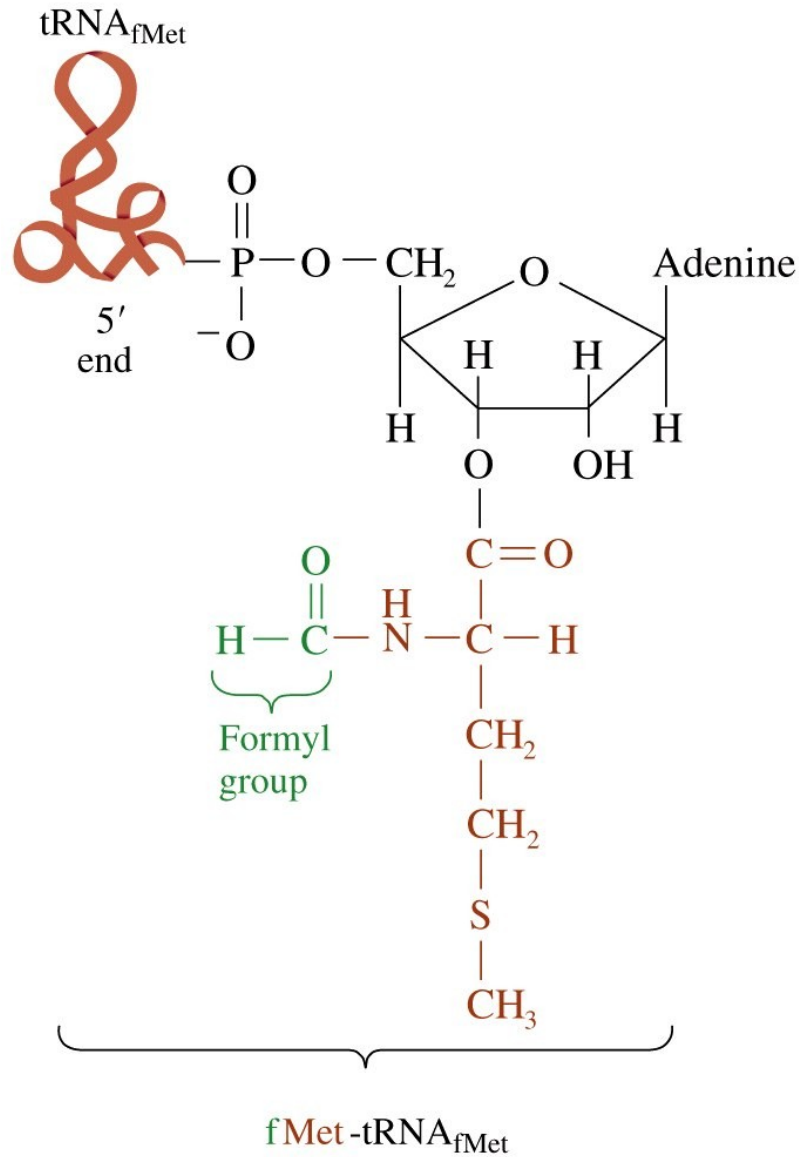


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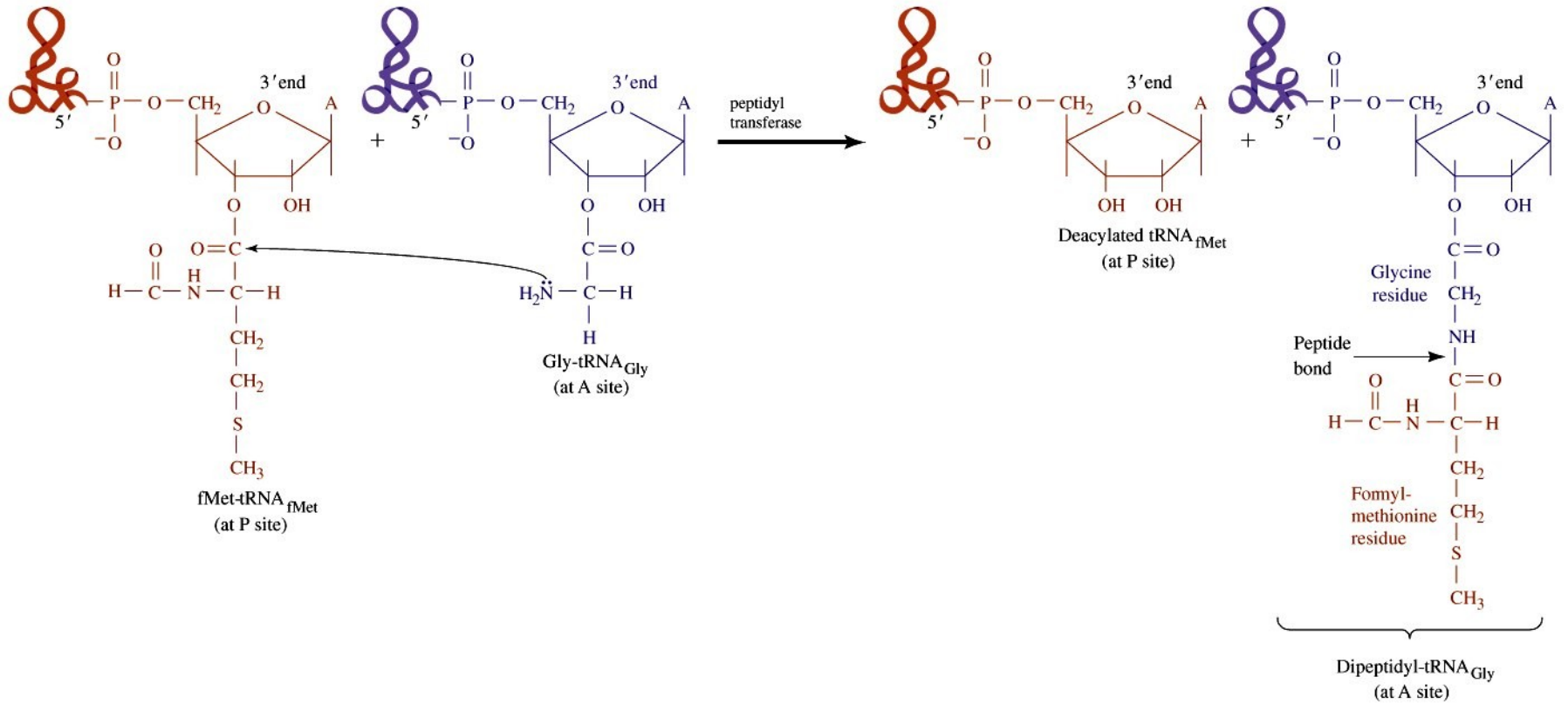
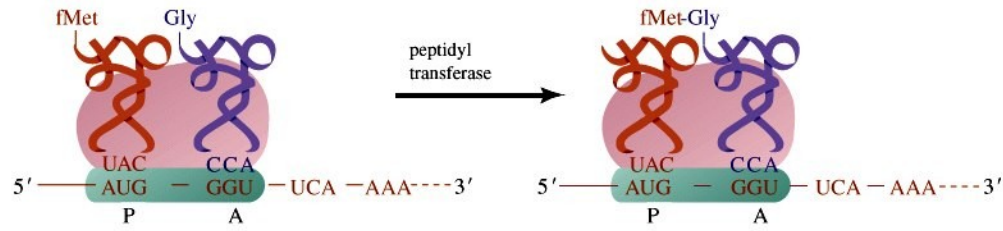


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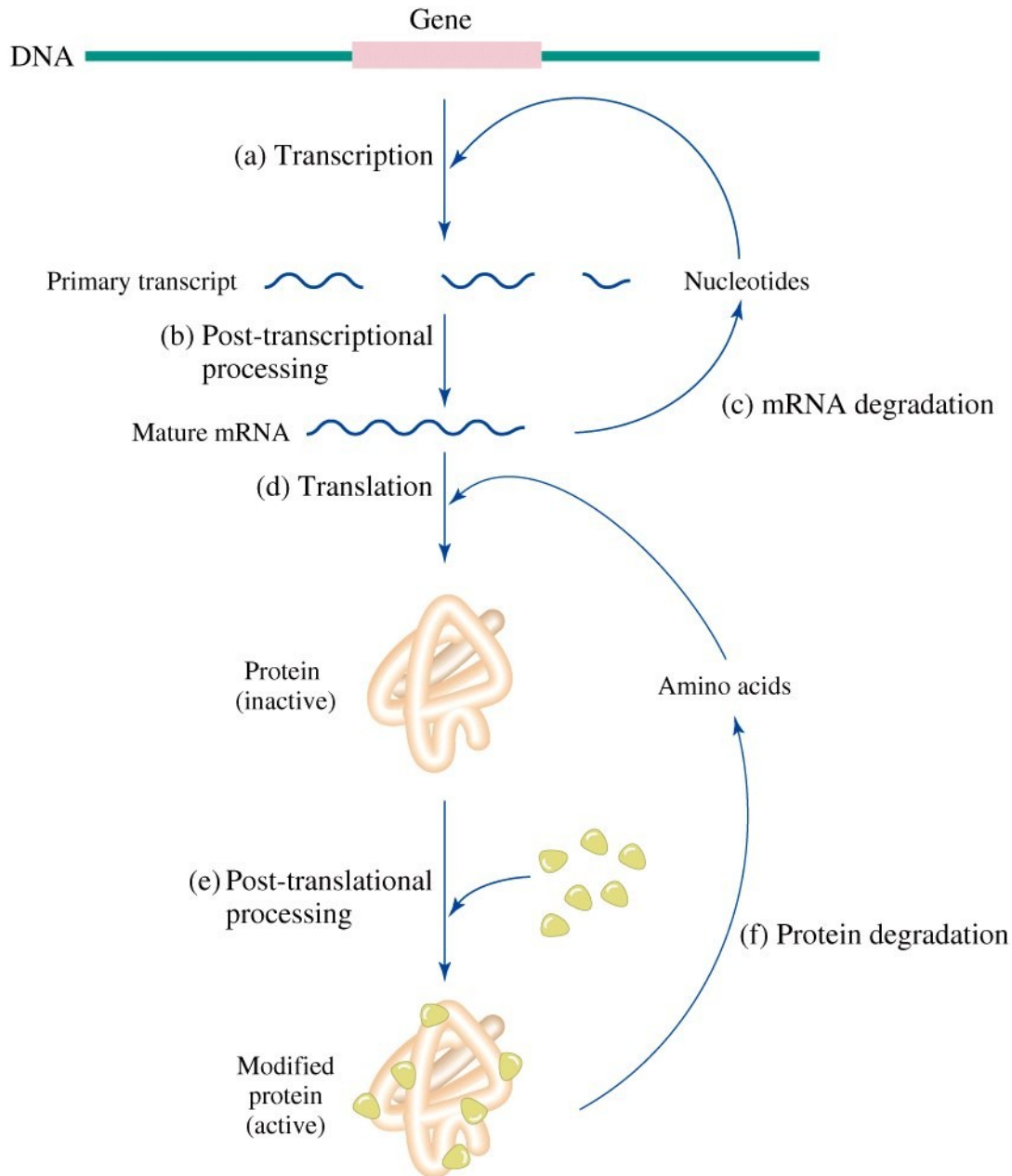


Figure 12-16 Concepts in Biochemistry, 3/e
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Kontrola exprese genu

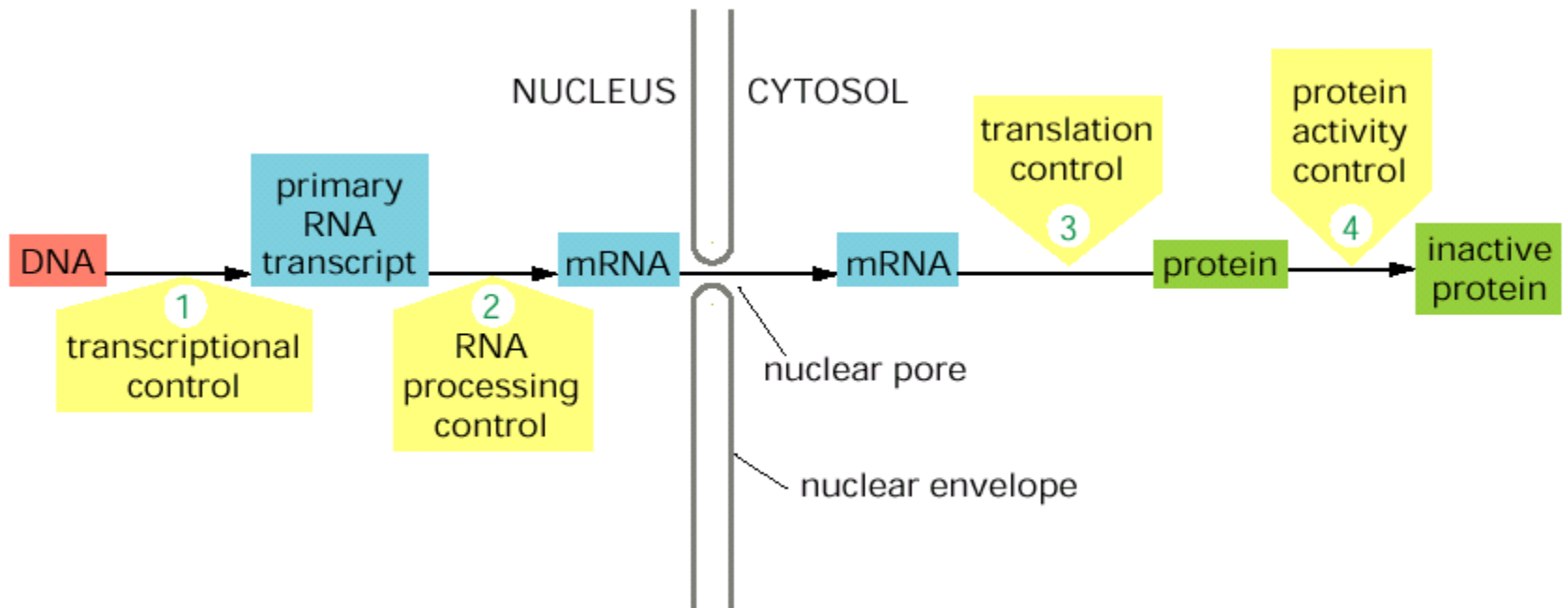
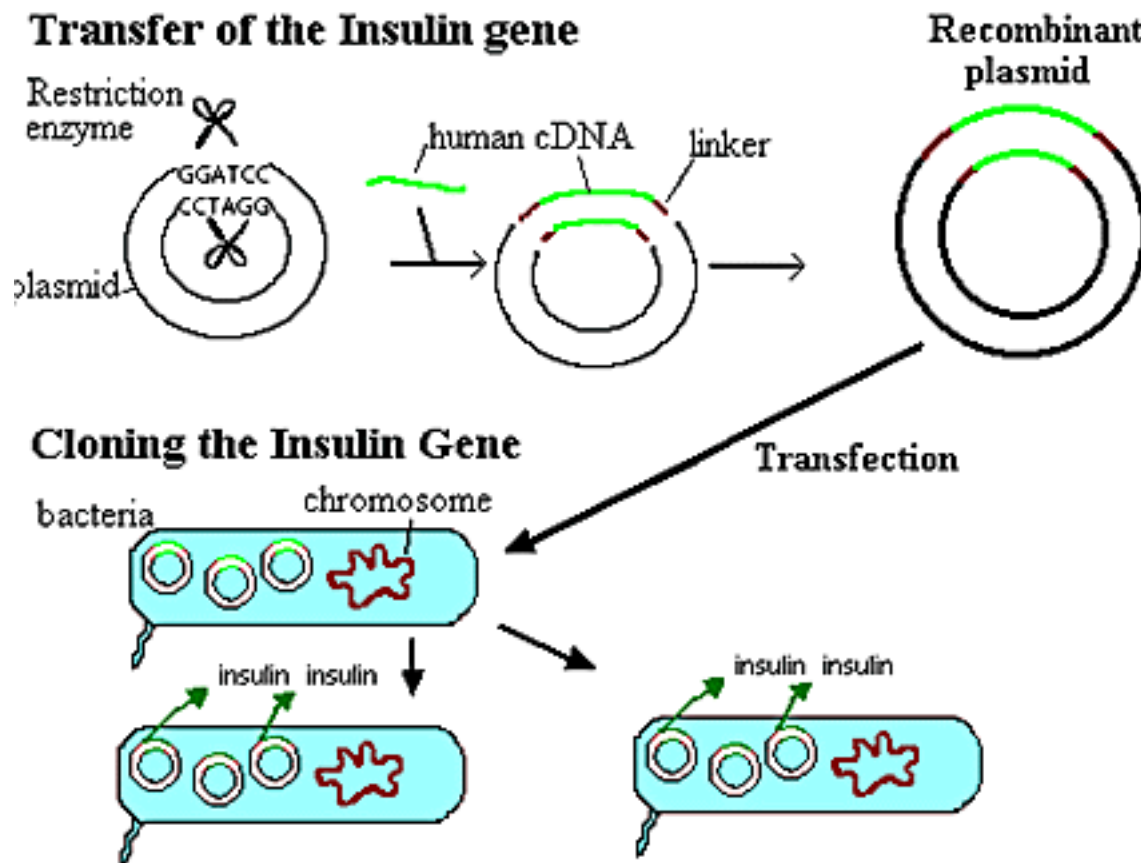


Table 12.4**Antibiotic inhibitors of protein synthesis**

Antibiotic	Mode of Action
Puromycin	Causes early termination by mimicking the action of an aminoacyl-tRNA; acts on prokaryotes and eukaryotes
Streptomycin	Causes misreading of mRNA and inhibits initiation; acts on prokaryotes
Tetracycline	Binds to the A site of ribosomes and blocks entry of aminoacyl-tRNAs; acts on prokaryotes
Erythromycin	Binds to ribosome and inhibits translocation; acts on prokaryotes
Chloramphenicol	Binds to 50S subunit and inhibits peptidyl transferase; acts on prokaryotes
Cycloheximide	Inhibits translocation of eukaryotic peptidyl-tRNA
Linezolid	Blocks formation of 70S initiation complex in prokaryotes

Table 12-4 Concepts in Biochemistry, 3/e**© 2006 John Wiley & Sons**

Genetické inženýrství



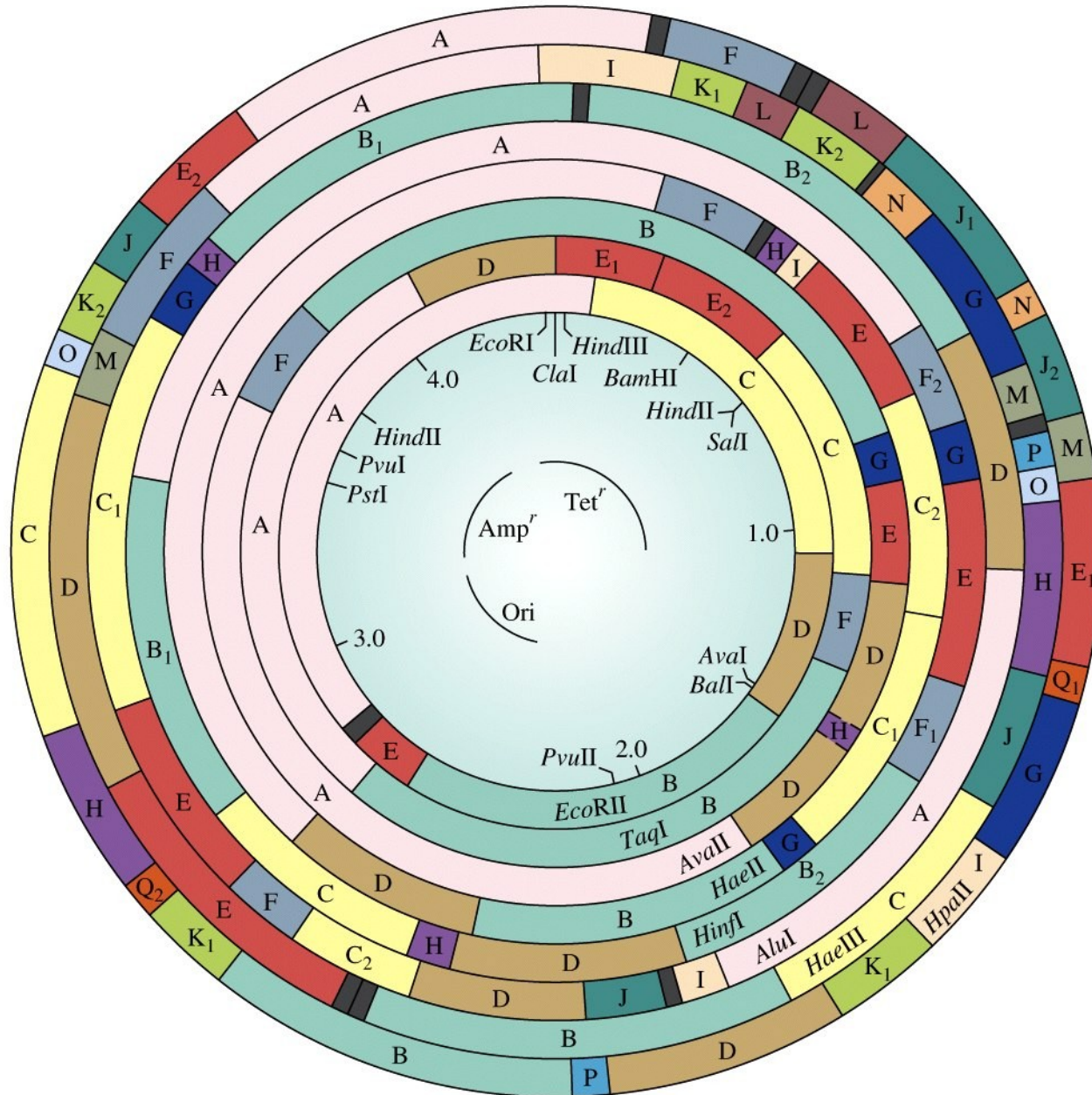


Figure 13-8 Concepts in Biochemistry, 3/e
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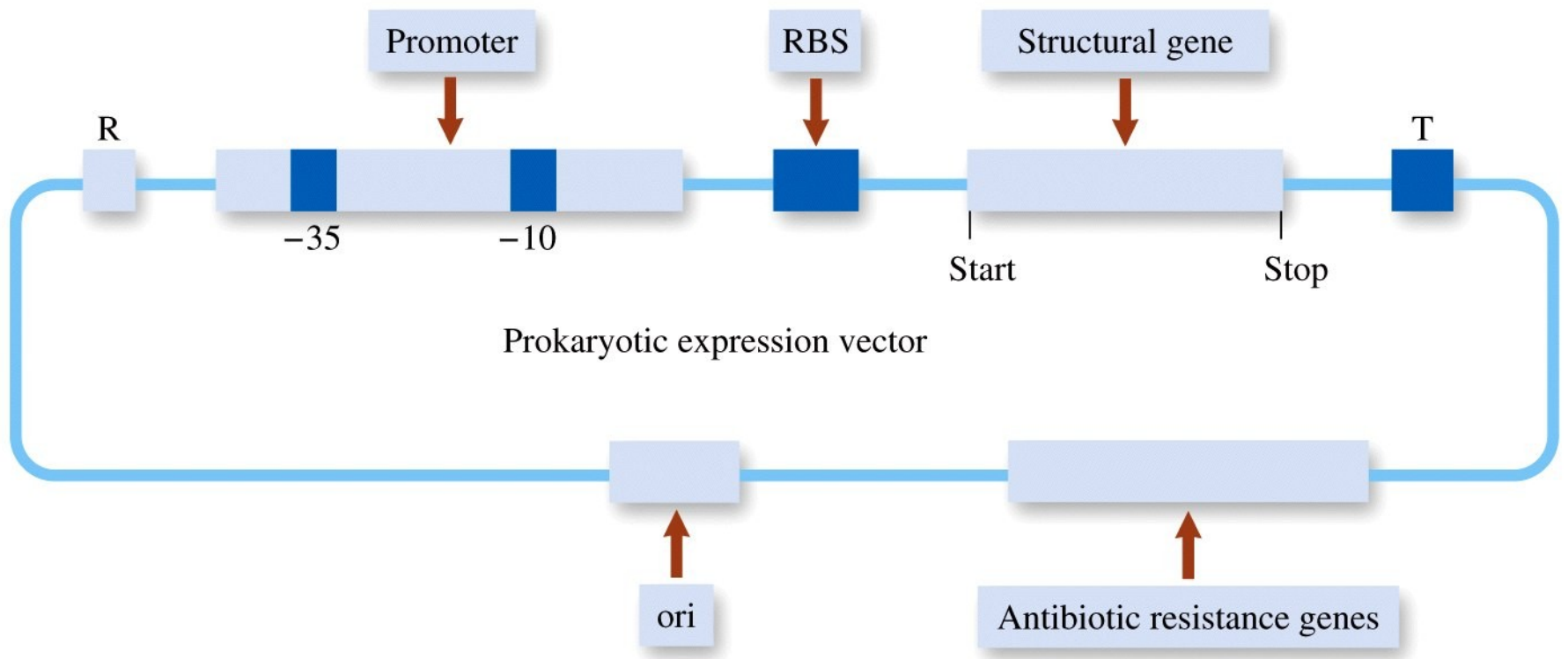


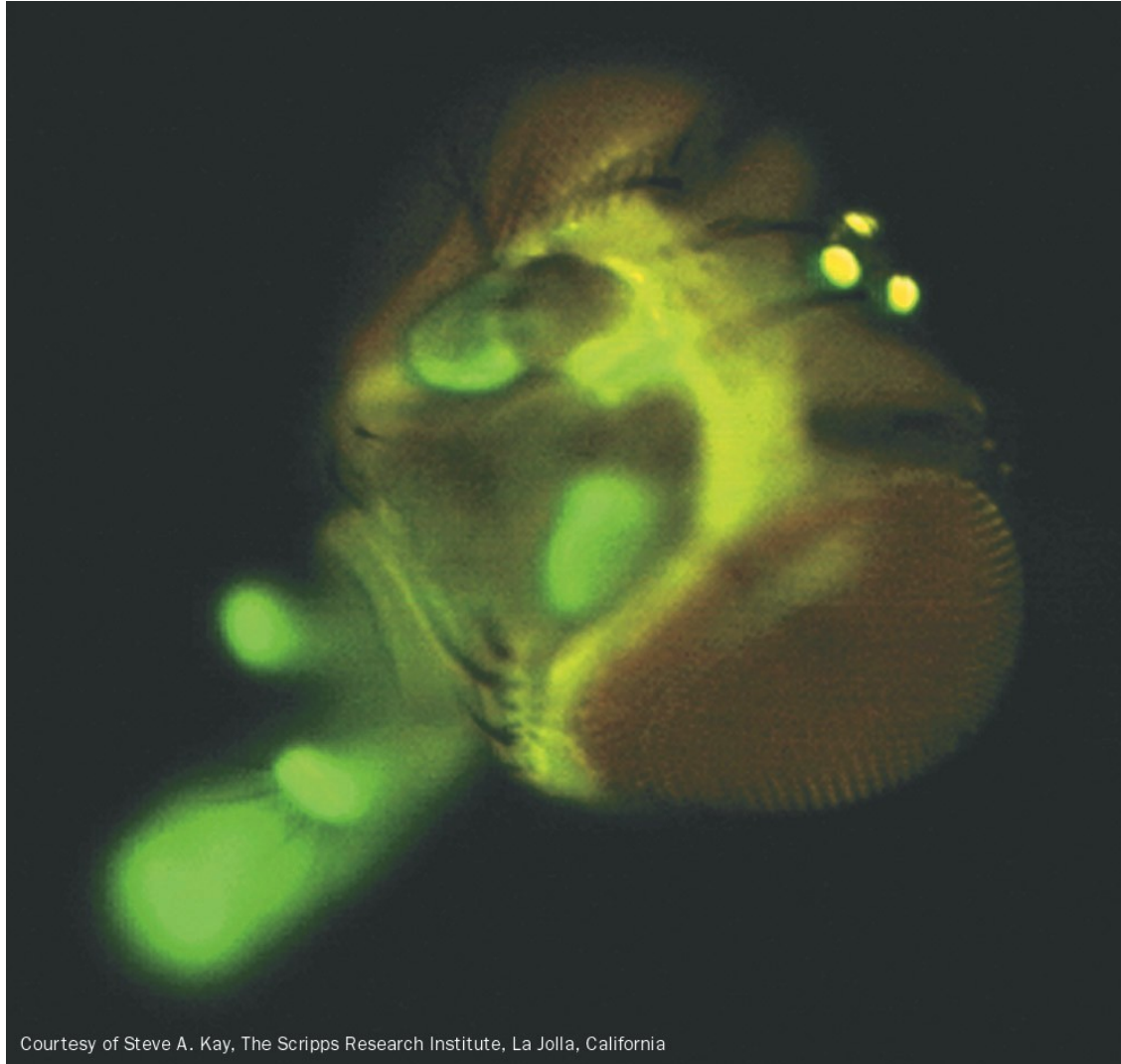
Figure 13-3 Concepts in Biochemistry, 3/e
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Inklusní tělíska



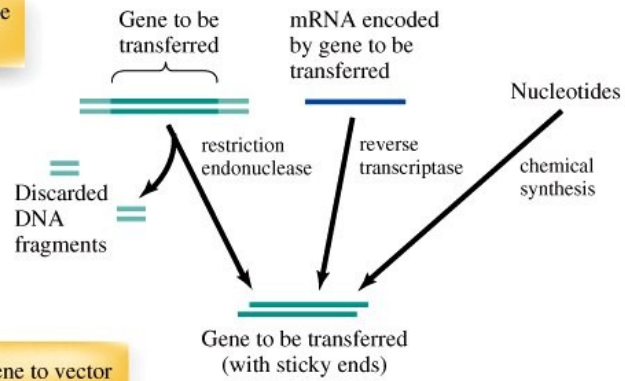
Courtesy of Teruhiko Beppu, Nihon University, Japan

Green Fluorescent Protein

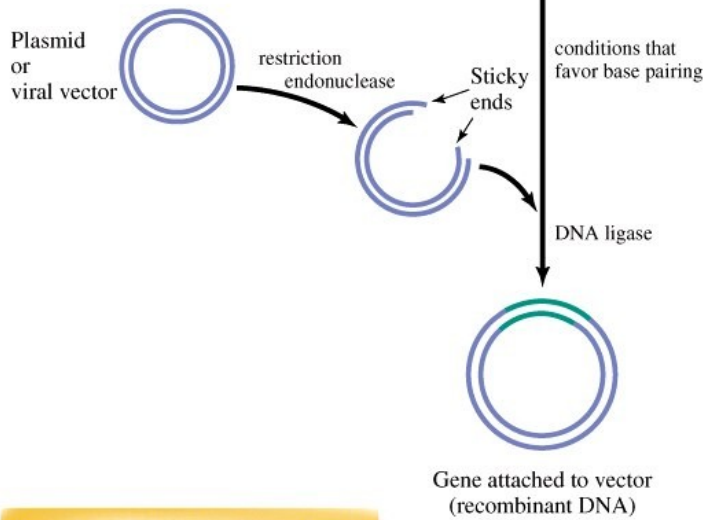


Courtesy of Steve A. Kay, The Scripps Research Institute, La Jolla, California

Step 1. Obtain gene to be transferred.



Steps 2 and 3. Attach gene to vector



Steps 4 and 5. Introduce recombinant DNA into host cell.

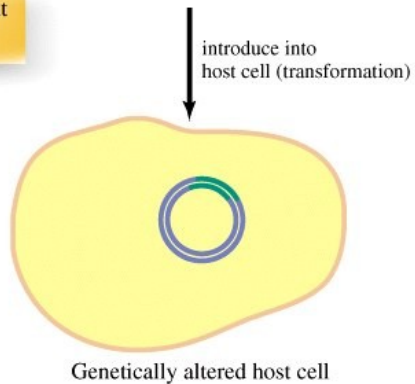


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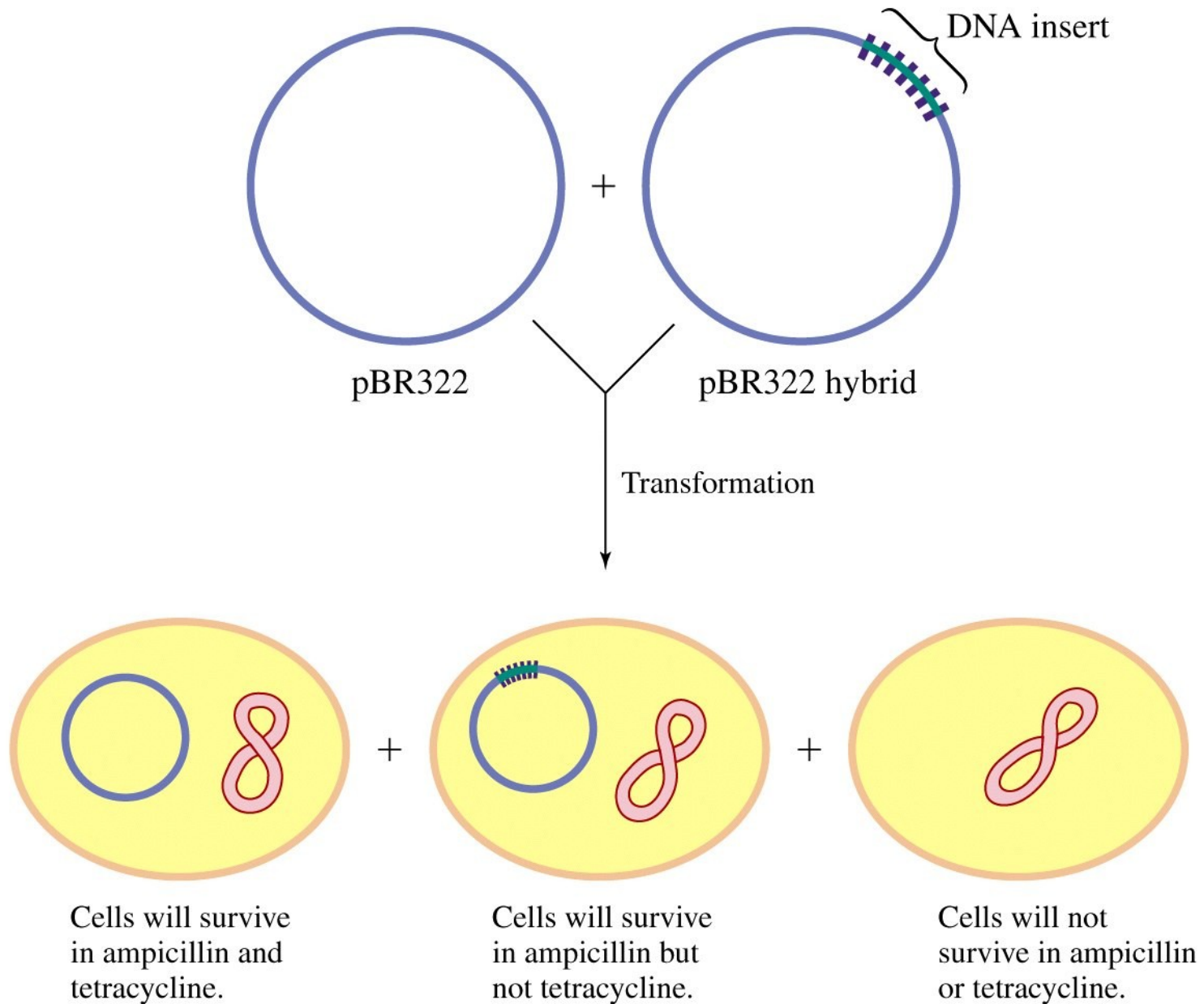


Figure 13-6 part 3 Concepts in Biochemistry, 3/e
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Table 13.1**Recombinant proteins and their use**

Protein	Use
Human insulin	Treatment of diabetes
Human somatotropin (growth hormone)	Treatment of dwarfism
Bovine somatotropin (BST)	Enhances milk production in dairy cattle
Porcine somatotropin	Enhances growth in pigs
Pulmozyme (DNase)	Treatment of cystic fibrosis
Tissue plasminogen activator (TPA)	Treatment of heart attack, stroke victims; dissolves blood clots
Erythropoietin	Stimulates erythrocyte production in anemia
Interferons	Antiviral agent; treatment of cancers
Atrial natriuretic factor	Reduces high blood pressure
Leptin	Treatment of obesity
Hepatitis B vaccine	Treatment of hepatitis
Herceptin	Monoclonal antibody to treat metastatic breast cancer
Superoxide dismutase	Destroys reactive oxygen species; treatment of arthritis

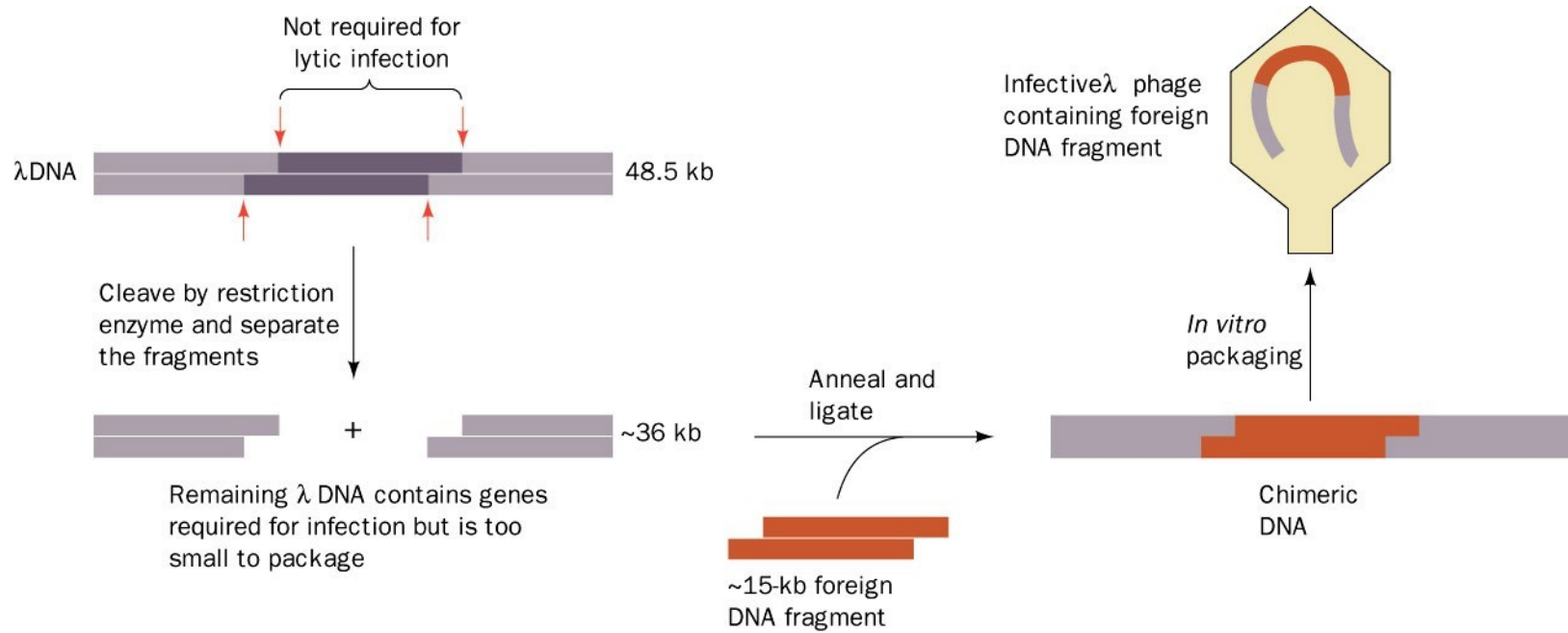


Table 13.2**Human gene therapy projects currently in or preparing for clinical trials**

Disease	Defective Protein, Gene, or Inserted DNA
Lesch-Nyhan syndrome	Hypoxanthine–guanine phosphoribosyl transferase (Section 19.5)
Amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease)	Superoxide dismutase
Adrenoleukodystrophy (ALD)	Very long chain fatty acid synthetase transporting protein
Severe combined immunodeficiency (SCID)	Adenosine deaminase
β -Thalassemia	β -Globin, a polypeptide of hemoglobin
Familial hypercholesterolemia	Liver receptor for low density lipoprotein (LDL) (Section 18.5)
Hemophilia	Blood-clotting factors
Duchenne's muscular dystrophy	Dystrophin
AIDS	The gene to produce a ribozyme that cleaves HIV RNA
Inherited emphysema	α_1 -Antitrypsin
Cystic fibrosis	A product that unclogs lung mucus is inhaled in a nasal spray
Cancer	E1A and p53 tumor suppressor genes

PCR Mullis

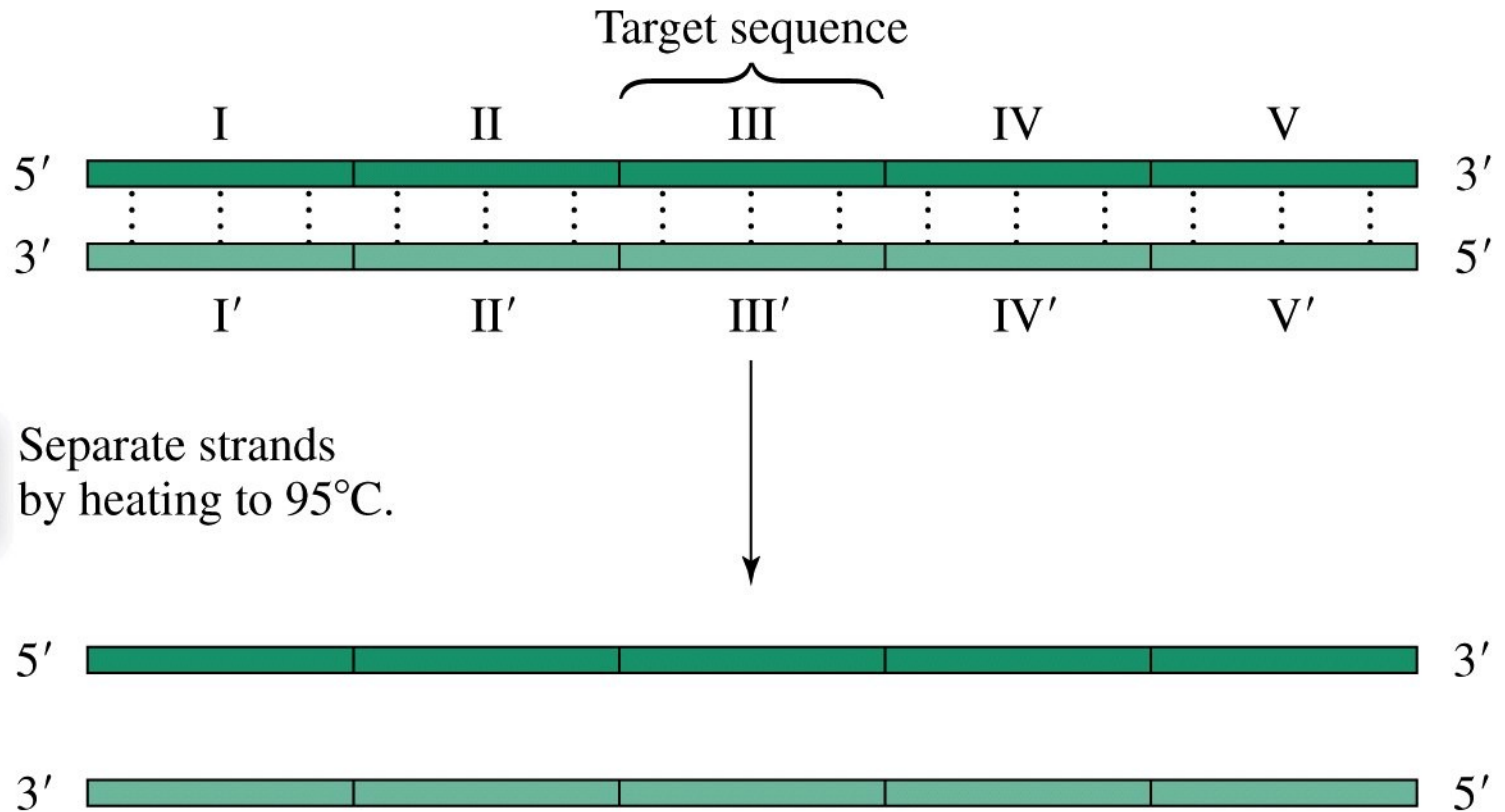


Figure 13-11 part 1 Concepts in Biochemistry, 3/e
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Step 2

Hybridize primers by cooling to 50°C.

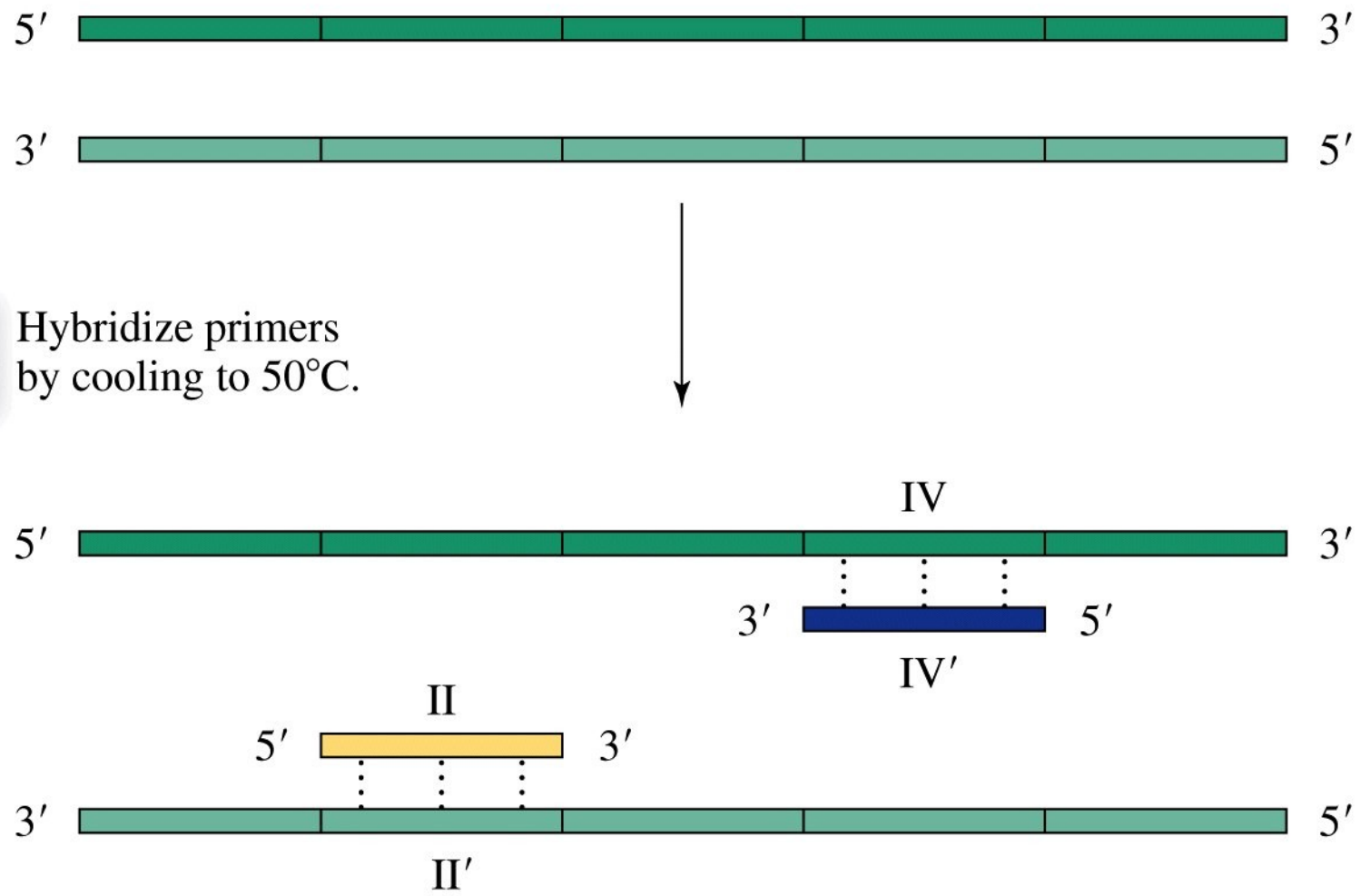
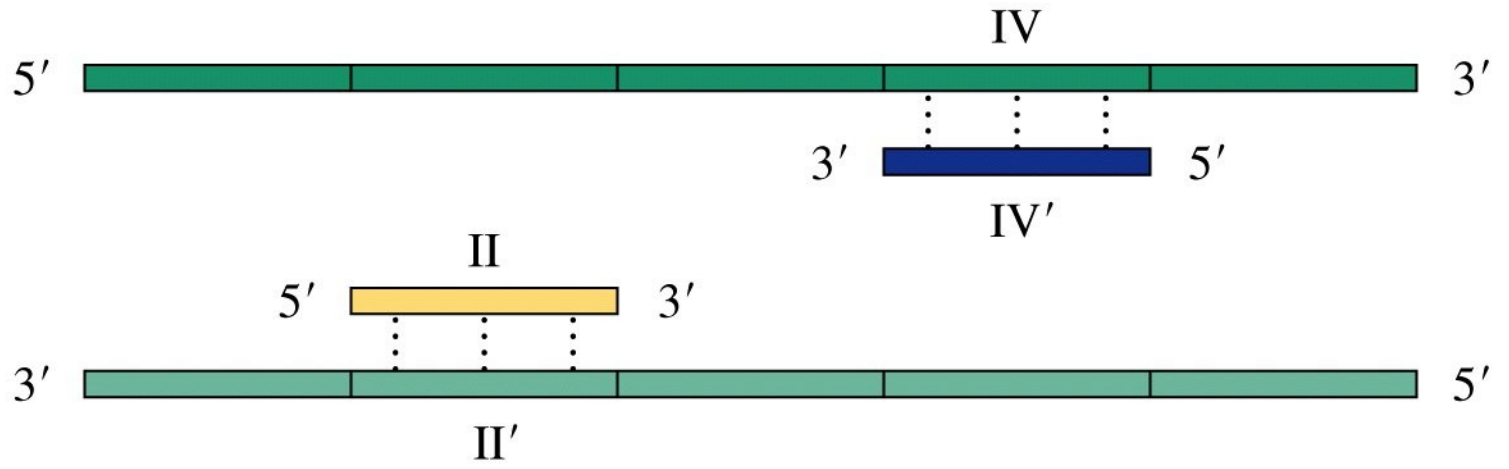


Figure 13-11 part 2 Concepts in Biochemistry, 3/e
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Step 3

DNA is synthesized by extending the primers at 72°C.

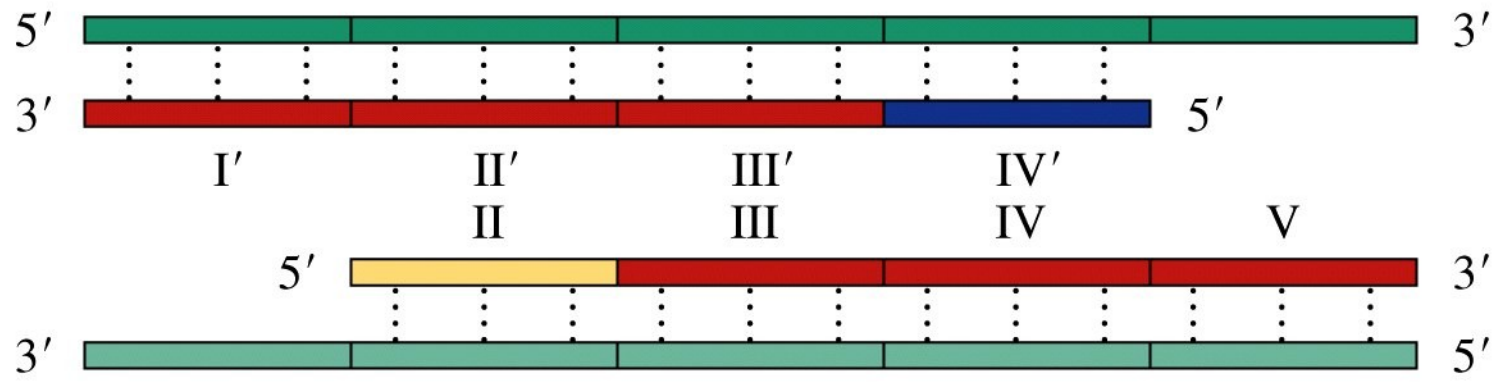
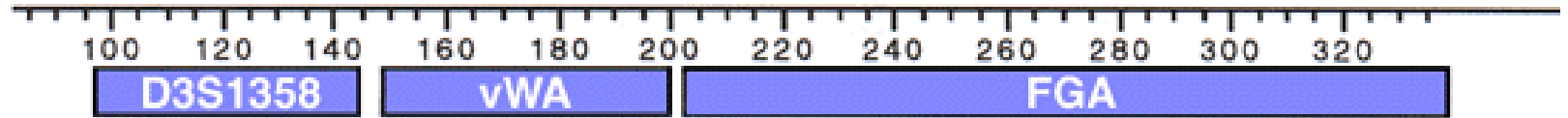
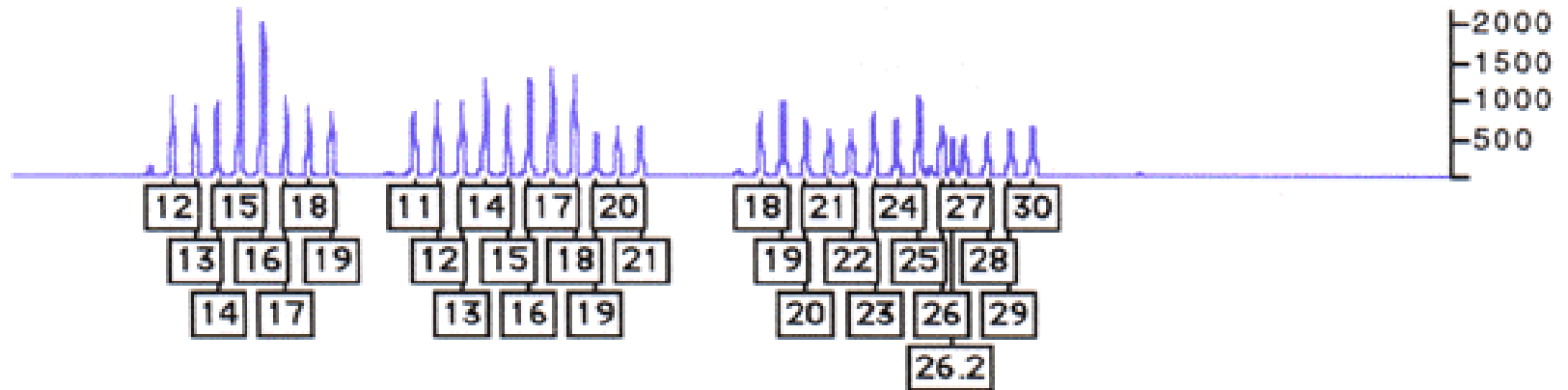


Figure 13-11 part 3 Concepts in Biochemistry, 3/e
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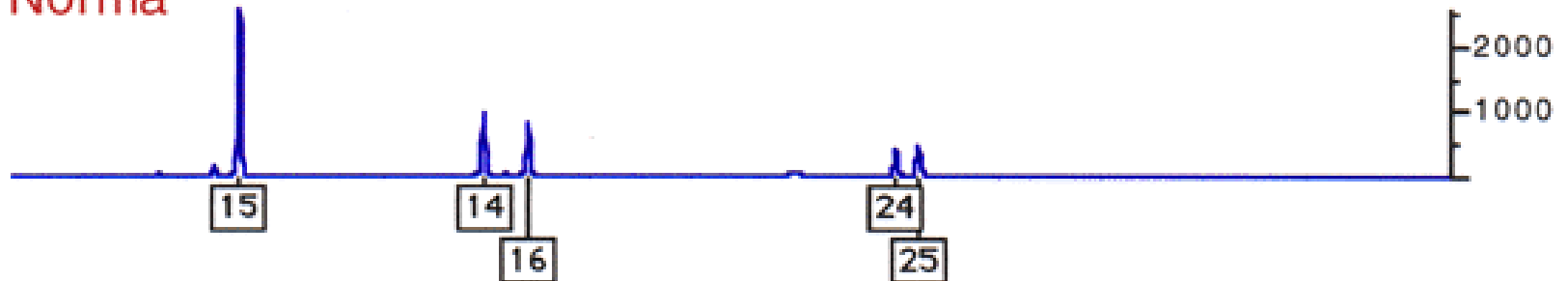
Short tandem repeats



• A5*PROFI...US LADDER 4 Blue PROFILER PLUS LADDER



Norma



DNA Array

